Peak Bone Mass, Lifestyle Factors and Birth Weight: A study of 25-year old women

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Abstract

Background: Osteoporosis is a common bone disease, which does not give symptoms until the ultimate outcome, the fragility fracture occurs. Regulation of bone mass is controlled by genetic, environmental and nutritional influences. Peak bone mass, defined as the maximum bone mass accrued, is usually reached by the third decade of life and is an important determinant of future osteoporotic fracture. A number of lifestyle factors, among them physical activity and smoking and even weight at birth are associated with bone mass. However, to what extent these factors are associated to bone mass in young age, peak bone mass, are less clear.

Aims: To evaluate peak bone mass and its association with birth weight, recreational levels of physical activity and smoking. To evaluate how bone mass in Swedish young adult women compares with other similarly aged populations and the DXA manufacturer supplied reference values.

Methods: 1061 women, aged 25 years at inclusion, were recruited to the PEAK-25 cohort. All participants were measured with DXA. In addition a comprehensive lifestyle questionnaire was completed, including detailed data on physical activities and smoking. Birth anthropometrics were obtained from the Swedish National Board of Health and Welfare.

Results: The BMD values of the PEAK-25 cohort were generally higher than equivalently aged European and North American cohorts and the reference cohort incorporated for reference in the DXA scanner. Women with lower birth weight had lower bone mineral content and lower birth weight appears to have a greater negative influence on bone mass than the positive influence of higher birth weight. Recreational levels of physical activity were found to be associated with higher peak bone mass and BMD gains were maximized through regular, high-impact exercise. We found that the quantity of cigarettes consumed, but not smoking duration, is negatively associated with peak bone mass. BMI increases with longer smoking duration and may partly reduce the adverse effects of smoking on bone.

Conclusions: If available, ethno-geographically obtained reference data should be used in order to receive more appropriate results from DXA scanning and improve diagnostic accuracy. We have identified risk factors associated with peak bone mass which have the potential for modification. Promoting physical activity, even on recreational level, will have beneficial influence on peak bone mass. Further, if not complete cessation of smoking, a reduced number of cigarettes may have beneficial effects on bone health. For bone promoting measures, children with low birth weight ought to obtain additional support.

Key words
Peak bone mass, normative values, birth weight, physical activity, smoking, bone mineral density, bone mineral content, dual energy X-ray absorptiometry

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Pappa

To Filippa, Fedon and Kassandra

Contents

List of Publications

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

I. M. Callréus, F. McGuigan, K. Åkesson. Country-specific young-adult DXA reference data are warranted for T-score calculations in women: Data from the PEAK-25 cohort. Accepted for publication in Journal of Clinical Densitometry.

II. M. Callréus, F. McGuigan, K. Åkesson. Birth weight is more important for peak bone mineral content than for bone density: The PEAK-25 study of 1,061 young adult women. Osteoporos Int 2012; DOI: 10.1007/s00198-012-2077-8.

III. M. Callréus, F. McGuigan, K. Ringsberg, K. Åkesson. Self-reported recreational exercise combining regularity and impact is necessary to maximize bone mineral density in young adult women: A population-based study of 1,061 women 25 years of age. Osteoporos Int 2012; 23(10): 2517–2526.

IV. M. Callréus, F. McGuigan, K. Åkesson. Adverse effects of smoking on peak bone mass may be attenuated by higher BMI in young female smokers. Submitted for publication.

Abbreviations

The following abbreviations, listed in alphabetic order, are used in this thesis:

BMC	Bone mineral content
BMD	Bone mineral density
BMI	Body mass index
BUA	Broadband ultrasound attenuation
COMB-RP	Combined score of RAL and PSS
DXA	Dual energy X-ray absorptiometry
ELBW	Extremely low birth weight
FN	Femoral neck
GRF	Ground reaction force
LBW	Low birth weight
LS	Lumbar spine L1 – L4
LS2	Lumbar spine L2 – L4
PBM	Peak bone mass
pDXA	peripheral Dual energy X-ray absorptiometry
pQCT	peripheral Quantitative Computed Tomography
PSS	Peak Strain Score
QCT	Quantitative Computed Tomography
QUS	Quantitative ultrasound
RAL	Recreational Activity Level
SEK	Swedish crown (currency)
SI	Stiffness index
SNBHW	Swedish National Board of Health and Welfare
SOS	Speed of sound
TB	Total body
TH	Total hip
TR	Trochanter
VLBW	Very low birth weight
WHO	World Health Organization

Introduction

Osteoporosis, the silent disease with no symptoms until the first fracture, is a major health problem worldwide. Its major manifestation, fragility fractures, create significant human affliction such as pain, reduced independence, debility and increased morbidity and mortality.

Approximately 70,000 osteoporosis-related fragility fractures occur in Sweden every year with a high financial burden for society. An estimated first-year direct cost for a hip fracture is €12,870–19,667 and €2,048–14,219 for a clinical vertebral fracture (2010) [1, 2].

To reduce the risk of experiencing an osteoporotic fragility fracture, several measures exist which include pharmacological treatment of osteoporosis, nutrition, modification of environmental factors, fall prevention and different behavioral changes such as physical exercise [3]. Identification and early intervention is thus of great interest both for the individual and for society.

Peak bone mass (PBM) is the highest amount of bone mass reached for an individual and is considered to be attained around the third decade, although the exact timing varies depending on sex and skeletal site. Peak bone mass, which is 60–80% determined by genetic factors, plays an essential role for the future risk of osteoporotic fracture. Despite the extensive genetic influence, there is scope for modification of bone mass as the heritability of fracture decreases with age while the contribution from environmental factors increases [4]. This study focuses on bone mass and its determinants in young adult women, a time close to peak bone mass and a period in life that has not been particularly studied by others.

However, in order to have the opportunity to make a change or intervention for the individual, accurate methods are needed for bone mass examinations. Diagnosis is established with dual X-ray absorptiometry (DXA) using the T-score, which is a relative calculation from a reference population. It is therefore of the utmost importance that the reference data are adequate for the measured population, otherwise misdiagnosis may increase.

In the earliest phase of life, the intrauterine environment, which may be reflected in birth weight, plays a role in modifying the genetic influence on bone. Later in life, the individuals' own choices can affect the future outcome of osteoporosis and subsequent fracture. Physical inactivity has a detrimental influence on bone

mass and may already in early years significantly impair peak bone mass, which is an important factor for future osteoporosis.

In addition, long-term smoking in older populations is associated with decreased bone mass and higher hip fracture incidence [5], although knowledge about smoking and bone mass in younger population is scarce.

In order to identify subjects at risk of osteoporosis, an improvement of methods for assessment is needed. Also, for knowledge of how certain factors have either positive or negative influence on the early developed peak bone mass, further studies are needed on populations at an age representing peak bone mass.

In this thesis we used data from the PEAK-25 cohort in order to determine the role of birth weight, physical activity and smoking for peak bone mass. The cohort is comprised of 1,061 women, all 25 years old at inclusion.

Review of the Literature

Bone

The skeleton comprises the axial skeleton including head and trunk, and the appendicular skeleton consisting of the limbs and pelvic girdle belongs to it. Its main function as a dynamic connective tissue is to provide a mechanical entity for both locomotion and protection of the body's vital organs. In addition, bone is metabolically active and serves as a reservoir for minerals, mainly calcium, magnesium and phosphate [6]. Bone also acts as the primary site for hematopoiesis and recently also implications have been found that bone exerts feedback control of energy homeostasis [7, 8].

Bone, as a composite connective tissue consists of both organic and inorganic matter. Approximately 60% of its weight is composed of inorganic matter, mainly a form of impure hydroxyapatite ($Ca_{10}[PO_4]_6[OH]_2$) and 8–10% water. The remainder consists of organic matter [9] where collagen dominates.

The outer side of bone is lined by a membrane, the periosteum, which covers the entire bone surface except articular surfaces and tendon and ligament insertion points. On the inner side of bone the endosteum resides, even though it is not a morphologically recognizable layer of tissue. It is composed of bone surface cells, including osteoblasts and bone lining cells.

It was identified early on that bone was influenced by mechanical loading associated with normal daily living. This concept was accepted and during the 19th century the theories were elaborated [10-12]. Julius Wolff postulated early in his hypothesis that the stress directions affecting bone led to the alignments of the trabecular bone [13]. This theory was later named Wolff's Law. However, even if some errors have been identified in Wolff's work, the main principle that the trabecular bone alignment is created by a functional adaptation process remains even today.

As regards the microarchitecture, the skeleton consists of two types of bone, cortical and trabecular, with cortical bone accounting for 80% of the total skeletal mass. The trabecular bone is located mainly within the axial skeleton and in the metaphyses of the long bones. The distinction between these types of bone is based on their porosity, which is a major determinant of both stiffness and strength

[14, 15]. The trabecular bone is more associated with the metabolic capabilities, whereas cortical bone stands mainly for the mechanical strength. Cortical bone has a high resistance to rotational and bending forces, while trabecular bone has higher shock absorption ability [16]. Bone is continuously remodeled, which consists of resorption and deposition of bone mineral. Age is one factor influencing remodeling. During the first two years of life, remodeling of cortical bone can be as high as 50%, while it decreases to 2–5% per year in the elderly. Trabecular bone has a 5–10 times higher remodeling rate than cortical bone [17].

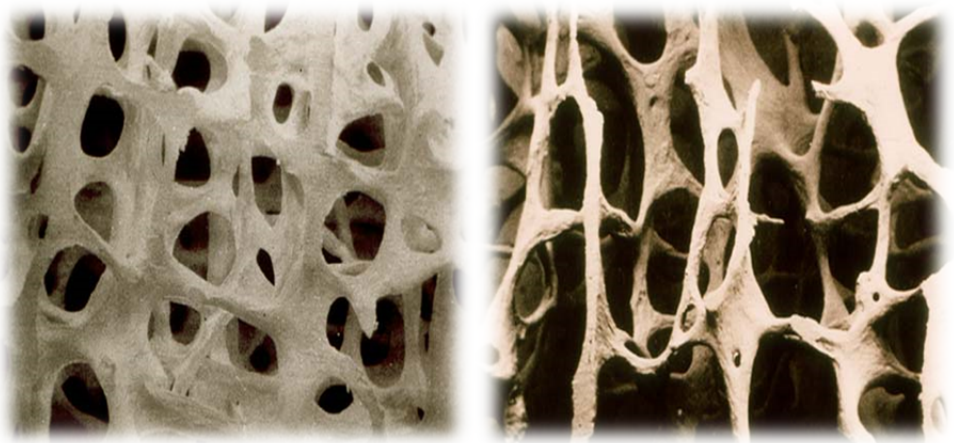

Figure 1 Normal trabecular bone (left) and osteoporotic trabecular bone (right) (*Courtesy of iofbonehealth.org*).

The results of the changes in the formation and resorption processes can be estimated by measuring bone mineral density (BMD). Disorders in remodeling, where bone resorption exceeds bone deposition, create imbalances. These imbalances increase, mainly influenced by age, and lead to osteopenia and osteoporosis (Figure 1).

Osteoporosis

In the mid-19th century the book *A Treatise on Dislocations and Fractures of the Joints* was published by Sir Astley Cooper (1768–1841). At this time the term osteoporosis was unknown but the complication in the form of hip fracture was well recognized as a sign of ageing. In his book he stated concerning aging: “That regular decay of nature which is called old age, is attended with changes which are easily detected in the dead body; and one of the principal of these is found in the bones, which become thin in their shell, and spongy in their texture; hence the

light soft bones of old persons may be cut with a pen-knife…" [18]. This terminology indicated the signs of osteoporosis.

However, the state of osteoporosis was later evaluated and is considered to originate in the work by Pommer in 1885, where he demonstrated that rickets and osteomalacia were due to lack of calcification of new bone tissue, whereas osteoporosis was merely a deficiency of bone [19].

The term postmenopausal osteoporosis was first used in the 1940s, as Fuller Albright, an American endocrinologist, recognized the drop in estrogen levels at menopause and associated it with bone loss [20, 21]. For primary osteoporosis it was later proposed by Fuller Albright together with Reifenstein [22], that it actually consisted of two separate conditions: bone loss related to drop in estrogen at menopause and bone loss related to aging.

Definition of osteoporosis

Osteoporosis is a multifactorial disease of the skeleton, controlled by genetic, environmental and nutritional influences, with genetics the most important, determining approximately 50–70% of variance in bone mass [23, 24] and environmental factors contributing 30% [25].

