Physical activity, bone gain and sustainment of peak bone mass

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Dedicated to my wonderful parents – Orvokki and Risto

"All the reasons that prevent us from exercising on a regular basis are the excuses."
Urho Kaleva Kekkonen, president of Finland 1956-1981

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Abstract

Weak and osteoporotic bones are an increasing cause of mortality and painful physical impairment among the elderly, especially in the Western world. Bone mineral density (BMD, g/cm^2) accrual during childhood and adolescence is thought to influence an individual's risk of osteoporosis and the related fractures.

A main aim of this thesis is to investigate the effects that various types of weight-bearing physical activity have on bone accretion in young males during their active sports careers and to study the effects that detraining has on BMD. The results suggest that bone is sensitive to loading after puberty in males, and important gains in BMD stemming from physical activity were observed during the 12-year follow-up period (papers I-III). These gains seem to be site-specific and related to the type and amount of physical activity in which individuals participate (papers I-III). For example, badminton, a sport that is characterized by jumps and rapid versatile moments in multiple directions was associated with greater gains in BMD than ice hockey was. In addition, our results indicate that with reduced training, exercise-induced bone benefits decline, predominantly at trabecular sites (paper II). In contrast, high bone density attained from previous physical loading was partially preserved at cortical bone sites after about eight years of reduced activity (papers I-II). In study IV, the associations between self-perceived health, BMD, and other lifestyle factors were studied in a well-defined group of women and men of varying ages. We found that self-perceived health was related to several lifestyle factors, such as physical activity, which were also related to BMD at the femoral neck.

In summary, BMD in young males seem to be especially sensitive to activities associated with supposed high strains in unusual directions at specific bone sites. A high bone density stemming from previous weight-bearing physical activity is largely lost at trabecular bone sites with reduced physical activity levels. Finally, self-perceived health seems to be associated with several lifestyle factors that are also associated with BMD at the femoral neck.

Preface

This thesis is based upon the following papers, referred to in the text by the following Roman numerals:

I. Tervo T, Nordström P, Neovius M, Nordström A. Constant Adaptation of Bone To Current Activity Level: A 12- Year Longitudinal Study in Males. J Clin Endocrinol Metab 2008; 93(12): 4873-9.

II. Tervo T, Nordström P, Neovius M, Nordström A. Reduced physical activity corresponds with greater bone loss at the trabecular than the cortical bone sites in men. Bone 2009; Jul (22).

III. Tervo T, Nordström P, Nordström A. Effects of badminton and ice hockey on bone mass in young males: a 12-year follow-up. Submitted for publication.

IV. Tervo, T, Nordström P, Nordström A. Association between self perceived health, physical activity and BMD in middle age men and women. Submitted for publication.

Abbreviations

ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
BMD	Bone Mineral Density
vBMD	Volumetric BMD
BMU	Basic multicellular unit
BUA	Broadband ultrasound attenuation
CTX	Carboxy terminal telopeptide of type 1 collagen
CV	Coefficient of variation
DXA	Dual energy X-ray absorptiometry
IDSC	International DXA Standardization Committee
MRI	Magnetic resonance imaging
OC	Osteocalcin
PBM	Peak bone mass
pDXA	Peripheral DXA
QCT	Quantitative computed tomography
QUS	Quantitative ultrasound
sBMD	Standardized BMD
SD	Standard deviation
SOS	Speed of sound
SBU	The Swedish Council on Technology Assessment in Health Care
VIP	Västerbotten Intervention Program
WHO	World Health Organization

Introduction

Osteoporosis is a major health problem worldwide that has a prevalence that is similar to that of other major diseases, such as cancer and cardiovascular disease [3, 4]. Because the fractures resulting from osteoporosis are associated with pain, debility, reduced independence, and an increased risk of death, prevention of this disease is highly important. The risk of experiencing an osteoporotic fracture can be reduced by medical treatment for osteoporosis, behavioral changes, and minimizing the risk of falling. The ability to perform these interventions is, however, dependent on the accurate identification of persons with low bone quality or a high risk of falling before the fractures occur. Even so, only about 15% of subjects with fractures have osteoporosis, thus, the sensitivity of fractures for diagnosing osteoporosis is poor [5]. A possible alternative to the early detection and treatment of osteoporosis is early prevention. Is has been suggested that a high peak bone mass from previous physical activity might reduce the risk of osteoporosis later in life [6]. However, to justify recommending physical activity to increase peak bone mass and possibly thereby reduce the risk of osteoporosis, more research is necessary to obtain information concerning the following questions:

1. Which bone sites are affected by physical activity?
2. Are bone sites rich in trabecular bone and bone sites rich in cortical bone affected by physical activity to a similar extent?
3. During what period of life is the bone most sensitive to physical activity?
4. What type and amount of physical activity is most effective?
5. Are there any sustained benefits in BMD with a subsequent reduced amount of physical activity?

This thesis is based on observations in athletes and controls during a 12-year study period, and its aims were to gain more knowledge regarding the questions above. In the final study, associations were investigated between life style factors, such as physical activity, and BMD in a cohort of men and women of different ages.

Osteoporosis and fractures

Osteoporosis is a systemic skeletal disease characterized by reduced bone mass and microstructural deterioration of bone tissue, leading to bone fragility and an increased risk of fractures [7] (**Figure 1**). Osteoporosis is a silent disease and fractures often develop before the diagnosis of the disease is made. The societal burden of these minimal trauma fractures, which includes reduction in quality of life and survival as well as increased societal costs, makes osteoporosis a major public health problem [8]. In Sweden, the lifetime risk of an osteoporotic fracture has been estimated to be approximately 50% for women and 25% for men, with an increase in incidence observed after the age of 50 years [1, 9].