The accepted definition of osteoporosis is that it is a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture [26]. The risk of fracture has to be included in the definition of osteoporosis as its outcome often is a fracture resulting from low-impact trauma. Large prospective cohort studies have been performed demonstrating that a decrease of one standard deviation in BMD may increase the risk of a fracture by a gradient of risk ranging from 1.5–3 [27-29]. The specific gradient of fracture risk varies as it is dependent on technique used, the type of fracture and the site measured [30].

The ability of the bone to resist fracture caused by trauma is related to the bone strength, which may be estimated through BMD, hence 75–90% of the variance in bone strength is related to BMD [31]. However, other factors help determine bone strength, which includes volumetric bone density, cortical thickness, microarchitecture and the intrinsic bone quality [32].

Diagnosing osteoporosis in a patient may be done in one of three different ways: the presence of a fragility fracture without the explanation of other causes; BMD measurements with DXA; or bone biopsy [30, 33, 34], with DXA providing the operational diagnosis of osteoporosis currently used.

As osteoporosis is defined as lower BMD, predictors for BMD at older ages are important to identify. What may affect future BMD are factors that have both neg-

ative and positive influence and some factors are subject to influence by the individual herself while others are not. However, the most significant determinants of future BMD, which is under influence of negative and positive factors, are the peak bone mass and the rate of subsequent bone loss [35].

Peak bone mass

Peak bone mass is a cornerstone in the physiology of the skeleton, defined as the amount of bone present at the end of skeletal maturation. It is an important determinant of osteoporotic fracture risk [36], and an estimate is that an increase of peak bone mass by one standard deviation may decrease the risk of future fracture by 50% [37].

Peak bone mass is considered to be reached approximately 10 years after the end of skeletal growth, that is to say, between 20 and 30 years of age. Some studies have identified more accurate intervals for the occurrence of peak bone mass, different depending on region, where hip BMD is reached at an age of 16–19 years and after 30 years of age in the lumbar spine [38]. A narrower range has also been presented, with attainment of peak bone mass at the end of the second or early in the third decade [39]. Even older ages have been discussed when it comes to total body BMD, as in some studies only slight changes have been observed in the age range 18–50 years [40], which may vary with ethnicity and geography.

Bone mass at older ages is principally determined by two factors: the amount of bone attained at young age and the subsequent loss of bone in the following years. The bone loss which occurs after the menopause may be either fast or slow, indicating a variation in bone turnover rates between different individuals, fast and slow losers of bone [35, 41]. Peak bone mass has been suggested to account for more than 50% of the variance of BMD at older ages [42]. Figure 2 shows a schematic illustration of the skeletal growth trajectory, peak bone mass and subsequent bone loss.

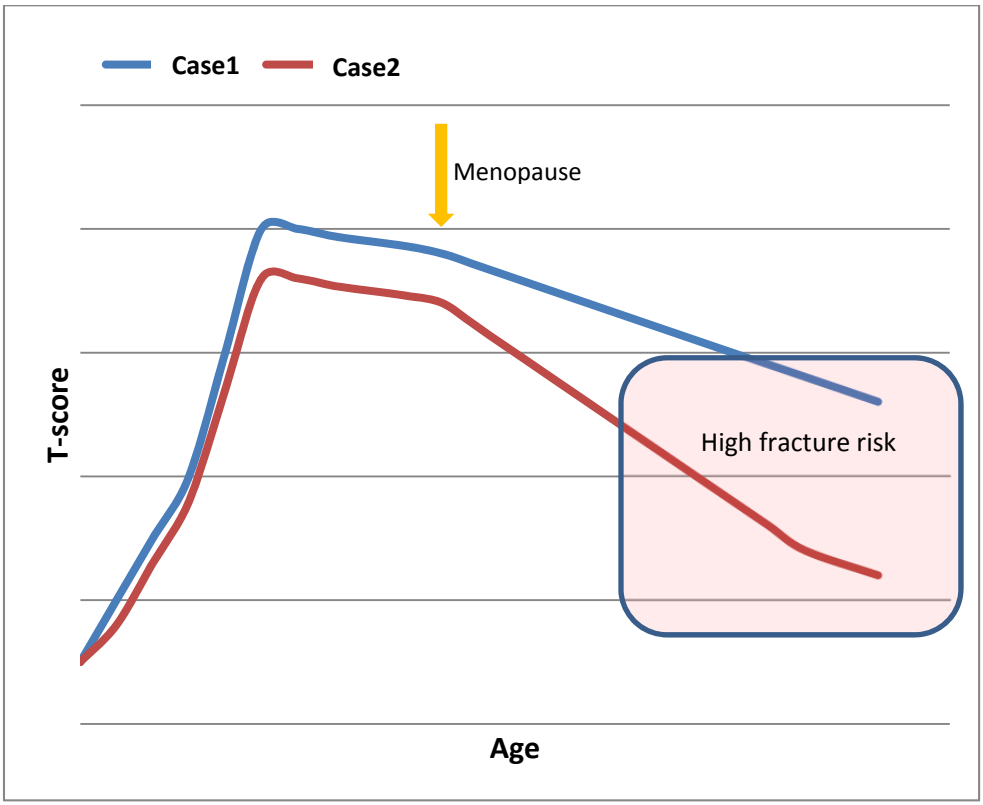

Figure 2 A schematic representation of bone mass during life. The red line (Case2) represents the outcome resulting from lower peak bone mass and faster bone loss after menopause, resulting in entering the zone of high fracture risk at an earlier age. Case1 shows the opposite.

The genetic influence on peak bone mass may be regulated by a multitude of nutritional, hormonal and other environmental factors which are to a greater or lesser extent modifiable [4, 43].

Epidemiology and outcomes

Life expectancy is increasing due to improved hygiene, vaccinations, better nutrition and improved treatments for certain diagnoses during recent decades [44] but better living circumstances such as housing have also contributed. However, with increasing age come problems associated with the musculoskeletal system, with osteoporosis as one of the most common diseases. And the ultimate outcome of osteoporosis is the fragility fractures caused by higher skeletal fragility, which develops: (1) when the bone mass is decreased; (2) due to inefficient architecture of bone; and (3) when bone quality decreases [32].

Osteoporosis increases with advancing age, a phenomenon that is seen all over the world. It is then followed by an increase in fracture rates. As the elderly are the fastest-growing segment in the world population, this is estimated to make a considerable impact on fractures in general and especially hip fractures. If the age-adjusted incidence of hip fractures remains unchanged, there will be an estimated increase from 1.7 million hip fractures in 1990 to 6.3 million in 2050. However, as there are indications of increasing fracture rates all over the world, a rise of 1% per year in fracture rates would result in 8.2 million hip fractures in 2050 [45]. In the UK, the burden of osteoporosis, estimated by a disease model which is validated with Swedish data, is expected to increase by approximately 20% by the year 2020 [46]. Recent studies with age-adjusted data demonstrate a general decline in fracture rates. Therefore, the general increase may be attributed to increased number of individuals and not the incidence, which many studies have shown to be unchanged or even decreasing [47].

In addition to personal suffering caused by fracture, there is also a significant economic burden on societies. Within the European Union the annual cost for all osteoporotic fractures is estimated at $30 billion and in the US approximately $20 billion [45, 48].

There are regional differences in the occurrence of fragility fractures, with the USA and the Scandinavian countries standing out compared to Central Europe and Britain [49]. In Sweden, a total of 304,000 hospital days were dedicated to the care of osteoporosis and its related fractures [50]. The annual cost of fracture was estimated to be 5.6 billion SEK, which represents 3.2% of the total health care costs in Sweden, where community care accounts for 66% of the total cost. When the value of QALY (quality-adjusted life-years) is included, the sum rises to >15 billion SEK annually. This amount is expected to increase dramatically over the years, an estimation is >26 billion SEK in 2050, assuming no change in fracture risk [51].

After the early years in life when fractures are predominantly due to e.g. sports and trauma, fracture incidence starts to increase around the age of 50 in women, and the incidence is twice that in men [52, 53]. At this age, osteoporosis becomes the major underlying cause of fractures, commonly striking the hip, forearm, proximal humerus, pelvis and vertebral column [54] (Figure 3) and there is a 50% risk that women at this age will have a fragility fracture during their lifetime [45]. Hip fractures have an exponential increase with age as the rate is 2/100,000 person-years in women under age 35, but this explodes to 3,032/100,000 person-years at 85 years of age. The corresponding number of fractures for men is 4 and 1,909, respectively. The higher susceptibility to fracture in women is due to the larger bone loss in women when they age compared to men. Other reasons are that women have a greater tendency to fall and also to live longer [49].

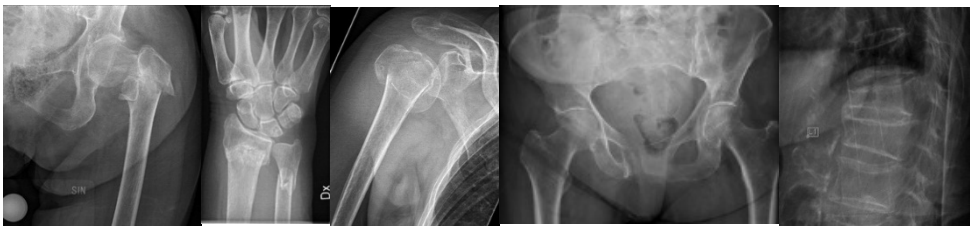

Figure 3 X-rays for the most common osteoporotic fragility fractures. From the left, hip, distal radius, proximal humerus, pelvis and vertebral fractures.

The cause of the fractures is mainly low-energy trauma. For hip fractures, a majority are caused by falling from standing height or less, whilst approximately 10% originate from more severe trauma [55]. Predictors such as poor health, decreased balance, low activity level and fall history have been connected to increased falling rates [56].

Survival depends on the type of fracture with hip fracture being most strongly associated with mortality. Some 10–20% more women than expected for age die within the first year [48] and the five-year survival of a hip fracture is 82%, with most deaths occurring within the 6 months following the hip fracture [57]. Even vertebral fractures have shown to cause excess mortality compared to the population [58]. Some studies have ascribed 30% of the excess mortality to the fracture event [1], and although some reports indicate a decline in hip fracture mortality, an increase in comorbidity afflicting life quality has been noted [59, 60].

The forearm and proximal humerus fractures do not present an association with mortality as hip fractures do, but still they contribute to disability and suffering for those affected. However, some studies have reported increased mortality after fracture of the proximal humerus [61]. Furthermore, a prior fracture, such as an uncomplicated distal forearm fracture, may be an indicator of future hip fracture [62-64], which calls for attention in this group of patients when it comes to preventive measures.

Although measures may be taken to decrease the risk of fracture in the elderly, intervention should perhaps start earlier, as achieving a high peak bone mass has been suggested to be of great importance in giving protection against fragility fractures later in life [65].

Assessment of bone mineral density

Traditional radiographic techniques cannot identify osteoporosis until it is severe, which means that it can be diagnosed, but no evaluation of the degree of osteoporosis is possible. Historically, osteoporosis was a clinical diagnosis that necessitat-

ed a history of one or more low-trauma fractures, mainly of the vertebra. However, this approach precluded the possibility of early intervention. Not until the last few decades have accurate noninvasive methods been available, which mainly concentrate attention on BMD.

In 1994, a working definition of osteoporosis was created by senior authorities in this field, acting on the behalf of the World Health Organization (WHO) [66, 67]. Here, a cutoff value at 2.5 standard deviations below the average of young adults was suggested in order to set the diagnosis osteoporosis (Table 1). These diagnostic criteria were based on measurements with dual energy X-ray absorptiometry (DXA), which is the technique validated for assessing bone mineral density [34, 66].

The clinical consequence of lower bone mass is fracture, which is why the focus has been on the ability of the different techniques to predict the probability of fracture. For hip BMD, measured with DXA, it provides a strong indicator of fracture risk, especially for computing the long-term fracture probabilities [29].

Table 1 Definitions of normal BMD, osteopenia, osteoporosis and established osteoporosis for DXA.

Diagnostic category	Definition	T-score
Normal bone mass	BMD < 1 SD below the average of young adult mean	> –1 SD
Osteopenia	BMD 1 to 2.5 SD below the average of young adult mean	–1 to –2.5 SD
Osteoporosis	BMD > 2.5 SD below the average of young adult mean	< –2.5 SD
Established osteoporosis	BMD > 2.5 SD below the average of young adult mean and the presence of one or more fragility fractures	< –2.5 SD

DXA

DXA (dual energy X-ray absorptiometry) (Figure 4) is the standard method for assessment of BMD. The technique is relatively young, with the first clinical scanners introduced at the end of the 1980s [68]. The results produced by the DXA scanners are usually presented as T- and Z-scores, which are calculations of standard deviations in relation to a built-in reference population.