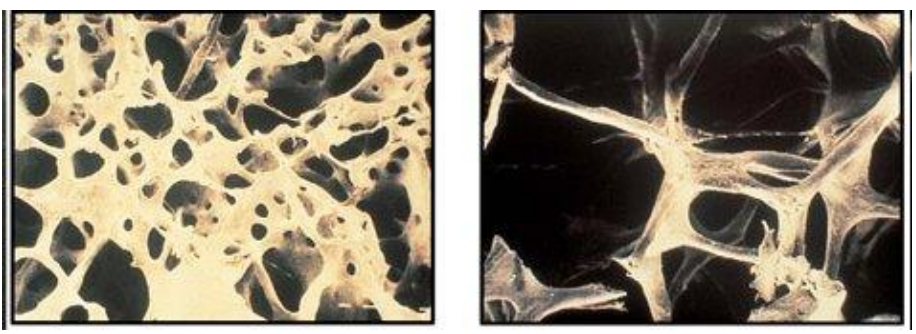


Figure 1. The figure shows a bone with normal bone mass and an osteoporotic bone with decreased bone mineral content and microstructural deterioration.

In Sweden, it has been estimated that 70 000 osteoporotic fractures occur annually, including 18 000 hip, 15 000 vertebral, and 25 000 forearm fractures [1]. The total cost of fractures are estimated to be as high as 5.6 billion Swedish crowns per year (2007), which is approximately 3.2% of the total health care costs in Sweden [8]. The different fractures are associated with different outcomes. The incidence of forearm fractures usually starts to increase between the ages of 40 and 65 years. In Sweden, the lifetime risk of wrist fracture is 21% for women at the age of 50 and 5% for men of the same age [3]. Vertebral fractures represent an underestimated fracture type that can cause back pain, limitations in activities of daily living, and psychosocial impairments [10]. Furthermore, thoracic or lumbar vertebral fractures have been suggested to be a risk factor for long-term morbidity (especially in women) and even mortality (in both genders) [3, 11]. Osteoporosis-related hip fractures cause a degree of high morbidity and lead to impaired quality of

life. Moreover, they account for most of the costs associated with and deaths due to osteoporosis [3].

Definition of osteoporosis

Today, the diagnosis of the osteoporosis is based on measurements of bone mineral density as laid out by the World Health Organization (WHO) guidelines in 1994. The assessments of bone mineral density (BMD, g/cm^2) are preferably made at the hip using dual energy x-ray absorptiometry (DXA) [2, 12].

The WHO proposed quantitative threshold values for the diagnosis of low bone mass and osteoporosis that were subsequently modified by the International Osteoporosis Foundation [13] (**Table 1**). BMD has an approximately normal distribution and therefore BMD values are expressed in relation to a reference population in standard deviation (SD) units (T-score). The reference population consists of young adult females in the same population. Osteoporosis in postmenopausal Caucasian women is defined as a BMD of 2.5 SD or more below the average value for young adult females. Despite the fact that these threshold values were defined in women, these values are also used in men [2, 12, 14].

Diagnostic category	Definition	BMD T-score
Normal bone density	Bone density is less than 1 SD below the average young adult value	>- 1
Osteopenia	Bone density is between 1 SD and 2.5 below the average value for young adults	-1 to -2.5
Osteoporosis	Bone density is at least 2.5 SD below the average value for young adults	<- 2.5
Established osteoporosis	Bone density is at least 2.5 SD below the young average adult value and the person has had at least one osteoporotic fracture	<-2.5

Table 1. Definition of osteoporosis.

Risk factors for osteoporosis

Osteoporosis is a multi-factorial disease in which genetic factors are estimated to determine 50- 70% of the variance in bone mass [15, 16]. Although BMD is a good predictor of osteoporosis-related fractures, there are other important risk factors that contribute to fracture risk that are independent of BMD, such as older age, physical inactivity, and low body weight [1, 17]. The clinical risk factors for osteoporosis and fractures are often divided in risk factors that cannot be modified and those that can be modified. These are shown in **Table 2**.

Risk factors that cannot be modified	Risk factors that can be modified
Age	Physical inactivity/immobilization
Previous fracture	Low body weight/anorexia nervosa
Female sex	Glucocorticoid medication use
Premature menopause	Cigarette smoking
Family history of fractures	Poor vision
Ethnicity	Alcohol abuse
Height	Vitamin D deficiency
Neuromuscular disorders	Calcium deficiency
	Hypogonadism
	Hyperparathyroidism
	Malabsorption
	Renal disorders
	The use of certain pharmaceutical agents
	Propensity for falling

Table 2. Risk factors for osteoporosis and fractures**.** Adopted from SBU [1] and Kanis et al. [2].

Bone structure

The bone is an organ that has several functions, including protecting vital organs from trauma, serving as a calcium storage, providing mechanical support for soft tissues, facilitating locomotion by providing attachments for muscles that allow them to acts as levers, and supporting hematopoiesis [18]. Bone can be divided into the following five types on the basis of shape: flat, short, irregular, sesamoid, and long bones. Long bones, such as the humerus, tibia, and femur, have two epiphyses with a midshaft (diaphysis) and a metaphysis (developmental zone) between them. Flat bones, such as the skull bones, scapulas, sternum, ribs, and ilium, provide protection for the internal organs and provide sites for muscle attachment. Bones like the vertebral bodies and the calcaneus are classified as irregular bones. Short bones, such as the carpal and tarsal bones, are defined as such because they are nearly equal in length and width. Sesamoid bones, such as the patella, protect tendons from excessive wear and tear [19].

All bones consist of two types of bone tissue, cortical and trabecular. The denser outer surface, or cortex, is composed of compact bone, cortical bone, and the inner region of bone are braced by narrow plates or trabecula, called trabecular bone [19]. The ratio of trabecular to cortical bone varies by skeletal site. Long bones consist almost solely of cortical bone, whereas about 75% of vertebral bodies consist of trabecular bone [1]. Although trabecular and cortical bone have different appearances, both contain the same materials; organic bone matrix, bone mineral, and several types of bone cells [18, 20].

The majority of the bone matrix is composed of collagen fibers and non-collagenous proteins. The matrix constitutes of more than 25% of the bone [18]. There are several types of collagen in bone, but adult bone is mainly composed of type I collagen, which is produced by osteoblasts (the bone-forming cells) [20]. This fibrous organic matrix gives bone its resistance to tractional and torsional forces [18]. The most important non-collagenous proteins that comprise part of the organic bone matrix include proteoglycans, glycoproteins, osteopontin, osteocalcin, and osteonectin. Proteoglycans, osteonectin, and glycoproteins are molecules that are presumably involved in the binding of bone minerals to the collagenous matrix [18]. Osteocalcin is a protein that has been associated with bone mineralization [21] (please see the “Bone turnover markers” section, p. 28).

The inorganic component of bone consists primarily of calcium and phosphorus. These minerals are organized into hydroxyapatite crystals ($Ca_{10}(PO_4)_6(OH)_2$) [18] that are elongated and hexagonal in shape, and lie

closely to the orientation of the collagen fibrils [20]. In the inorganic component of the bone matrix, there are also other trace elements, such as fluoride, strontium, and magnesium. The total body calcium content is about 1300 g in adults. The inorganic component of bones is the greatest source of calcium in the body, comprising 99.9% of the body's calcium stores. These minerals give bone its stiffness and resistance to compressive forces [18].

Bone cells

Bone is an active tissue that renews continuously. This process is maintained by three types of bone cells: osteoblasts, osteoclasts, and osteocytes. Osteoblasts, which are mesenchyme-derived, are the bone-forming cells [22, 23]. These cells synthesize and secrete type I collagen and alkaline phosphatase, an enzyme that plays a key role in the mineralization process [22]. In addition, osteoblasts form an unmineralized bone matrix called osteoid. They also synthesize collagenase, prostaglandin E_2 (PGE_2), and the bone-associated proteins osteocalcin and osteonectin [18].

Bone resorption is carried out by hematopoietically-derived, large, multinucleated cells called osteoclasts that break down calcified bone or cartilage [18, 23]. These cells are derived from a stem cell precursor of the monocyte/macrophage lineage [18]. Osteoclastogenesis begins when a hematopoietic stem cell is stimulated to generate mononuclear cells. After that these mononuclear cells become committed preosteoclasts they are entering into the circulation. Some of the circulating preosteoclasts attach to bone and develop into osteoclasts [18, 22]. Osteoclasts are very efficient cells that break down mineralized bone by attaching to the surface of a bone and secreting acids and lysosomal enzymes between their surface and the bony surface. The bone is dissolved by this chemical process [20].

Osteocytes are the third type of bone cells that play an important role in bone renewal. These cells are terminally differentiated, inactive osteoblasts. While active osteoblasts are secreting unmineralized matrix, a small number of osteoblasts fall behind and become incorporated into the matrix [22]. These osteoblasts go through a morphological change and develop into osteocytes with cytoplasmic processes that connect them to other osteocytes and osteoblast-derived bone-lining cells, which are found on the bone surface [24]. Osteocytes communicate with each other and lining cells through a network of canaliculi [24]. It has been speculated that osteocytes act as mechanosensors [22, 25] (**Figure 2**).

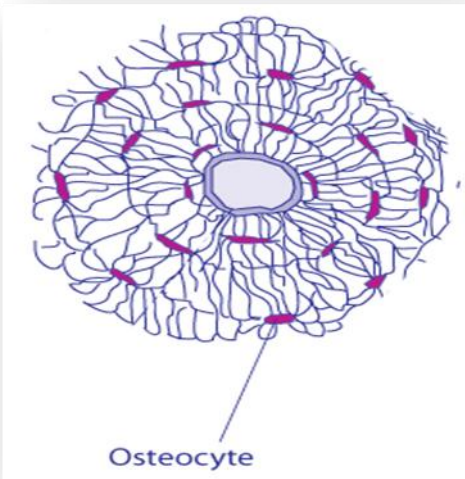


Figure 2. The majority of bone cells are osteocytes that are embedded in the calcified bone matrix. They have contact with each other and with cells on the bone surface through a network of canaliculi.

Bone modeling and remodeling

Both cellular mechanisms, modeling and remodeling, are responsible for the adaptation of the bone structure. Bone modeling occurs during period of growth and it produces a change in the size and shape of bone when new bone is deposited without previous resorption [26]. In the adult skeleton, the bone is being continuously remodeled as osteoclasts break down micro-damaged bone and osteoblasts form new bone in its place [18]. The purpose of the modeling process is to establish a high peak bone mass during periods of growth and the purpose of the remodeling process is to maintain bone strength [24]. During the remodeling process, osteoblasts and osteoclasts work together in teams and create a temporary anatomic structure, a basic multicellular unit (BMU) [22] **(Figure 3)**. Osteoclast teams are always followed by osteoblast teams, and the entire structure moves as a single unit. Thus, bone resorption and formation are coupled to each other in most cases, at least under normal physiological conditions. Resorption of bone by osteoclasts takes about three weeks and bone formation by osteoblasts takes about three months [22] .