The reference population used for calculations of T-scores is extracted from NHANES III database and consists of white females, aged 20-29 years (n=409).

The NHANES III is a part of NHANES (National Health and Nutrition Examination Survey), which is a research program conducted by the National Center for Health Statistics (NCHS) with the purpose of assessing health and nutritional status in the US population. Several surveys have been conducted and the third survey, NHANES III [69, 70], was collected during the years 1988-1994 including 16,573 men and women.

The treatment guidelines issued for osteoporosis are mainly based on results from DXA measurements. Central DXA is the method of choice for measuring hip and lumbar spine BMD. In general, the reason for the use of these specific regions of interest (ROI) is that hip BMD has the highest reliability when it comes to predicting hip fracture risk [27, 29], while evaluating spine BMD is best for diagnosing spinal osteoporosis and for monitoring osteoporosis treatment response [71].

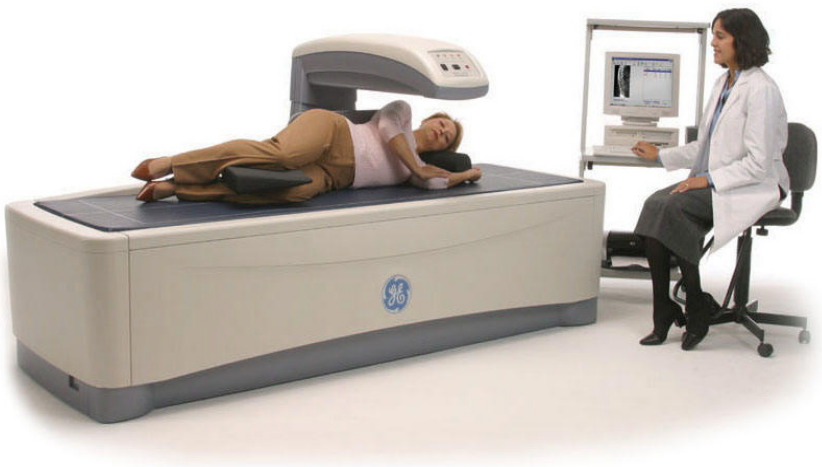

Figure 4 The GE Lunar Prodigy DXA scanner which was used to assess bone mass and body composition in the PEAK-25 cohort (*Courtesy of GE Healthcare*).

To establish the osteoporosis diagnosis, the reference ROI is the femoral neck, which should be used. In addition, diagnosis may be done in the lumbar spine and total hip in postmenopausal women and men aged 50 or older, but other hip regions such as Ward's triangle or trochanter are not recommended [69]. For assessment of osteoporosis specifically in the spine and evaluation of the risk of vertebral fracture, measurement of BMD in the lumbar spine is preferred. This relates to the increased trabecular bone loss seen in middle-aged postmenopausal women [72]. However, in older women, misdiagnosing may increase due to the degenerative changes in the vertebral column [73].

The DXA technique uses radiation and the first-generation scanners employed a narrow beam of radiation, the so-called pencil-beam scanners. A scanning session could take as long as 5–10 minutes for a hip or spine. However, with the introduction of the newer scanners which use a different technique, fan-beam scanners, the examination time was reduced to 10–30 seconds for a hip or spine and the image resolution was increased [74, 75]. The radiation beam is sent across the measurement site and a detector measures the attenuation of radiation which then relates to bone mineral content (BMC), which is measured in grams (g). The area measured is then determined by a computer (Figure 5) and hence the BMD can be calculated from these two factors [76].

The DXA technique has two major drawbacks. Firstly, the scanning is a projection of a two-dimensional image where measurement of the areal density is affected by the bone size and the true three-dimensional volumetric density. Secondly, the human body consists of three types of tissue – bone, lean muscle and fat – and DXA is limited in its ability to distinguish bone from soft tissue [77].

However, the advantages outweigh the disadvantages as the technique involves low radiation, short scan time, proven ability to predict fracture risk, stable calibration and high precision [77]. For in-vivo scans the precision, expressed as a coefficient of variation (CV), is 1–2.5% [78]. Today, the market for DXA scanners are dominated by three manufacturers: Lunar, Hologic and Norland.

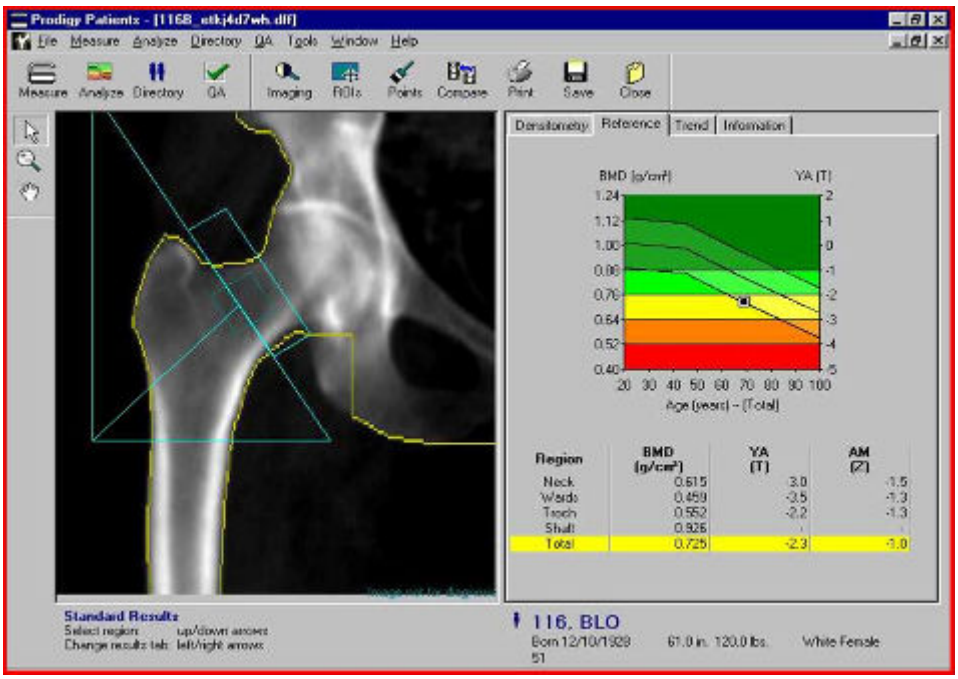

Figure 5 Screen capture from a hip scan with DXA Lunar Prodigy.

T-scores and Z-scores

The T- and Z-scores generated by the DXA scanners have different applicability and are used and calculated differently. The T-score is the preferred value for reporting BMD in postmenopausal women and in men age 50 or older, and the WHO densitometric classification (Table 1) applies. For calculation of hip T-scores, the measured BMD value is compared with the built-in reference population, which is recommended to be the white female, aged 20–29 years, NHANES III data [69]. It is calculated according to the formula:

$$T - score = \frac{Measured\ BMD - Young\ adult\ mean}{Young\ adult\ population\ SD}$$

The T-score used for the WHO classification of osteoporosis may only be applied to DXA in the femoral neck, total femur, lumbar spine and the one distal third of radius [69].

The Z-score is less frequently used as it is not applicable to the WHO diagnostic classification. Instead a standard deviation of –2.0 is considered the major breakpoint, where values above this limit are considered to be "within the expected range for age" while values below are "below the expected range for age". For the general calculation of Z-scores, the same formula as for T-score applies, but here the comparison is instead a mean BMD which is matched for age. The target groups for Z-score usage as BMD reporting are females prior to menopause, males under age 50 and children. However, osteoporosis may not be diagnosed in men under age 50 on BMD values alone.

$$Z - score = \frac{Measured\ BMD - Aged\ matched\ mean\ BMD}{Aged\ matched\ population\ SD}$$

For an older individual the T- and Z-scores may demonstrate a large difference because the calculations are based on different reference values, hence a low T-score may exist together with a normal Z-score. On the other hand, at the age of presumed peak bone mass, the difference between the T- and Z-score ought to be zero. This depends on the calculations, where both of the scores are calculated based on similar reference values.

Both the T- and Z-scores are adjusted for sex and ethnicity but the Z-score is also adjusted for body weight in Lunar but not Hologic scanners.

This can create larger differences between T- and Z-scores which can be significant [79] and as no standardization for Z-calculations exists; this may therefore render comparisons between the DXA manufacturers more difficult [80].

QUS

Even if DXA is the standard for assessing BMD, a quest for a cheaper and portable solution is in progress. Quantitative ultrasound (QUS) has come up as an alternative fulfilling these criteria, also by having no ionizing radiation. Measurements of the ultrasonic wave passing through cortical and trabecular bone are made and reported as the variables broadband ultrasound attenuation (BUA) and speed of sound (SOS). BUA is greater in a more complex bone structure, which means that normal bone has a higher BUA value than osteoporotic [81]. SOS is higher in bone with greater connectivity, which is the case in normal bone [82]. In addition, for Lunar Achilles® scanners, stiffness index (SI), a composite of BUA and SOS, is calculated according to the formula:

$$SI = 0.28 \times SOS + 0.67 \times BUA - 420$$

However, QUS may not be used to diagnose osteoporosis according to the WHO definition or to monitor the efficacy of treatment [83]. Even if the only validated skeletal site in clinical use of QUS is the calcaneus, it has been shown that QUS of the calcaneus has the ability to predict hip fractures and osteoporotic fractures in elderly women [27, 84, 85].

pDXA

Smaller DXA scanners, peripheral DXA (pDXA) have been developed for increased portability, and the scanned regions are the forearm and calcaneus. The forearm scans are considered to be predictive of wrist fractures and the calcaneus scans to be predictive of spine fractures [86, 87]. However for monitoring the effects of medical treatment the pDXA is not clinically established [69].

QCT

One of the shortcomings of the DXA technique is its inability to measure in a three-dimensional manner, giving a true volumetric density, but also the lack of ability to distinguish between cortical vs trabecular bone. Quantitative computed tomography (QCT) is a technique resolving these issues, although at a higher cost, both financially but above all with higher dosage of ionizing radiation. These disadvantages of QCT make the technique less applicable in clinical practice for BMD assessment, so it is mainly used for research purposes [88, 89]. For research purposes, the peripheral QCTs (pQCT) have become more widespread and are primarily used for volumetric measurements of bone density in the forearm and tibia.

Pharmacological therapy

Different approaches are available in the treatment of osteoporosis, aiming at different mechanisms and stages of the disease. In broad terms, the pharmacological treatments can be described according to their main target: antiresorptive targeting osteoclasts and anabolic targeting osteoblasts. In addition, specific pharmacological treatment is used together with calcium and vitamin D. The latter counteracts the secondary hyperparathyroidism caused by the negative calcium balance, whereby resorption may be reduced. Even if the effect of this supplementation has been uncertain, meta-analysis has demonstrated a 12% risk reduction of fracture [90] and also a risk reduction of falls [91].

The ultimate purpose of osteoporosis therapy is to reduce the risk of fractures. A recent meta-analysis of 34 studies [92] demonstrated differences between the marketed substances in their ability to prevent fractures in the hip and vertebral column. All of the included substances reduced the risk of new vertebral fractures, except etidronate, compared to placebo. Denosumab was more efficient than strontium ranelate, raloxifene, alendronate and risedronate in preventing new vertebral fractures. In addition, denosumab reduced the risk of hip fracture, together with risedronate, alendronate and zoledronic acid.

Antiresorptive therapy

Biphosphonates

This group constitutes today the most important and well-documented substances in the antiresorptive therapy. In Sweden, five of the substances are registered for the indication osteoporosis, namely: alendronate, risedronate, zoledronic acid, ibandronate and etidronate. Alendronate and risedronate are best documented and first choices. For cases with adverse side effects or lack of therapeutic response, the second choice is zoledronic acid. It is administered as an intravenous injection once a year. The bisphosphonates cause apoptosis in the osteoclasts, thereby reducing bone resorption [93, 94].

Raloxifene

Raloxifene is a selective estrogen receptor modulator (SERM), which acts like an estrogen agonist on bone tissue. It is considered to be the secondary choice in the treatment and its anti-fracture effect is mainly on vertebral fractures [95]. It is not associated with an increased risk of breast cancer as is estrogen, but may exacerbate climacteric symptoms and increase the risk of venous thromboembolism.