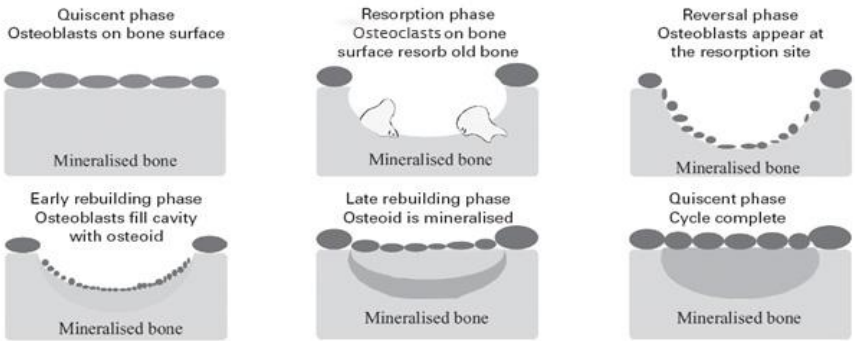


Figure 3. Bone remodeling process.

Bone structure and mechanical loading

Bone is a dynamic tissue that is able to adapt its structure in response to mechanical loading [22]. This was discovered in 1892 by the German anatomist Julius Wolff. He and his colleagues saw that mechanical loading can affect bone architecture. Under these conditions, a bone remodels itself to become stronger, and when the loading is decreased, the bone will lose strength [27]. This is called Wolff's Law. The bone remodeling process can occur on all four surfaces of the bone: the periosteal, endocortical, trabecular, and intracortical surfaces. The bone remodeling process, which is stimulated by mechanical loading, is thought to be initiated by osteocytes because these cells may be strain-sensitive and therefore may transduce mechanical loading signals to the osteoblasts and the osteoclasts [22].

The majority of bone cells are osteocytes, and they are distributed throughout the bone tissue [24] (**Figure 2**). They are always oriented in a way that enables them to maintain gap junction connections with the nearby osteocytes and osteoblast-derived lining cells on the bone surface [24]. Through this mechanism, mechanical and metabolic signals can be sent between different osteocytes and between osteocytes and bone lining cells. It has been suggested that osteocytes may not react directly to strain, but rather do so indirectly as a result of changes in the extracellular fluid flow that occur with loading. This fluid flow causes shear stress on the membranes of bone cells that is proportional in magnitude to the applied bone tissue strain rate, and is enhanced at higher loading frequencies [28, 29]. In sum, osteocytes detect changes in mechanical loading and transmit

signals through bone lining cells to osteoblasts and osteoclasts. This complex process leads to new bone formation [22].

Both animal and human studies have shown that activity must be weight-bearing and dynamic to increase bone formation [30-33]. In contrast, static strains do not produce an adaptive response in the bone [30]. Also, strain rate and frequency seem to be an essential determinant of bone adaptation [29, 34]. Thus, a high strain rate is related to a greater osteogenic response than a low strain rate [35]. This has been demonstrated in many exercise studies that have shown that there is a higher BMD among athletes taking part in high-impact sports (such as racket sports and gymnastics) than among athletes taking part in low-impact sport sports (such as swimming and cycling) [31, 36, 37]. In addition, strains in unusual loading directions have been suggested to be most effective for inducing bone remodeling [38].

Predictors of peak bone mass

Peak bone mass is defined as the amount of bone tissue present when skeletal maturation is completed [39]. It has been suggested that peak bone mass may account for at least 50% of the variation in the bone mass even among very elderly individuals [40]. There is now strong evidence that peak bone mass is achieved at different skeletal sites just before and at the end of the second decade of life. However, there are great gender and skeletal site differences related to when peak bone mass is attained [41-44].

Genetic factors affecting peak bone mass

Results from twin and family studies have suggested that variation in peak bone mass is genetically determined and depends on age and skeletal site [45-47]. Genetic factors may account for up to 50-70% of inter-individual variation in peak bone mass [15, 16] but the rest can be influenced by environmental factors, such as physical activity and nutritional status [48].

Physical activity

Exercise has been shown to be crucial for maximizing peak bone mass, and high peak bone mass is thought to be of great importance in terms of reducing bone loss later in life [49, 50]. During growth periods, the skeleton is responsive to mechanical loading and the bone adapts its structure to the loading environment to which it is exposed during this time. This has been confirmed in randomized exercise intervention studies that have demonstrated training-induced increases in both BMC and BMD [51-57]. Participation in high-impact sports, including jumping and running, seem to be especially effective at optimizing peak bone mass [58-62]. However, information regarding the exact exercise regimen (including dose, frequency, intensity, and duration) that must be undertaken to optimize peak bone mass is still lacking. According to the results of randomized studies in which different exercise programs were tested, the physical activity should be high-impact, weight-bearing, should last at least 10-20 min, and should be repeated three days a week. Although physical activity seems to be effective at inducing bone accrual, it must be kept in mind that an excessive physical training may have detrimental effects on bone mass [63, 64]. Excessive training in young adult women, especially in combination with restricted calorie intake, probably partly promotes bone loss by interfering with the menstrual cycle [63, 64]. Also, in young male endurance athletes, bone mass can be affected regardless of testosterone levels, suggesting that other factors such as low

calcium intake, energy deficits, weight loss, and low body fat may be associated with reduced bone mass [65].

Nutritional status

A good nutritional status is a prerequisite for good bone health during growth and adolescence. Since the bone gain during growth periods is dependent on adequate amounts of dietary calcium intake, calcium deficiency might lead to lower peak bone mass. However, there is conflicting evidence about the role of calcium supplementation has in increasing bone mass. In 1992, a twin study that included a cohort of prepubertal twins showed that a rise in mean daily calcium intake from 900 mg to 1600 mg increased bone mineral density in the radius, the lumbar spine, Ward's triangle, and the greater trochanter [66]. A subsequent meta-analysis examining the effect of calcium supplementation on bone mass in healthy children showed that calcium supplementation had no effect on BMD at the femoral neck and lumbar spine. This meta-analysis included 19 randomized studies involving 2859 children [67]. It might be that "normal" daily calcium intake during growth is sufficient for optimizing bone growth, and therefore calcium supplementation may not further increase peak bone mass. The Swedish National Food Administration recommends 900 mg of calcium a day for boys and girls between 10 and 17 years of age [68]. Although nutritional status and calcium intake play an important role in bone health, a 15-year prospective longitudinal study showed that weight-bearing activity and having a normal age-related weight are more important predictors of peak bone mass in lumbar spine than calcium intake [48].

Vitamin D deficiency may influence a patient's risk of fractures by influencing their bone mass, muscle strength, and balance [69, 70]. The role of vitamin D is primarily to maintain serum calcium and phosphate levels by directly influencing the intestinal absorption of these ions. Vitamin D is present in fish oils and dairy products, but the most important source of vitamin D in humans results from its endogenous production in the skin as a result of exposure to sunlight [71]. It is known that severe vitamin D deficiency causes osteomalacia and rickets, but there is little evidence of any relationship between vitamin D and peak bone mass [72]. However, in Northern latitudes, low vitamin D intake during the winter may have negative effects on the acquisition of peak bone mass since essentially no vitamin D is synthesized in the skin during this time [72].

Smoking

There is some evidence suggesting that tobacco smoking is associated with lower bone mass in adolescents and young adult smokers [50, 73]. It has also been demonstrated that young male smokers have a reduced BMD of the total body, lumbar spine and trochanter and also a reduced cortical thickness both in the radius and tibia compared to non-smokers [73]. In older men and women, continuous smoking has been shown to increase the risk of hip fracture [74, 75]. In women, a study suggested that one out of eight hip fractures is attributable to smoking [74]. Various mechanisms have been proposed to explain the effect of smoking on bone mass. These include toxic effects on bone cells through direct effects of nicotine on osteoblast cell proliferation [76], a decrease in levels of endogenous estrogen [77], a reduction of calcium absorption [78, 79], and other unhealthy lifestyle factors that may be related to smoking, such as physical inactivity [73].

Methods for investigating bone density

Measurement of bone mass or bone density is of central importance to fracture prevention because different measures of bone density have been proven to be a good estimate of bone strength and a strong predictor of the future risk of fractures in both men and women [80]. Several non-invasive methods have been developed to measure bone mass. Nowadays, the most commonly used method to measure bone mass in clinical practice and research is dual energy x-ray absorptiomentry (DXA). Other techniques include peripheral DXA (pDXA), quantitative ultrasound (QUS), and quantitative computed tomography (QCT). Furthermore, magnetic resonance imaging (MRI) has been used to investigate the microstructure of the bone.

DXA

Table model DXA machines were introduced in the late 1980s [81] and DXA is currently the most widely used bone densitometry technique [80] (**Figure 4**). Accordingly, the WHO criteria for osteoporosis and osteopenia are based on bone mineral density measurements made by DXA [12]. DXA machines can measure the amount of mineral in certain parts of the skeleton, e.g., at the hip or spine, or the total amount of bone mineral in the entire body. Moreover, DXA also measures lean body mass and fat body mass (**Figure 5**). Measurements made at one site have a strong correlation with measurements made at other sites, [82] and it has been shown that the BMD of the hip is a great predictor of not only hip fractures, but also fractures at other sites [83].

Figure 4. Table model DXA machine. (Photo by Fredrik Eklund)

The first DXA scanners used a pencil beam of radiation that took 5-10 minutes to scan a patient's hip or spine. With the newer fan-beam DXA scanners, scan times have been shortened and imagine quality has improved significantly [80]. With a fan-beam scanner, it takes only 10 to 30 seconds to measure the BMD of the hip or spine [84].

The advantages of DXA include a good measurement precision, a short scan time, and a low radiation dose. In DXA scans, precision is usually expressed as a coefficient of variation (CV). For in vivo scans, the precision is 1-2.5% [82]. The radiation dose for spine, hip, or whole body scans ranges from 1 to 70 µSv, depending upon the equipment and the scan mode that are used [80]. In contrast, the average background radiation to which an individual is exposed is about 2400 µSv per year [85].

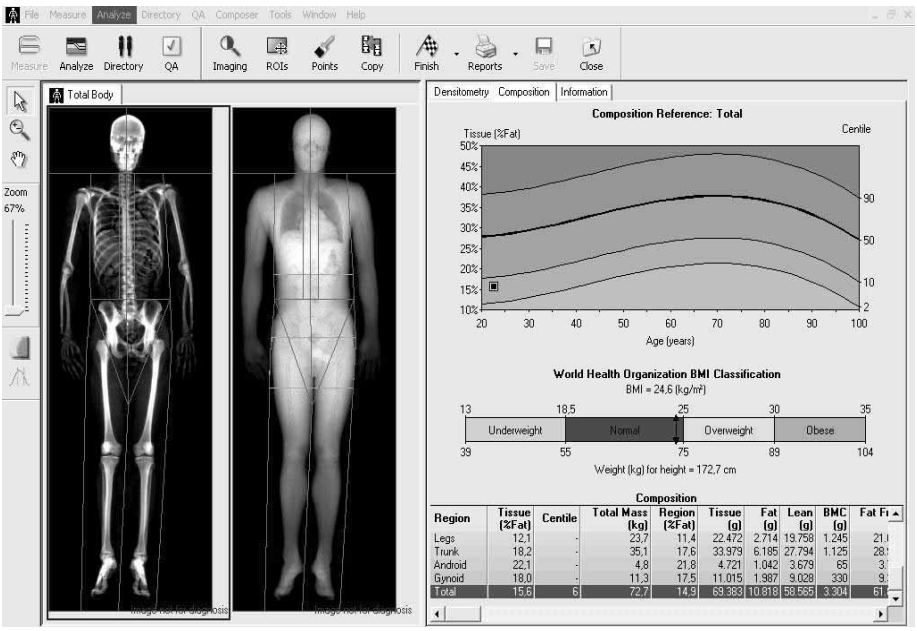
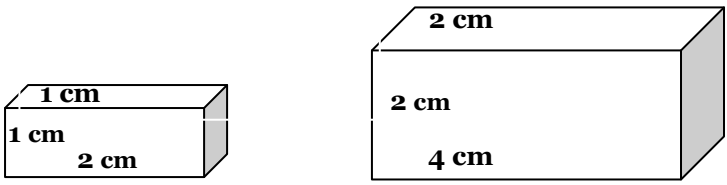


Figure 5. DXA measures bone mineral density as well as also lean and fat body mass.