Strontium ranelate

Strontium ranelate has a dual action on bone by inhibiting bone resorption and stimulating bone formation, thereby improving bone microarchitecture [96, 97]. The nucleus of strontium is very near the size of calcium, so it is incorporated in the mineral phase into bones in the place of calcium.

Denosumab

One of the latest contributions to the arsenal of pharmacological treatment for osteoporosis is denosumab, which is a human monoclonal antibody. It acts by blocking the binding of RANKL (receptor activator of nuclear factor κB ligand) to RANK, thereby inhibiting the development and activity of osteoclasts. In this manner bone resorption is decreased and subsequent bone mass is increased. It is administered through subcutaneous injections every 6 months [98].

Anabolic therapy

Instead of inhibiting the resorption, stimulation of bone formation is the mechanism behind anabolic therapy. The agent used today is parathyroid hormone (PTH), either truncated (1–34, teriparatide) or full-length (1–84) which is administered through daily injections. PTH increases the trabecular bone, mainly in the vertebral column [99, 100], and is associated with a clear reduction in fractures. It is an expensive treatment.

Risk factor evaluation

The fragility fracture, is influenced by several factors. They may act somewhat differently, e.g. increasing BMD, reducing the risk of falls and decreasing the susceptibility to osteoporosis and avoidance of situational risks. The risk factors [3, 50, 91, 101, 102] in general can be divided into non-modifiable and modifiable factors (Table 2). Other risk factors not included in the table are comorbidities such as rheumatoid arthritis.

Table 2 Modifiable and non-modifiable risk factors for osteoporosis, falls and fractures.

Modifiable	Risk factor	Osteoporosis	Fall	Fracture
No	Higher age	+	+	+
No	Female	+	+	+
No	Previous fracture	+	+	+
No	Menopause (premature)	+		+
No	Body height (tall)		+	+
No	Heredity (of fracture)			+
No	Ethnicity (white/Asian)			+
Yes	Alcohol consumption	+	+	+
Yes	Physical activity (low)	+	+	+
Yes	Smoking	+	+	+
Yes	Low exposure to sun/vit D	+	+	+
Yes	Low dietary calcium	+		+
Yes	Cortisone treatment	+		+
Yes	Low weight/BMI	+		+
Yes	Predisposition to falls		+	+
Yes	Impaired vision		+	+
Yes	High caffeine intake			+
Yes	Low BMD			+

The influence of environmental risk factors is more pronounced at the hip than the lumbar spine [103], which gives lifestyle intervention more potential for reducing the incidence of hip fractures.

Fracture Risk Assessment by FRAX®

The specific risk factors (e.g. previous fracture, parent fractured hip, smoking, glucocorticoids, rheumatoid arthritis, secondary osteoporosis, alcohol and BMD) for each individual can be added up in a model in order to obtain an estimation of the individual's specific risk of fracture, and thereby enabling therapeutic actions.

The WHO Fracture Risk Assessment Tool (FRAX®) is a web- or desktop-hosted application producing an algorithm which is developed for fracture risk evaluation of patients (http://www.shef.ac.uk/FRAX/). It is based on several large international cohorts where individual patient models are combined with clinical risk factors and BMD at the femoral neck. The result produced by the application is the 10-year probability of a hip fracture or a major osteoporotic fracture in the shoulder, hip, forearm or spine. The result, given as an absolute risk, may be included in specific guidelines in order to help the clinician to decide whether the fracture risk for the patient is sufficiently high to benefit from pharmacological therapy for risk reduction [104], although the FRAX® is not suitable for monitoring the treatment response [105].

Risk factors and BMD assessment

Below follows an introductory outline of the topics addressed in the thesis.

BMD assessment

Bone mineral density is the most important predictor of low-energy fractures [27-29], which is why accurate methods are important for identifying individuals at risk. Its accuracy is partly determined by the use of correct reference data in the analyses. If the reference data are not appropriate, the method itself may be a source of error in the identification process.

DXA is the diagnostic tool of preference and the standard when diagnosing osteoporosis [69]. As described before, relative scores are used clinically; T- and Z-scores, and they are calculated based on reference values. Therefore is it of the utmost importance that the reference values are relevant for the measured population, for both clinical and research use.

The NHANES III database [70] is the recommended choice for calculation of T-scores in the hip. For the spine the recommendation is to use Caucasian, non-race-adjusted normative data for women and men, independently of ethnic group [69]. The reference data incorporated by the manufacturers of the DXA scanner, may not always correspond well to the measured population [106].

Several studies report bone mass data from different countries and regions where the regional and ethnic differences in bone mass are obvious. In Asian populations the BMD is lower than in Sweden, but at the same time the fracture rate in Sweden is 70% higher than in Asian settings [107, 108]. So even if BMD is the standard for prediction of fracture, BMD alone does not explain the high hip fracture rates

in Sweden, as other factors explaining the variation are e.g. body weight, degree of industrialization or level of physical labor, latitude, diet, reproductive history and femur anatomy [108].

However, in order to increase the precision of DXA for fracture prediction, the reference databases used have to be scrutinized in relation to the measured population for a better understanding of the results from the DXA scanner. This can be done through establishment of regional normative reference databases.

Birth weight

Birth weight is partly a result of the intrauterine living conditions, i.e. mother-fetus malnutrition, and has been related to several medical conditions such as depression, cancer, hyperthyroidism, type 2 diabetes, coronary heart disease, hypertension and osteoporosis [109-111]. The latter is important when it comes to discussions about bone mass, as several studies have been performed in order to confirm the relationships between birth weight and later bone mass [112-114], and also its associations with future fracture incidence. Adverse factors already in the fetal life or preterm birth may induce permanent negative effects on the skeleton [115]. While some studies have identified an association with BMD, more studies have found BMC to be more related to birth weight [112-114].

Although BMD is a better predictor of future fragility fracture, BMC has important clinical implications as a predictor of fractures, as decreased BMC has been associated with an increased risk of fracture in several studies [116, 117].

Not only the birth weight per se is the predictor, but also growth in childhood, which is linked to birth weight. The growth trajectory is programmed during intrauterine or early postnatal life [49, 118]. It is one of the most important determinants of the bone envelope and has been demonstrated to predict later fracture in the hip [119-121]. A retarded growth trajectory in infancy is seen in lower body weight at one year, which is associated with lower adult BMC [122].

Even if the growth trajectory is retarded, a catch-up in growth is usually seen from early infancy until 2–3 years of age, but may continue into adolescence. The catch-up is usually incomplete, which results in shorter and lighter bodily constitution [123], hence lower bone size and BMC.

Adult bone mass is a combination of both density and bone size, which both influence the fracture risk. Also, the bone geometry and properties constitute the bone strength [117]. A better growth trajectory is associated with greater bone size and bone strength and hence decreased fracture risk [121, 124].

Physical activity

Many studies have reported beneficial effects of physical activity on bone, but several of them are either based on athletes or focus on a specific sport, while less is known about normal populations taking part in physical activities on a recreational basis.

Physical activity during the first three decades of life may increase peak bone mass and hence decrease the incidence of future osteoporotic fracture events [49]. Even when the athlete retires from sport activity, the benefits remain although in a reduced manner, but may contribute a to lower risk of sustaining a fragility fracture in older age [125].

For the maximization of peak bone mass, physical activity is one major contributor [43], thereby establishing a better starting point for bone health later in life.

When bone is exposed to mechanical load, both dynamic and static, a response is generated as deformations of the bone matrix occur. The response consists of fluid flow within the bone, which is proposed to be the factor by which the bone cell network senses mechanical loading [126]. However, the dynamic mechanical stimuli on bone is more powerful than static loading, as it only requires a short duration of loading, preferably with high magnitude but few repetitions to initiate the osteogenic response [127-129] described above.

The mechanostat theory [130] is one of the classical works by Frost, originally proposed in 1964, where he stated the need for a strain on bone. There is a minimum level of strain on bone in order to generate an adaptive response. Strain below this level would cause bone loss, but strain above evokes bone modeling (Figure 6).

In addition to the activity level, the type of activity deciding the amount of strain imposed on the bone is of importance. Sport activities with greater axial load produce higher impact on bone, measured as GRF (Ground Reaction Forces, multiples of body weight) [131, 132]. The response from high-impact activities seems to be more efficient in optimizing peak bone mass than low-impact activities [133-135]. Despite the beneficial bone effects caused by the high-impact forces, excessive exposure to high-impact training may be injurious to bone in line with the mechanostat theory [130, 136].

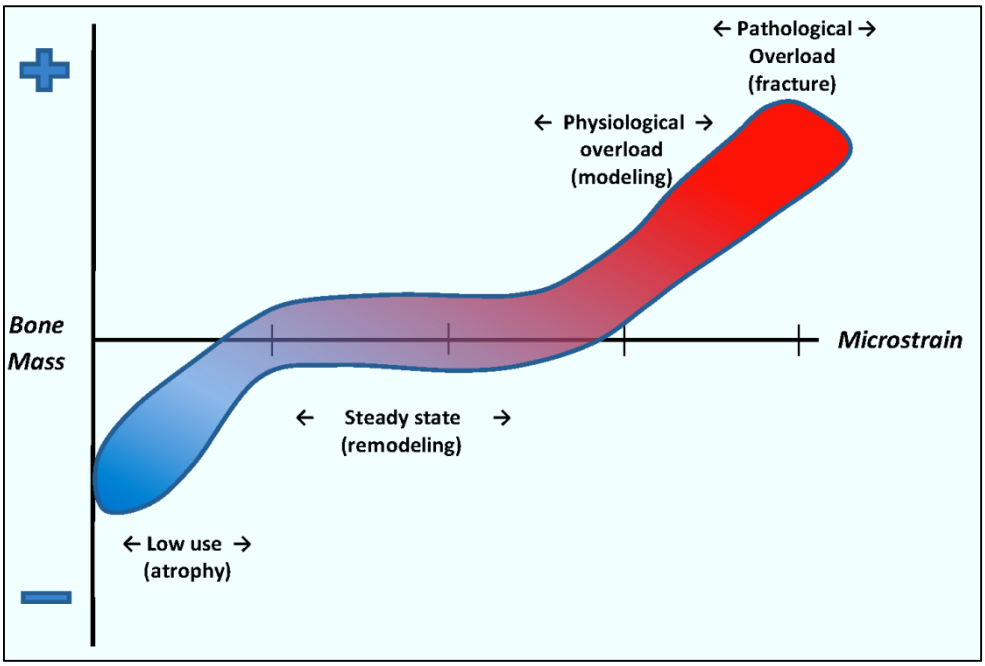

Figure 6 Graphical illustration of the "Mechanostat theory" [130]. Physical inactivity causes atrophy and bone loss, while physical activity within physiological limits generates modeling with an increase in BMD.

A major challenge in evaluating physical activity is the estimation of the strain produced, as this may vary within the same activity. Objective measurements of strain in certain activities are also scarce, and both these insecurities necessitate caution when evaluating results.

Enjoyment of physical activity is partly determined by behavioral patterns early in life, and for most individuals physical education in school plays a significant role in determining and maintaining healthy habits of exercising [137-141].

Smoking

Many epidemiological studies concerning smoking and osteoporosis originate from Daniell's report [142] in 1972 on the connection between smoking and osteoporosis. Despite extensive education concerning the hazards of smoking, the prevalence of smoking is expected to increase from today's 1.3 billion to 1.5 billion smokers in 2040–2050 [143]. Tobacco-related mortality is expected to increase as in the year 2000 approximately 5 million people were estimated to die from direct or indirect consequences of smoking. Trend analyses project that this number will increase to 10 million smokers yearly [144].

An increase in fractures associated with smoking has been demonstrated for both women and men [5, 145-147]. However, the pathways leading to higher risk of fracture are not solely dependent on BMD as BMD accounts for only 23% of the smoking-related increase in fracture risk [146]. Other important causal associations with increased fracture risk in smokers include higher risk of falls, lower average BMI, direct toxic effects on bone, reduced calcium absorption, elevated cortisol levels, lower calciotropic hormones and lower estradiol levels [56, 148-151] (Figure 7). Nicotine acts directly on bone cells, decreasing the osteogenic activity, but also through other factors important for bone remodeling such as angiogenesis [152]. Another bone-related factor affected by smoking is impaired collagen metabolism [153].