A drawback to DXA scanning is that the BMD measurements it makes are affected by bone size. Thus, if a large and small bone have the same volumetric BMD (vBMD g/cm^3), the larger one will appear to have a higher BMD [82] (**Figure 6**). Another disadvantage of DXA is its inability to discriminate between cortical and trabecular bone. Finally, different manufacturers of DXA equipment use different analysis algorithms, calibration standards, and region of interest (ROI) definitions. Thus, scans from different DXA machines cannot be directly compared. For that reason, the International DXA Standardization committee (IDSC) performed a cross-calibration study on three DXA scanners made by three different manufacturers in order to allow physicians to calculate a standardized BMD value (sBMD) regardless of the DXA machine that was used [86].



	Small	Large
Projected area, cm ^{2}	2	8
Volume, cm ^{3}	2	16
vBMD, volumetric bone density**,** g/cm ^{3}	1	1
aBMD, areal bone density, g/cm^2	1	2
BMC, bone mineral content, g	2	16

Figure 6. Bone size influences DXA measurements. This figure illustrates the effect that bone size has on BMD measurements. Both samples have an identical vBMD, but the measured BMD of the larger sample is twice that of the smaller sample.

Peripheral DXA

Peripheral DXA (pDXA) measures bone density at peripheral sites, such as the forearm or calcaneus. Due to the smaller size of the device, it is portable, cheaper, and easier to use than table DXA scanners [87]. One drawback of pDXA is a lack of standardization between different manufacturers and between pDXA and whole body DXA. The diagnosis of osteoporosis, i.e., a T –score of -2.5 or below, is used to diagnose osteoporosis despite the lack of evidence that BMD at peripheral sites correlate with BMD measurements in the axial skeleton, such as the hip and spine [88]. The measurements might also be misleading since there are differences in age-related bone loss at the different skeletal sites [89]. However, a peripheral test has been shown to be related to the future risk of fractures, although BMD of the hip is generally a better predictor of this [87].

QUS

Quantitative ultrasound techniques have been developed in recent years and the use of QUS is rising. The QUS technique has been shown to be a useful tool that provides information on bone status and fracture risk. QUS uses sound waves rather than radiation to assess properties of bone that are related to density and bone strength [82]. QUS measurements include

measurements of speed of sound (SOS) through bone as well as the absorption of sound by bone, called broadband ultrasound attenuation (BUA). BUA is influenced by bone structural parameters and also by bone density. On the other hand, SOS is affected by bone density and elasticity. It has been demonstrated that calcaneal QUS measurements predict the risk of hip fracture and non-spine fractures in older men, [90] and osteoporotic fractures, particularly hip fractures, in women [91-93]. However, DXA should be considered the gold standard for evaluating the fracture risk because it has been validated in several populations, and there are published normative data as well as well-defined and widely available quality control procedures to ensure the accuracy and reliability of the results [90]. Calcaneal ultrasound measurements seem to be highly reliable and sensitive to longitudinal changes in BMD over time, even in the elderly [94]. Even if all QUS devices are based on the same principles, there are differences when it comes to the precision, accuracy, and skeletal site measured by the different devices [95]. Therefore, when analyzing data derived from different techniques, it must be kept in mind that not all ultrasound techniques have reached the same significance level with regard to clinical results and thus they are not all equally reliable in the clinical setting. Consequently, a better standardization of instruments is needed if QUS is to be used for the diagnosis of osteoporosis [94]. Advantages of QUS include its low cost, the lack of exposure to ionizing radiation, and its portability.

QCT

Quantitative computed tomography (QCT) is the only radiological method that can be used to measure true volumetric bone density (g/cm^3). This is an advantage when studying growing children and adolescents, in whom DXA scanners might underestimate or overestimate the true bone density due to growth-related variation in bone size [96]. It also allows separate measurements to be made of the trabecular and cortical bone compartments, increasing the ability to identify specific effects that certain diseases or treatments might have on bone structure [97]. Since trabecular bone is more metabolically active than cortical bone, QCT can be more sensitive to changes in the bone than BMD measured by DXA [97]. In addition, QCT is less influenced by degenerative diseases of the spine than DXA [80]. It is a very precise method for investigating bone mass and structure [98]. QCT is not widely used in clinical practice because it is more expensive than DXA and also exposes patients to a higher radiation dose than DXA. Another limitation of QCT is that the WHO has not defined thresholds for diagnosing osteoporosis using QCT measurements like it has for DXA measures. It is currently mainly used as a research tool [97]. Peripheral QCT is used to

estimate bone density at peripheral sites of the body, such as the radius and tibia. Peripheral QCT of the distal radius have been shown to predict hip fractures, but not vertebral fractures, in postmenopausal women [97]. Advantages of peripheral QCT include that the method is relatively inexpensive, it is easy to use, and the radiation dose to which patients are exposed is negligible [97].

MRI

Magnetic resonance imaging (MRI) appears to be a useful method for measuring trabecular bone micro-architecture and the structural components of bone in vivo [99]. Using high-resolution MR imaging, the trabecular network can be analyzed in both two and three dimensions. This makes it possible to analyze the bone volume/total volume ratio, trabecular thickness, trabecular number, and trabecular separation of the bone that is being imaged. These parameters can be used to assess for osteoporosis [80]. Disadvantages of MRI include the high cost and the time-consuming nature of the test. Both MRI and QCT can measure trabecular bone micro-architecture, but the advantages of MRI include that it does not rely on ionizing radiation and allows for multi-planar image acquisition [99].