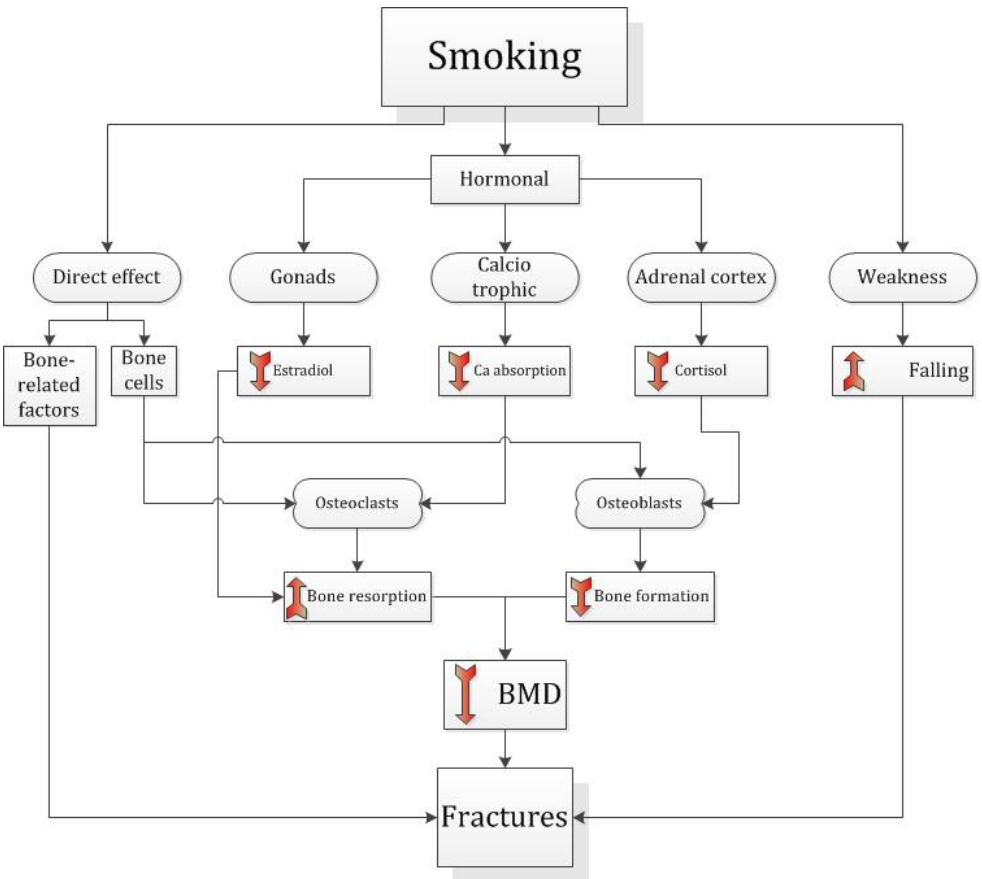

Figure 7 A schematic flowchart of causal associations between smoking and increased fracture risk.

Smoking is considered to be an independent predictor of low BMD [145, 146], but most studies have focused on older populations with longer smoking duration. The few studies available on smoking at an early age need to be supplemented with additional similar studies as early smoking has been associated with lower BMD, which might be an effect of the vulnerable peak bone mass acquisition [154].

Passive smoking has also been identified as a risk factor [155, 156]. However, quantifying the effect of passive smoking presents a difficult problem. Other factors complicating the evaluation of smoking as a contributory factor to decreased BMD are socio-demographic traits in smokers, as lower awareness of personal health and unhealthy lifestyle are themselves confounders of low BMD [157, 158].

Few studies have investigated the reversibility of smoking cessation but cross-sectional studies have demonstrated a catch-up in BMD in former smokers. However, extended time is needed; improvements are seen within 10 years, but it may take more than 30 years for former smokers to reach the levels of the never smokers [159]. Other studies indicate a quicker recovery, as improvements for markers of bone formation and resorption are seen within 6 weeks after cessation, followed by an improvement of BMD after 1 year [160, 161].

Despite the timeframes discussed above, cessation of smoking is an important step towards better bone health and reduces the risk of fracture even if a remaining risk is seen in former smokers [145, 146].

Aims of the Thesis

The general aim of this thesis was to evaluate the influence of established risk factors for osteoporosis on the attainment of peak bone mass in young adult Swedish women in order to understand the relationship between bone health in youth and osteoporotic fracture risk in old age.

The specific aims of the study were:

I. To provide normative DXA data at peak bone mass for young adult Swedish women to facilitate comparison with DXA scanner reference values and equivalently aged European and American populations.

II. To evaluate the association between birth weight and bone mineral density, bone mineral content and body composition in young adulthood at peak bone mass.

III. To investigate the associations between recreational physical activity and attainment of peak bone mass.

IV. To investigate the association between smoking and peak bone mass and fracture, in particular the influence of age at starting, duration, time since cessation, the daily consumption and total exposure.

Subjects and Methods

Malmö is the third biggest city in Sweden with 302,835 inhabitants (January 1, 2012) [162] and its population is served by only one hospital, Skåne University Hospital – Malmö. The Department of Orthopaedics is thereby the major clinic serving its population with treatment of orthopedic diseases and the only place for fracture treatment within the catchment area.

In this thesis young women at the age of 25 years, living in Malmö, were selected to be included in the PEAK-25 cohort, focusing on bone health.

PEAK-25 cohort

The purpose of the cohort was to include women at an age closely representative of peak bone mass, based on the findings from other studies [38-40, 163]. The PEAK-25 cohort was used for all four of the included studies.

During the years 1999 until 2004, letters of invitations were sent to women living in Malmö, inviting them to participate in our study on bone health in young adult women. The invitations were sent out continuously throughout the year in order to decrease the risk of seasonal bias. The subjects were randomly selected through the computerized administrative population system.

Our goal was to reach women at the age of 25 within our catchment area. A total of 2,394 invitations were sent and 1,166 agreed to participate. This gave us a response rate of 49% [164]. However, 102 subjects were directly excluded as they were pregnant or had been pregnant during the last year. That left us with 1,064 women who completed the baseline examinations. The cohort was continuously analyzed upon recruitment, and three subjects were identified as being out of the age range at baseline (24 years, n=2; 26 years, n=1) and thus excluded, leaving a cohort of 1,061 women, all 25 years old.

Questionnaire

A comprehensive questionnaire, consisting of approximately 250 questions, was administered to all participants. They were asked to complete the questions pertaining to eight separate areas of interest: (a) environment during childhood/adolescence and relatives, (b) women's health issues, (c) diseases and medications, (d) education and working life, (e) lifestyle and physical exercise, (f) tobacco/alcohol/drugs, (g) nutrition, and (h) appearance and sleep.

The response reliability of the questionnaire was tested after 3 months (mean 12.2 weeks, range 10.6–13.4 weeks) by asking 20 participants to complete the same questionnaire again. The results from the two questionnaires were then compared with Sign test and no significant differences were identified (p value 0.125–1.0).

Bone mineral density assessment

Bone mineral density and body composition variables were assessed with dual energy X-ray absorptiometry (DXA, Prodigy, Lunar Corp., GE, Madison, Wisconsin, USA). Software versions 2.15–7.70 were used during the years of collection of the cohort and the results obtained from the scans were used in the four studies presented in this thesis. Regions of interest (ROI) and variables measured were BMC and BMD in total body (TB), femoral neck (FN), trochanter (TR), total hip (TH), lumbar spine L1–L4 (LS) and lumbar spine L2–L4 (LS2). In addition, lean and fat mass were included.

To measure total body, subjects were placed in a supine position with hands supinated. For lumbar spine measurements, hips and knees were flexed with a pillow in order to flatten the normal lordosis, and to measure the hip (left side), the legs were slightly abducted and internally rotated.

Reproducibility was checked daily by the use of a manufacturer-supplied phantom. A second scan was made in 15 women in order to establish the absolute precision error (CV%) of the DXA measurements in this cohort. The outcomes were 0.37% (TB), 0.90% (FN), 0.56% (TR), 0.50% (TH) 0.65% (LS; L1–L4) and 0.70% (LS2; L2–L4).

Standardized BMD

The different DXA scanners measure somewhat differently. That is why it is not possible to compare measured BMD values from different scanners with each other. Therefore, standardization has been attempted to overcome this difficulty by

calculation of standardized BMD (sBMD). We recalculated the BMD values from published studies in order to make comparisons of BMD between studies possible independently of the DXA scanner model. The sBMD value for each of the hip regions was calculated according to the formula [165, 166]:

$$sBMD = \alpha + \beta \times BMD$$

Where α and β are dependent on scanner manufacturer and sub-region of the hip. The coefficients are:

1. Hologic FN (α=0.019, β=1.087), TR (α=–0.017, β=1.105), TH (α=0.006, β=1.008)
2. Lunar FN (α=–0.023, β=0.939), TR (α=–0.042, β=0.949), TH (α=–0.031, β=0.979)
3. Norland FN (α=0.006, β=0.985), TR (α=0.057, β=0.961), TH (α=0.026, β=1.012).

Recalculations of the standard deviations (SD_{recalc}) [167] used the same β-values according to the formula:

$$SD_{recalc} = \beta \times SD$$

For calculation of LS-sBMD, the formula below was used [168, 169]:

$$LS\text{-}sBMD = \delta \times (LS\text{-}BMD - \varepsilon) + 1.0436$$

The scanner-dependent coefficients are as follows:

1. Hologic (δ=1.0550, ε=0.972)
2. Lunar (δ=0.9683, ε=1.100)
3. Norland (δ=0.9743, ε=0.969)

The standard deviations in the lumbar spine were recalculated according to the formula given previously (SD_{recalc}), and using β-coefficients suggested by Genant et al [168]:

1. Hologic 1.0755
2. Lunar 0.9522
3. Norland 1.0761

The LS-sBMD was originally developed for L2–L4, but we used it for L1–L4, as the recalculated values are relatively equal to their original values. Furthermore, this extrapolation has been used before [170]. The sBMD values are reported in mg/cm^2 compared to the regular BMD reported in g/cm^2. This is done only to make a distinction between the two variables.

Physical activity

In the comprehensive questionnaire completed by the participants, one section was dedicated to physical activity. They were asked to specify e.g. daily walking habits, types of exercises, amount of time spent, seasonal variations in their activity, if they had been more active earlier in their life and if they enjoyed physical education in school.

For estimation of the intensity and regularity of recreational activity, we created the recreational activity level (RAL), where the participants were asked to grade their level of activity on a scale from 1–6. Grade 1 represented "hardly any exercise at all" and 6 represented "active at a regular level with practice and competitions". A value of ≥4 was used as cutoff between high and low recreational activity levels as this value represents the lowest level of regularity of physical activity.

The RAL could not discriminate between different types of exercise and their varying strain on bone tissue. Therefore, in addition to RAL, we used the existing peak strain score (PSS) [131]. Based on the information in the questionnaire about the different activities performed, we provided a single peak strain score (PSS) for each activity. The PSS gives an estimation of the impact imposed on the skeleton when performing the specific activity, and the score is based on ground reaction forces (GRF), which are multiples of the body weight imposed on the skeleton when performing the activity. Running, for example, produces a GRF of 2.6 which then gives a PSS of 2 (Table 3).

Table 3 The PSS scoring model used for scoring the different activities, based on ground reaction forces (GRF) imposed on the skeleton [131, 132].

PSS	GRF	Estimation criteria	Examples
3	> 4	Activities including jumping actions	Basketball, volleyball
2	2–4	Activities including sprinting and turning actions	Badminton, tennis, running
1	1–2	Weight-bearing activities	Dancing, strength training
0	< 1	All other activities	Bicycling, swimming

To assign a PSS to the different activities, we mainly used the existing BPAQ (Bone-specific Physical Activity Questionnaire) quantification by Weeks [132]

and for those activities not reported in the literature, an estimation of GRF was made according to the same principles. Hence, each activity received a score from 0–3, depending on impact load, and the activities were summed up, giving a result from 0–16 points, where ≥5 was considered high impact.

As the two scores above measure physical activity somewhat differently, with RAL focusing on endurance and PSS on impact, a composite of RAL and PSS (COMB-RP) was created in order to get an estimation of the overall physical activity. This allowed us to extract individuals engaged in high impact sports on a frequent basis (RAL≥4 and PSS≥5) compared to subjects with lower impact and frequency (RAL≤3 and PSS≤4). These two groups were designated high-COMB-RP and low-COMB-RP.

Birth data

In Sweden, since 1973, data of all pregnancies leading to delivery are reported to the Swedish National Board of Health and Welfare (SNBHW). The information is collected from prenatal clinics and delivery wards at our hospitals and the data are recorded in a separate database (MFR, Medicinskt Födelseregister). The data includes information within three categories: (1) pregnancy (i.e. diagnoses, information about parents, complications related to pregnancy, earlier pregnancies); (2) delivery (i.e. medical complications, anesthesia, method of delivery); and (3) newborn (i.e. gestational age, weight, length, diagnoses, status at birth).