Methods for investigating bone metabolism

Bone turnover markers

The skeleton is constantly being remodeled by bone formation and bone resorption. As a result, bone is persistently being remade. Markers of bone turnover are released into the circulation during this process and reflect the activity of osteoblasts (the bone-forming cells) and osteoclasts (the bone-resorbing cells). Thus, these markers increase our understanding of the bone remodeling cycle. Biochemical assays that determine the serum or urine levels of bone markers may be useful in the study of skeletal metabolism [100]. Some of the markers of bone formation are enzymes or other proteins secreted by osteoblasts. These markers can be measured in the serum [101]. Markers of bone resorption are mostly produced during the breakdown of type I collagen, the primary protein that forms the bone matrix that is produced by osteoblasts. Bone resorption markers can be measured both in serum and in urine [102].

BMD changes take several months to be apparent on DXA, whereas bone turnover markers are very sensitive to changes in bone formation and resorption, and may reflect an early response to changes in bone metabolism, e.g., after initiation of anti-resorptive drug therapy. Consequently, bone markers can be used as a complement to BMD measurements to attain a more accurate picture of the bone metabolism. Assays for monitoring bone turnover are relative cheap, safe, and easy to perform [103]. The disadvantage of bone turnover measurements is the relatively poor precision of the assays as well as the existence of diurnal and day-to-day variation in the levels of bone turnover markers [104]. The interpretation can be especially difficult in children and adolescents because the levels of bone markers are also affected by age, pubertal stage, hormonal regulation, growth velocity, and nutritional status. An additional disadvantage is that the levels of these markers reflect the rate of bone turnover in the whole skeleton and not at individual skeletal sites [105].

Osteocalcin

Osteocalcin (OC), also called bone GLA protein, is a non-collagenous protein that is synthesized by differentiated osteoblasts as they deposit new bone matrix. Serum levels of osteocalcin can be used as a marker of bone turnover and osteoblast activity [18]. However, although osteocalcin seems to be a marker of bone turnover and has been associated with bone mineralization [21], its function with respect to bone structure is not really known. In a recent ground-breaking study, it was shown that osteocalcin influences both lipid and glucose metabolism in experimental models [106]. These data have been confirmed in clinical studies [107].

CTX

Carboxy terminal telopeptide of type 1 collagen, or CTX, is a peptide that is a degradation product of type I collagen. CTX levels primarily reflect osteoclast activity and therefore, bone resorption. CTX can be measured in the serum or the urine. Although CTX seems to be a sensitive and specific index of bone resorption in adults [100], there is no consistent data suggesting that CTX, or any other bone markers, can predict the risk of future fractures [108].

Parathyroid hormone

Parathyroid hormone (PTH) is a polypeptide hormone that is synthesized by the chief cells of the parathyroid gland. It is an important regulator of plasma calcium concentration. If the serum calcium level falls, PTH acts to increase the concentration of calcium through its actions on the bone, intestine, and kidneys. Therefore, some of PTH's effects are indirect. In the bone, PTH stimulates osteoclasts to resorb bone, most likely indirectly through osteoblast signaling, because osteoclasts do not have PTH receptors. PTH acts on the kidneys to increase the tubular resorption of calcium and decrease phosphate reabsorption in the proximal tubule. PTH also increases 1,25-dihydroxy-vitamin D synthesis in the kidneys leading to a subsequent increase in intestinal calcium absorption [18].

Physical activity

Effects of physical activity on BMD in children

The effects of physical activity on the growing skeleton have been studied broadly in cross-sectional trials [109], non-randomized interventional trials [110], and randomized trials [51-56, 111-113] (**Table 3a and b**). It has been recognized that physical activity during the growing years is a key determinant of peak bone mass, and that a higher peak bone mass accrual may influence fracture risk later in life [114]. Randomized controlled interventional studies have indicated that weight-bearing activity not only increases BMD and BMC, but also increases bone size in 6-12-year-old children during 7-20 month-long interventions [51-56] (**Table 3a and b**). It has also been observed that the effects of weight bearing activity are site- and region-specific because jumping sports primarily improve BMD in the lower limbs while racket sports lead to bone mass improvements in the playing arm. The intervention programs in these studies were conducted in school settings and ranged in duration from 10-30 min and in frequency from 3-5 times a week, emphasizing that only a limited amount of physical activity is needed to achieve significant effects on bone mass accrual during childhood. In summary, physical activity seems to have beneficial effects on both the bone mineral accrual and structural geometry in the growing skeleton of both boys and girls.

Table 3a. The bone mass response to physical activity observed in non-randomized controlled intervention study and randomized controlled interventional studies of children.

Author	Participants /Age	Intervention /Exercise	Study period	Measurements	Results
Non-randomized interventional study					
Morris et al. [110]	71 girls 9-10 years	High-impact 3 times a week	10 months	BMD, BMC, and BA of the TB, LS, PF, and FN	All BMC, BMD, and FN BA increased significantly more in the IG than in the CG
Randomized interventional studies					
Bradney et al. [56]	40 boys 8.4-11.8 years	Weight-bearing 3 times a week	8 months	BMC and BMD of the TB, LS, and legs	TB, LS, and leg BMD increased significantly more in the IG than in the CG
MacKay et al. [51]	144 children 6-10 years	High-impact 3 times a week	8 months	BMC, BMD, BA of the TB, LS, and PF	TR BMD increased significantly more in the IG than in the CG
Fuchs et al. [55]	38 girls, 51 boys 5.9-9.8 years	High-impact 3 times a week	7 months	BMC, BMD, BA of the FN and LS	FN, LS BMC, LS BMD and FN BA increased significantly

BMD= bone mineral density, BMC=bone mineral content, BA= bone area, TB=total body, LS=lumbar spine, FN=femoral neck, PF=proximal femur, TR=femur trochanter, IG=Intervention group, CG=Control group .