From this database information about weight and length at birth was obtained for the women in the PEAK-25 cohort. For unknown reasons, there were missing data, leaving us with information about weight in 1,047 cases (98.7%) and length in 1,034 cases (97.5%). The birth weight was used in statistical analysis categorized in two different ways. Firstly, they were grouped according to the WHO classification in normal (>2,500 g), low (LBW; 1,500–2,499 g), very low (VLBW; 1,000–1,499 g), and extremely low (ELBW; <1,000 g). A tertile grouping was also used, in order to obtain more equal groups in size for comparisons.

Tobacco smoking

In the questionnaire described above, one section focused on smoking, alcohol and drug use. For the smoking variables, there were 7 cases with missing data, leaving us with 1,054 participants with known smoking status. The questions asked concerning smoking habits gave us information about smoking status, age when smoking started and stopped, duration and amount of cigarettes consumed per day.

For practical purposes, to study the associations between bone mass and smoking, a clear definition of smoking is needed. All participants reporting cigarette consumption, even if just occasionally, were labeled current smokers (n=276). For comparison we used the category never smokers (n=591). The third group consisted of the former smokers (n=187).

To measure the amount of tobacco consumed, two different measures were used. Firstly, cigarettes/day, which measures the current consumption or historical consumption in the former smokers. The second measure is pack-years, which in addition to the tobacco amount includes duration of the consumption. This is calculated according to the formula:

$$pack-years = \frac{\left(\frac{Cigarettes}{day}\right) \times smoking\ years}{20}$$

Statistical Methods

For statistical analysis, SPSS software versions 17.0–19.0 (SPSS Inc., Chicago, Illinois, USA) were used.

Descriptive statistics are presented with mean, standard deviation (SD) and range (studies I–IV).

Pearson's correlation coefficients were determined in studies I and II for analysis of continuous variables. In study II, the results were presented with quadratic curve estimation and 95%CI.

Regression analysis was performed in studies II–IV in order to determine the effect sizes of factors and covariates on bone and presented with 95%CI and standardized β-values. In studies II and IV, the unstandardized β-values (β_{std}) were determined.

ANOVA, ANCOVA and t-test were used for group comparisons (studies I–IV), and for subgroup analyses Bonferroni (study II) and Fisher's least significant difference (LSD) (study IV). For group comparisons with non-parametric, dichotomous data, Chi^2 test was used (III–IV). In study IV, the risk ratio for fracture in current and ever smokers was calculated and reported with 95%CI.

Variables were tested for normal distribution with Shapiro-Wilks test. Sign test was used for testing the response-reliability of the questionnaire. The coefficients of variation (CV%) were determined for the DXA measurements at TB, FN, TR, TH, LS and LS2. This was done by remeasuring 15 individuals and the calculations were performed with ISCD Bone Densitometry Precision Calculating Tool, Version 2.1.

For all analyses, the level of significance was set at $p<0.05$.

Ethics

Studies I–IV all received ethical approval from the Institutional Review Board, Lund University, Lund, Sweden, and followed the ethical standards stipulated in 1964 in the Declaration of Helsinki. The database setup was approved by the Swedish Data Inspection Board. Written informed consent was obtained from all participating subjects and all data were treated confidentially.

Results

Paper I

Country-specific young-adult DXA reference data are warranted for T-score calculations in women: Data from the PEAK-25 cohort.

Aim

The primary aim of this study was to provide normative DXA-values for 25-year-old Swedish women. The secondary aims were to evaluate how the BMD values of the cohort compare to established reference values supplied by the DXA manufacturers and to compare with other similar populations published in the literature and furthermore to compare the concordance between the different measurement sites.

Subjects and Methods

The study was performed in the PEAK-25 cohort, consisting of 1,061 25-year-old women. BMD values from DXA were used from TB, FN, TR, TH, LS (L1–L4) and LS2 (L2–L4) and T- and Z-scores were reported for FN, TH and LS. A new Z-score was calculated based on the PEAK-25 cohort as reference. After recalculation of BMD to give standardized BMD (sBMD) values, BMD could be compared between PEAK-25 and cohorts of similarly aged women from nine other published studies.

Results

The mean BMD values, T- and Z-scores are presented in table 4.

Table 4 BMD values and the T- and Z-scores provided by the Lunar Prodigy DXA scanner in the Peak-25 cohort.

Variable	*N*	*Mean ± SD*	*Range*
BMD (g/cm^2)			
Total body	1060	1.174 ± 0.073	0.969 – 1.486
Femoral neck	1057	1.053 ± 0.123	0.746 – 1.604
Trochanter	1057	0.830 ± 0.108	0.537 – 1.357
Total hip	1022	1.061 ± 0.121	0.742 – 1.593
Lumbar spine (L1–L4)	1059	1.217 ± 0.128	0.824 – 1.868
Lumbar spine (L2–L4)	1060	1.239 ± 0.131	0.842 – 1.885
T-score			
Femoral neck	1057	0.61 ± 1.02	–1.95 – 5.20
Total hip	1022	0.50 ± 1.01	–2.15 – 4.94
Lumbar spine	1059	0.31 ± 1.07	–2.97 – 5.74
Z-score			
Femoral neck	1057	0.54 ± 0.98	–1.77 – 4.27
Total hip	1022	0.47 ± 0.96	–1.92 – 4.75
Lumbar spine	1059	0.32 ± 1.03	–3.15 – 5.54

The Z-scores were similar to the T-scores except in FN ($p<0.001$) and TH ($p<0.001$), where T-scores were higher.

When using the cohort's own calculated Z-scores instead of the scanner-derived Z-scores, the prevalence of subjects "below the expected range for age" (≤2SD), increased from 0 → 7 (FN), 0 → 16 (TH) and 12 → 17 (LS).

The sBMD values were generally higher in the PEAK-25 cohort than the corresponding values from NHANES III: FN (1.5%; $p=0.044$) and TH (5.4%; $p<0.001$) but lower at TR (–2.5%; $p=0.002$). Compared to other studies PEAK-25 values were either non-significantly different or generally higher than those reported: FN-sBMD (1.5–7.5%), TR-sBMD (–5.9%–2.9%), TH-sBMD (2.6–9.2%), LS (L1–L4) (4.7%) and LS2 (L2–L4) (3.4%–6.5%).

When the highest and lowest quartile of each BMD ROI were compared, the concordance was high where hip BMD identified the same subjects in 71–78% of the cases in the low quartile and 70–84% in the high quartile. Discordance was less than 1%. Concordance between hip and spine was 53–60% and discordance 3–4%. Correlations between LS and hip were lowest at 0.62–0.74, and highest between TH and TR ($r=0.92$).

Conclusions

This study indicates that BMD is generally higher than in equivalently aged European and North American cohorts. It also provides normative bone mass values in Swedish women aged 25. The concordance between different hip BMD ROIs are high. This study also emphasizes the importance of using ethno-geographical reference data in order to discriminate osteoporosis versus normal bone mass.

Paper II

Birth weight is more important for peak bone mineral content than for bone density: The PEAK-25 study of 1,061 young adult women.

Aim

The aim of this study was to evaluate the influence of birth weight on peak bone mass and body composition variables.

Subjects and Methods

In addition to bone mass measurement with DXA in the PEAK-25 cohort (n=1,061 women aged 25), birth anthropometrics were obtained from the Swedish National Board of Health and Welfare (SNBHW). Birth weight and birth length data were available for 1,047 and 1,034 subjects, respectively. Subjects were divided into birth weight tertiles (high/intermediate/low) and according to the WHO classification for analysis.

Results

The mean birth weight was 3,392±537 g and length at birth 50±2.3 cm. Within our cohort 95.7% were classified as normal birth weight, 3.8% as low birth weight and 0.5% as very low birth weight according to the WHO classification. 2.2% weighed >4,500 g.

Both current body weight and height were correlated to birth weight ($r=0.20$; $p<0.001$; $r=0.28$; $p<0.001$, respectively). Birth weight was positively correlated to all measured sites and strongest for bone mineral content (BMC) at TB ($r=0.24$; $p<0.001$), TH ($r=0.17$; $p<0.001$), FN ($r=0.16$; $p<0.001$) and LS ($r=0.15$; $p<0.001$). After body weight adjustments, birth weight was still associated with BMC at all sites.

For every change of 1 kilogram in birth weight, there was an estimated effect size of 151 g in TB-BMC, which corresponds to a difference of 0.4 SD.

For BMD variables, no significant correlations for birth weight remained after current body weight adjustments. Comparing the tertiles of birth weight, the largest differences were seen between the low vs. high tertile; TB-BMC (–7.2%), FN-BMC (–5.3%), TH-BMC (–6.0%) and LS-BMC (–6.3%). Differences in BMC were also evident between low and intermediate tertiles (–4.3% – –4.6%), but no differences were seen for BMC in intermediate vs. high tertiles.

Even when subjects within the WHO categories low birth weight (LBW) and very low birth weight (VLBW) were excluded, associations for birth weight and BMC were still seen, although weaker; TB-BMC ($r=0.14$; $p<0.001$), FN-BMC ($r=0.08$; $p=0.02$), TH-BMC ($r=0.09$; $p=0.004$), and LS-BMC ($r=0.09$; $p=0.003$).

Conclusions

Women with lower birth weight have lower BMC at the age of 25. Reduced quantities of fat and lean mass are also seen related to lower birth weight. The negative influence of lower birth weight on BMC is more pronounced than the positive influence of higher birth weight.

Paper III

Self-reported recreational exercise combining regularity and impact is necessary to maximize bone mineral density in young adult women: A population-based study of 1,061 women 25 years of age.

Aim

The primary aim of this study was to evaluate the effects of recreational physical activity on peak bone mineral density. The secondary aim was to get insight into how activity levels change from adolescence into the mid-third decade.

Subjects and Methods

From the PEAK-25 cohort, 1,061 25-year-old women were examined with focus on physical activity. In a comprehensive questionnaire the participants specified their patterns of recreational physical activity. This information was then analyzed using two different scores; RAL (recreational activity level, graded 1–6), which measured the subject's activity level and PSS (peak strain score), which measured the impact of physical activity on the subject. These scores were also combined (COMB-RP) in order to be able to identify subjects with high activity level and high impact. The top five activities were evaluated separately and compared to non-active women (RAL=1) for their effect on BMD. A constructed regional ratio of site-BMD/TB-BMD [171] was used for identification of site-specific bone gain.

Results

A total of 85 different sports were reported. Women with high RAL had higher BMD than less active women; TR-BMD 3.3% ($p<0.001$), FN-BMD 2.6% ($p<0.001$), and LS-BMD 1.5% ($p=0.0194$). Similar results were seen for PSS, where women with high impact scores had higher BMD than women exposed to lower impact; TR-BMD 3.5% ($p<0.001$), FN-BMD 2.9% ($p<0.001$) and LS-BMD 2.1% ($p=0.0039$).

Women with high RAL and high PSS (23.8%; n=246), adjusted for body weight, had even higher BMD; TR-BMD 5.5% ($p<0.001$), FN-BMD 4.7% ($p<0.001$) and LS-BMD 3.1% ($p<0.001$), than women with low RAL/PSS (44.8%; n=476). For the top five activities (running, strength training, aerobics (low and high intensity) and spinning) runners had the highest BMD difference (TR-BMD +8.5% and FN-BMD +7.2%) compared to non-active women. Spinning (TR-BMD +6.4% and FN-BMD +7.2%) and high-intensity aerobics (TR-BMD +6.5% and FN-BMD +5.8%) also produced significant differences, but no differences in BMD were seen for low-intensity aerobics or strength training. None of the activities produced any significant BMD gain in the lumbar spine.

The constructed regional ratio showed site-specific bone gain for the high-COMB-RP group, where the ratio was 1.4-3.5% higher than low-COMB-RP. This was further supported by the finding of Head-BMD/TB-BMD ratio with 3.3% lower ratio for high-COMB-RP, indicating redistribution (unpublished data).

In total 68% enjoyed physical education in school and 27% of them became active with high RAL and high PSS (high-COMB-RP). This may be compared to the 32% who did not like physical education, only 16% of whom continued to be active within the high-COMB-RP group. This difference was confirmed in the low-COMB-RP group, where 79% had been more active earlier, compared to only 46% in the high-COMB-RP group.

Conclusions

Bone mineral density in young adult women is influenced by the overall recreational physical activity, and specifically activities inducing high impacts on the skeleton are more beneficial to bone health. Women at the age of 25 who enjoyed physical education in school have a higher tendency to maintain their activity level and are thereby more likely to achieve and maintain a higher peak bone mass than women who are relatively inactive.

Paper IV

Adverse effects of smoking on peak bone mass may be attenuated by higher BMI in young female smokers.

Aim

The aim of this study was to evaluate whether smoking was associated with lower bone mass, and if so, to elucidate whether it is dependent on dose and whether the smoking duration, time at smoke start and time since cessation play a role in the outcome. A further aim was to study whether fracture prevalence was higher in smokers.

Subjects and Methods

A total of 1,054 subjects from the PEAK-25 cohort provided information on smoking. The participants were divided into current, former and never smokers. Smoking consumption was measured with both cigarettes/day and pack-years. In addition, smoking duration, age at smoke-start, time since smoking cessation and fractures were analyzed. ANOVA was used for group analyses and regression for analyses of continuous variables. DXA was used to assess BMD in FN, TR, TH and LS. Adjustments were made for BMI, physical activity and dietary calcium intake.

Results

Smoking status in the cohort was 26.2% (current), 17.7% (former) and 56.1% (never) smokers. Average age at smoke start was 15.5±2.3 (current) and 15.3±2.2 (former). Smoking status was not an independent predictor of BMD, which was the case for BMI and physical activity (both $p<0.001$). In the current smokers, the amount of cigarettes consumed, demonstrated dose-dependency and was an independent predictor of FN-BMD ($p=0.037$). A similar outcome was obtained for adjusted regression analysis, with significant outcome for FN-BMD ($\beta_{std}=–0.162$; $p=0.012$) but not for TR-BMD ($\beta_{std}=–0.114$; $p=0.076$), TH-BMD ($\beta_{std}=–0.099$; $p=0.129$) or LS-BMD ($\beta_{std}=–0.067$; $p=0.305$). For pack-years, differences were mostly pronounced in FN-BMD.

Among the current smokers, duration was not an independent predictor of BMD, neither continuously nor categorized. However, the longer the duration, the higher the BMI ($p=0.038$). Age at smoke start was not associated with BMD.

Time since cessation was not associated with BMD in the long term, as no difference was seen for <6 months after cessation compared to >5 years. In the short term, a decrease of BMD is seen up to 24 months after cessation ($p=0.027–0.050$;

TR, TH and LS) and then after >24 months after cessation, a recovery of BMD to levels of never smokers.

The number of women with fractures occurring in the smoker categories after the age of 15 were 27 (current), 20 (former) and 47 (never). The risk ratio for fracture for current/former smokers compared to never smokers was 1.276 (95%CI 0.868–1.876). For only the current compared to never smokers the risk ratio was 1.230 (95%CI 0.784–1.931).

Conclusions

Smoking status is not associated with BMD in young women. For current smokers, increased dose is associated with decreased BMD in the hip. Age at smoke start or duration of smoking are not associated with BMD. BMI is higher in longer smoking duration among current smokers, which then might partly counteract the adverse effects of smoking. Approximately 2 years after cessation, BMD has recovered to levels of never smokers. Current and former smokers do not have an increased risk of fracture compared to never smokers.

General Discussion

Peak bone mass is the maximum amount of bone acquired by an individual and reached during the third decade of life. It is assumed that a number of genetic, environmental and lifestyle factors contribute to peak bone mass as they do to bone loss later in life. Whereas factors contributing to osteoporosis have been extensively studied, those associated with peak bone mass are known in much less detail.

The purpose of this thesis was to investigate factors determining peak bone mass and in addition to determine whether young adult Swedish women reach a peak level similar to that of equivalent populations.

Reference data

In order to further understand the high fracture risk in Sweden, we wished to investigate whether a lower peak bone mass could be part of the explanation. However, we found that Swedish 25-year-old women generally have higher BMD than corresponding cohorts in Europe and North America. The most interesting finding was how the PEAK-25 values related to NHANES III, which is the established hip reference database for DXA scanners. For femoral neck, which should be used as reference site for diagnosing osteoporosis [69], the PEAK-25 BMD value was 1.5% higher. Similarly, the total hip value was 5.4% higher, findings suggesting that indeed a lower peak bone mass is not currently present in young Swedish women.

Furthermore, DXA scanners give Z-scores, calculated from the reference data provided by the manufacturer. Using these Z-scores, none of the women had femoral neck values below the "expected range for age" (prevalence 0%). However, when we used our own cohort as the locally derived reference data, the prevalence rose to 0.7% in the femoral neck. This means that, by scanning 100,000 women, a total of 700 would change classification category from having "normal bone mass" to "low bone mass", only by using locally derived reference data instead the reference data provided by the manufacturer. A similar increase was seen in the total hip where the prevalence of having a Z-score below the "expected range for age" rose from 0% to 1.6%.

This illustrates one built-in source of error which occurs when using the same reference data independently of geographic region. Only by using local reference data does the prevalence of the classification low bone mass increase. The reason for this is that using our own local reference data, whose average is higher, moves the breakpoints upwards, hence more cases are below the breakpoint for lower bone mass (Figure 8).

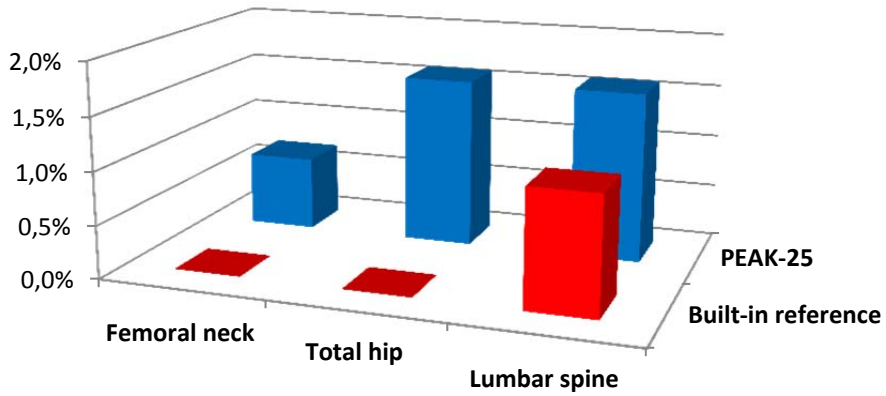

Figure 8 The change in prevalence of low bone mass (Z-score<–2SD), when using PEAK-25 (blue) as reference instead of the built-in reference data (red).

In addition to the issues concerning the reference data, the calculation of T- and Z-scores leads to a different question. With T-score derived from an average BMD value from what is considered the peak bone mass period in life, a large overlap and subsequently similar scores are expected when measuring a population at an age close to peak bone mass. However, in the PEAK-25 cohort the T-scores were higher than the Z-scores in the femoral neck and total hip. Similar discrepancies have previously been addressed [80] identifying the absence of standardization of Z-score calculations as the problem. The Z-score calculations may vary between the manufacturers of DXA scanners, and even within the same manufacturer. This calls for cautious interpretation of the scores, primarily the Z-scores.

Although the results indicate high peak bone mass in Swedish women compared to other similarly aged populations, the lifetime risk of osteoporotic fractures is high in Sweden. This leads us to wonder to what extent peak bone mass determines future fracture risk in Swedish women. However, it is difficult to draw any conclusion about future fracture risk based on today's BMD measurements performed in young adulthood, since this would assume that the fracture risk will be as high as it is among the elderly of today. The reason derives from the difficulties in comparing different generations as they have grown up under different living conditions, which may have affected the development of peak bone mass.

These results emphasize the risk of underdiagnosing low bone mass when using reference data from a setting which is not ethno-geographically appropriate. This also illustrates the need to complement NHANES III data with regionally derived reference data, when possible. In addition, a standardization of the Z-score calculation is indicated for more accurate comparisons of data from different scanners.

How early is future bone mass determined?

Birth weight is considered an indicator of prenatal health, and increasing evidence indicates its importance for future health status because of long-term effects on metabolism.

Prenatal health and birth weight may then also have implications for the development of bone mass. We show in our study that bone mineral content at all sites was positively associated with birth weight. However, bone mineral density was not.

Since birth weight has an association with BMC, birth weight indirectly determines the bone size which is related to higher fracture risk in adults [116, 117] including hip fractures [172]. Even early in childhood this connection is suggested, with higher fracture risk among those with low birth weight [173].

Birth weight was also positively associated with lean mass, which has implications for peak bone mass. The mechanism by which lean mass is suggested to contribute to BMD is by being part of increased BMI but also by increased strength and muscle contractions [174, 175]. By this association another path contributing to higher peak bone mass is added.

Similarly, fat mass was also positively associated with birth weight. However, when adjusted for body weight the relation changed direction, becoming negatively associated instead. A possible explanation for this is that individuals with higher body weight have proportionally greater body fat mass and a relatively lower proportion of BMC and lean mass. After adjustment for body weight this lower proportion of BMC and lean mass became apparent and was associated with lower birth weight. Similar observations in fat mass have also been seen when adjusting for bone size [174].

The differences in BMC were more pronounced for participants in the lower birth weight tertile, and our results indicate that low birth weight was more negatively associated with BMC than high birth weight was positively associated. A possible explanation for this could be a limit in bone dimension, which has a maximum size beyond which it cannot increase.

These findings enhance our knowledge regarding the importance of the prenatal environment with its most common manifestation – low birth weight as a potential contributor to reduced peak bone mass.

Furthermore, it illustrates the need for awareness and preventive measures for low birth weight children. The knowledge of skeletal risks of low birth weight could well be administered through the child welfare centers (BVC), giving information to parents so that steps can be taken to promote bone health, such as more physical activity.

Physical activity – how much?

Bone health is dependent on mechanical signaling which results from all weight-bearing physical activities, whether occurring from activities of daily living or through special training. Exercise and training have therefore been extensively studied in relation to bone health. However, most studies focus on athletes and specific sport activities while less is known about the associations in normal populations taking part in physical activity on a recreational level.

In this study of young women we show a positive association between recreational physical activity and peak bone mass. In general physical activity with high strain on bone (e.g. high intensity aerobics) was more beneficial than low-strain activities, and higher bone mass was seen in both the hip and the spine.

Furthermore, specific sports increase BMD on sites exposed to weight-bearing, which indicates a site-specific association between physical activity and BMD [133, 176-178]. This site-specific bone gain was also obvious in our study where women with higher activity had higher bone mass in sites exposed to mechanical load, which supports earlier studies. Interestingly, the women with higher activity levels simultaneously had lower bone mass in the head, which we interpret as redistribution of bone mass (unpublished data); this also supports site-specific bone gain since the head is a non-loaded site.

The most frequent activities were analyzed separately; running, spinning and high-impact aerobics were associated with greater bone mass in the hip of active compared to non-active women. Running and high-impact aerobics have axial impact on the skeleton, but for spinning it is probably the exerted muscular strain imposed on the hip girdle that creates the increase in bone mass [179]. Also these individuals are more commonly involved in more than one physical activity, which may contribute to the positive associations with bone mass.

From most studies the results for BMD and physical activity in this age group point in the same direction, with higher BMD as a response to physical activity,

even though some studies are more cautious in their statements about the beneficial effects of moderate physical activities [180]. And it is not only the level of activity that matters, as the type of sport also seems to play a role.

A direct link between physical activity and decreased rate of osteoporotic fractures is arguable [181], but our results indicate that even physical activity at a recreational level early in life is positively associated with a determinant of future fracture risk, namely peak bone mass.

From a lifestyle perspective physical activity in childhood, here represented as physical education in school, has clearly demonstrated a positive association with future level of physical activity [140, 141]. This emphasizes the need for early motivational efforts among children in order stimulate physical activity and thereby contribute to better bone health in the future.

In summary, even activity at a recreational level, will have a beneficial influence on peak bone mass, preferably activities with higher impact and a regularity of activities every week, on a whole-year basis.

The adverse effects of smoking on bone

Long-term smoking during life is a well-known contributor to several medical conditions and also to decreased bone mass and higher risk of fracture [145, 146]. We show that smoking may have adverse effects on peak bone health at an early age.

The amount of consumed cigarettes was the clearest indicator of smoking and bone mass differences in the hip, demonstrating a clear dose-response. However, this was not seen in former smokers, which indicates the reversibility of the adverse effects. Hence, we specifically addressed the question of whether the adverse effects were reversible after cessation of smoking. In this analysis we observed lower BMD at all sites after cessation, but BMD returned to the levels of never smokers after more than two years prior to the investigation. Similar time intervals have been observed for improvements of BMD in postmenopausal women upon smoking cessation [161]. The explanation for this temporary decrease in BMD is unknown but opens up for speculations on the impact of BMI and other biological responses to smoking cessation. However, our data did not permit us to evaluate this further.

Smoking duration has been associated in many studies with lower BMD, mainly in older populations [182]. In the PEAK-25 cohort, smoking duration was not associated with lower BMD. Several reasons could contribute to the explanation. One is the young age of the cohort. At this young age, the women may not have accumu-

lated enough duration in order to impose significant BMD change on bone, probably because a certain threshold in smoking duration has to be reached.

Furthermore, in these young women there was a simultaneous increase in BMI with duration, which is opposite to older smokers, who generally are thinner with lower BMI [146]. This increase in BMI exerts a higher mechanical load on bone, which may partly compensate for the negative associations of smoking. Another BMI-related mechanism is the estrogen balance. Increased extra-ovarian estrogen is produced in the fat mass and may hence compensate partly for the hypoestrogenic state which smoking women attain compared to non-smokers [183, 184]. However, this relationship mainly concerns postmenopausal women, while it is not known to what extent this contributes in young women.

The possible contribution of smoking to fracture risk has mainly been shown in older populations [5, 146, 147] and less in younger individuals, mainly males [185]. We could see a trend in the Peak-25 cohort of increased prevalence of fracture in both current and ever smokers. However, even if there is an association between smoking and fracture in young women, the causal relationship is not ascertained because other factors, such as less personal health awareness, may be more common among smokers [157, 158].

In summary, the clear dose-response association with bone mass seen in smokers already at an early age suggests that even a decrease in cigarette consumption has beneficial effects on peak bone mass, although a total smoke stop is preferable.

Strengths and Limitations

Strengths

Our studies have both limitations and strengths which require some considerations.

The most considerable strength of these studies is the size of the cohort. It is to the best of our knowledge, the largest population-based cohort of young adult women with a narrow age focus closely representing peak bone mass. The recruitment of the participants was population-based, from the same catchment area and not through advertising, which may reduce the selection bias.

The single age and sex focus of the cohort may also minimize the contribution from confounders. The amount of missing data in the cohort is low, which promotes the credibility of the presented results.

The birth weight data were retrieved from validated national databases, rather than recalled information from their mothers, thereby increasing the accuracy of the data.

Limitations

One limitation was the response rate of 49% in the PEAK-25 cohort, which may give a selection bias, although we did not identify specific reasons for non-participation. One reason could be the age group. In the questionnaire, recall bias is possible in some questions and also a social desirability where the participants might overestimate positive factors, such as physical activity, as well as underestimating negative factors, such as alcohol and smoking.

Another limitation in self-reported data applies to fractures, which were self-reported and not radiographically confirmed. However, the response reliability of the questionnaire was good according to the recompletions of the questionnaire made by a selection of participants [186].

We used the standard peak strain score, widely used in other studies, which measures the impact of an activity. Generally when estimating physical activity there are inherent limitations when giving scores to different activities, as the energy expenditure within the same activity may vary. However, the differences are probably small with a limited effect on the results.

It is an unresolved question how to best quantify and classify smoking over time. For the classification of smokers, e.g. a participant who quit smoking a short time before the examinations was classified as a former smoker, independently of the amount consumed. Secondly, when using the classification pack-years it cannot differentiate between someone who smoked a few cigarettes for a very long period and someone who smoked a very high number for a short period of time. In addition many studies only apply a dichotomous answer for smoking, which may be more suitable in older populations, but less so in the young. We tried to avoid these sources of error by studying the associations of smoking and bone mass mainly between the groups of never smokers and current smokers.

Furthermore, we relied on standardized BMD values which are an approximation and may not fully compensate for the differences between various DXA scanners. Cross-calibration between the different scanners was not possible, but nevertheless, the consistent finding of higher BMD in the PEAK-25 cohort, compared to the other cohorts measured with different types of scanners, indicates accuracy.

Despite having access to validated information on birth weight, we lacked such data as gestational age, which could have provided additional information on heritable traits of body size. Furthermore, for Swedish populations using the birth

weight classification according to WHO, although well established, created very small numbers with low weight, which then reduces the possibility of making robust conclusions for this group. The WHO classification is hence more relevant in developing countries, where low birth weight is more common, or in studies focusing specifically on low birth weight.

Discussion summary

Many factors have an impact on peak bone mass. From the earliest stages in life, where a low birth weight might negatively influence peak bone mass, both directly through bone mineral content and also indirectly through decreased lean mass. Later in life decisions are made either pro or con bone health. Physical activity, even on a recreational level, influenced peak bone mass and participation in activities with high impact increased the positive influence even more. Smoking, on the other hand, was associated with lower peak bone mass, especially with an increasing amount of cigarettes consumed. The duration of smoking, however, did not seem to be of importance, probably attenuated by a simultaneous increase in BMI, which might partly protect against the negative effects of smoking on bone.

Although the changes in BMD and BMC associated with the risk factors are small and maybe not clinically relevant by themselves, an additive effect may result. Using two extreme scenarios, one scenario is a woman, born with lower birth weight does not enjoy physical education in school and starts to smoke in her teens. To a greater likelihood, she will take less part in physical activities when she becomes older and might also develop other risk behaviors. This has to be compared to another extreme scenario involving a woman with normal birth weight who enjoyed physical education in school and hence continued to take part in physical activities. Through this increased health awareness, she did not start to smoke and avoided other risk behavior associated with smoking. Some parts of this additive effect have been examined, e.g. men with low birth weight might be more vulnerable to the adverse effect of smoking [187].

The factors evaluated in this thesis give small changes in BMD already at the age around peak bone mass. They might be small but together with other genetic and environmental factors at this age, they may create leverage, giving substantial effect when needed – in the elderly at risk of osteoporosis and subsequent fractures.

Clinical implications

Understanding the epidemiology of risk and protective factors may increase the possibility of identifying individuals at risk of developing osteoporosis and subsequent fracture, and thereby make early intervention possible.

Individuals with low birth weight might be a risk group for impaired bone health, so pointing out risk and promoting preventive factors may decrease future personal suffering from fractures.

Participation in recreational physical activity is common among young women, with approximately 70% regularly participating in various training/exercise activities. This study indicates that these activities are important for bone health. It also highlights that previous positive exposure to physical education in school enhances the motivation to continue as a young adult, which firstly says it should be fun to exercise in school and secondly raises the question about giving grades when the purpose is for health interventions.

Smoking is negative for bone health, but as a clear dose-dependency exists, by decreasing the number of cigarettes consumed, the harm on bone may be reduced. However, major advantages of quitting smoking are indisputable; in bone a recovery of bone mass to the levels of never smokers is seen after a couple of years.

Future perspectives

Further studies are needed also among elderly people in order to investigate the differences in the outcome of T-scores when using the NHANES III vs local reference data. The question includes what it means for the prevalence of osteoporosis and how many more or fewer individuals will be subject to pharmacological treatment for the right or wrong reason. Would we have more or fewer fractures if we used locally derived reference data?

The majority of studies on birth weight and other lifestyle factors and later bone mass are cross-sectional retrospective, but there is a need for longitudinal prospective studies. In this manner it would increase the possibility to more accurately investigate when in time different factors play the biggest role in future bone mass.

We know how some risk factors are associated with peak bone mass, but what is needed is increased knowledge of how these risk factors interact. A simulation model would be desirable, for young individuals where they could e.g. decrease the amount of cigarettes, increase their physical activity, change their weight etc. The outcome could be bone mass which could be extrapolated into older age and

thereby increase the motivation for redirecting the efforts towards better bone health.

Conclusions

- Peak bone mass in young adult Swedish women is generally higher than in other similar populations in Europe and North America.
- The use of reference data supplied by the manufacturers of DXA scanners, affects the outcome of the relative scores used for diagnosing osteoporosis. Therefore, if possible, locally derived reference data should be used to increase diagnostic validity.
- Bone mineral content is lower in young Swedish women who weighed less at birth and low birth weight has a more negative effect on bone mineral content than high birth weight has a positive effect.
- Physical activity at a recreational level is positively associated with bone mineral density, and especially high-impact activities have greater influence on peak bone mass. Activities with lower impact, but high muscular strain, such as spinning, may also positively influence peak bone mass. The bone gain associated with physical activity is mainly site-specific.
- Cigarette smoking is associated with lower bone mineral density in the hip and it is dose-dependent on the amount of cigarettes smoked.
- Young women, in contrast to elderly women, who have smoked longer, have a higher BMI and this may partly counteract the adverse effect of smoking on bone.

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Sammanfattning på svenska

Peak Bone Mass, livsstilsfaktorer och födelsevikt
En studie av 25-åriga kvinnor

Bakgrund

Osteoporos kallas ”den tysta sjukdomen” då den vanligtvis inte ger sig tillkänna förrän skelettet blivit så pass skört att endast en mindre skada eller ansträngning orsakar en smärtsam fraktur. Frakturerna drabbar framför allt höft, bäcken, rygg, axel och handled.

Varje år inträffar c:a 70 000 frakturer i Sverige som är osteoporosrelaterade och åsamkar lidande hos den drabbade individen samt stora kostnader för samhället.

För att ställa diagnosen osteoporos gör man en mätning av skelettets täthet med en sk dual energy X-ray absorptiometry (DXA). Definitionen av osteoporos är att bentätheten är mindre än -2.5 standardavvikelser (SD), jämfört med unga, mätt med DXA.

Tidpunkten när benvävnaden nått sin maximala täthet och styrka är olika i höft och rygg, men genomsnittsåldern bedöms ligga kring 25-30 år och benämns peak bone mass (PBM). Peak bone mass har en stor betydelse för den framtida benmassan för ju större den är, desto bättre är utgångsläget när man sedan under åren tappar benmassa och risken för frakturer ökar.

Att ett flertal faktorer påverkar benmassan vet vi, men hur dessa riskfaktorer ger utslag i benmassan redan i åldern kring peak bone mass är mindre känt, varför behov av ytterligare kunskap kring detta är nödvändigt.

Målsättning

Förutom den aktiva behandling som insättes vid diagnosticerad osteoporos kan en stor del av följderna av osteoporos, fragilitetsfrakturerna, minskas. Detta genom att man identifierar riskfaktorer och riskindivider tidigt för att kunna sätta in adekvata åtgärder.

I denna avhandling har vi studerat några riskfaktorer för osteoporos och huruvida dessa faktorer redan i tidiga år ger ett avtryck i den maximala benmassan som kan uppnås, peak bone mass. Vi har även studerat metoden, DXA, som används vid diagnosticerandet av sjukdomen.

Material och metod

Totalt sett ingår 1061 kvinnor i den kohort, PEAK-25, varifrån vi använt data ifrån bentäthetsmätningar samt frågeformulär. Samtliga kvinnor som inkluderades var 25 år vid tidpunkten för undersökningarna. Åldern 25 år valdes då den kan anses representera en genomsnittsålder då den högsta benmassan uppnås, peak bone mass.

I delarbetena I-IV användes data från bentäthetsmätningarna. Utöver detta insamlades födelsedata från Medicinska Födelseregistret till delarbete II. För delarbete III användes data från frågeformuläret avseende utövandet av fysisk aktivitet och nya och etablerade skattningsskalor för fysisk aktivitet användes. Delarbete IV koncentrerades kring frågeformulärdata avseende faktorer kring rökning i kombination med bentäthetsdata.

Resultat

Slutsats i delarbete 1 är att benmassan hos svenska 25-åriga kvinnor generellt sett är högre eller jämförbar med andra likande populationer.

Slutsats i delarbete 2 visar att låg födelsevikt ger ett lägre benmineralinnehåll i skelettet (bone mineral content, BMC). Skillnaderna är tydligare för låg födelsevikt än för hög.

Slutsats i delarbete 3 är att vanlig motionsträning är gynnsam för skelettet, där både löpning och spinning givit ökning av benmassa. De som gillade skolgymnastiken hade en större tendens att bibehålla en högre aktivitetsnivå senare i livet och därvidlag troligen öka sin peak bone mass.

Slutsats i delarbete 4 visar att ju mer man röker desto sämre benmassa får man i höften men ej i ryggen. Ålder då man började röka eller tiden man rökt spelar mindre roll, men för de som slutar har skelettet återhämtat sig efter c:a 2 år.

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