Table 3b. The bone mass response to physical activity observed in randomized controlled interventional studies in children.

Author	Participants /Age	Intervention /Exercise	Study period	Measurements	Results
MacKelvie et al. [53]	87 girls 8.7-11.7 years	High-impact 3 times a week	7 months	BMD, BMC of TB, LS, PF, FN, TR, vBMD of the FN	FN, LS BMC, BMD, and FN vBMD increased significantly more in the ErPG than in the CG
MacKelvie et al. [52]	75 girls 8.8-11.7 years	High-impact 3 times a week	20 months	BMC of the TB, LS, and PF	FN and LS BMC increased significantly more in the IG than in the CG
MacKelvie et al. [54]	64 boys 8.8-12.1 years	High-Impact 3 times a week	20 months	BMC and BA of the TB, LS and PF	FN BMC increased significantly more in the IG than in the CG
MacDonald et al. [111]	281 boys and girls, 129 controls 10.2±0.6 at baseline	High-impact 5 times a week	16 months	BSI of the DT and SSIp of the TMS as assessed by pQCT	BSI increased significantly more in prepubertal boys than postpubertal boys and girls

BMD= bone mineral density, BMC=bone mineral content, BA= bone area, TB=total body, LS=lumbar spine, PF=proximal femur, LS=lumbar spine, FN=femoral neck, TR=trochanter, DT= distal tibia, vBMD= volumetric BMD, TMS=tibial midshaft, BSI= bone strength index, SSIp= polar strength strain index, pQCT= peripheral dual-energy X-ray absorptiometry, IG=Intervention group, CG=Control Group, ErPG= early pubertal group.

Effects of physical activity on BMD in adolescents

Data from one interventional study [112] (**Table 4a and b**) and two cross-sectional studies indicate that physical activity is more beneficial for growing girls early in puberty than it is after puberty [115, 116].

However, cross-sectional studies have demonstrated a higher bone mass in athletes involved in weight-bearing activities than in sedentary controls in both genders [31, 37, 60, 117, 118]. One cross-sectional study also found a larger bone size and higher bone mineral content in adolescent boys participating in high-impact activities lasting one hour or more a day than less active adolescent boys [119]. Effects on bone mass have mostly been observed in weight-loaded regions. The limitations of these studies include their small sample sizes, their cross-sectional study designs, and the risk of selection bias due to a genetic predisposition to higher BMD in athletes.

Results from interventional studies evaluating the effects of physical activity on bone mass are conflicting [48, 112, 120-129] (**Table 4a and b**). Two studies failed to show that physical activity had any effects on bone mass in adolescent girls [121, 122]. In contrast, four other studies found that there was a higher BMD in the lumbar spine and proximal femur and a higher total body and femoral neck BMC in physically active boys and girls compared to control subjects. These differences in results might be explained by the variations in the type and intensity of training (resistance training vs. high-impact training) assessed in these studies, too high activity of the control group, too short interventional duration, and a lack of power. More consistent results that have been obtained from observational longitudinal studies suggest that participation in weight-bearing activities increases bone mass in both boys and girls [48, 124-127]. One three-year observational study showed a higher bone mass in athletes taking part in badminton or ice hockey compared to sedentary controls [128]. Consequently, it seems that weight bearing, high-impact activity during adolescence is important for skeletal mineralization like it is in childhood.

Table 4a. The bone mass response to physical activity observed in interventional studies in adolescents.

Author	Participants /Age	Intervention /Exercise	Study period	Measurements	Results
Randomized intervention studies					
Snow-Harter et al. [120]	52 girls 19.9+0.7 years	Running or weight lifting 3 times a week	8 months	BMC and BMD of the FN and LS	LS BMD increased significantly more in runners and weightlifters than in the CG
Weeks et al. [123]	46 boys, 53 girls 13.8 years	High Impact 2 times a week	8 months	Calcaneal BUA, BMC, BMD, and BA of the FN, TR, LS, and TB	Calcaneal BUA as well as FN, TB, and LS BMC increased significantly more in the IG than in the CG
Kato et al. [129]	36 young women 20 years	10 maximal jumps/day 3 times a week	6 months	BMD of the LS, FN, Ward´s, and TR	FN and LS BMD increased significantly more in the IG than in the CG
Non-randomized interventional studies					
Blimkie et al. [121]	36 girls 14-18 years	Resistance training 3 times a week	6 months	BMC and BMD of the TB and LS	NS
Witzke et al. [122]	53 girls 13-15 years	High impact 3 times a week	9 months	BMC of the LS, PF, and TB	NS
Heinonen et al. [112]	139 girls 10-15 years	Step aerobics + extra jumping 2 times a week	9 months	BMC of the LS and PF	LS and PF BMD increased significantly more in the Pre than in the Post and CG

BMD= bone mineral density, BMC=bone mineral content, BA= bone area, LS=lumbar spine, FN=femoral neck, TB=total body, PF=proximal femur, TR=trochanter, Ward´s= Ward's triangle IG=Intervention group, CG=Control group, Pre= premenarcheal group, Post=postmenarcheal group, BUA=broadband ultrasound attenuation, NS=non-significant.

Table 4b. The bone mass response to physical activity observed in interventional studies in adolescents.

Author	Participants /Age	Intervention /Exercise	Study period	Measurements	Results
Observational longitudinal studies					
Welten et al. [48]	84 boys, 98 girls 13-27 years	Interview evaluating exercise levels	15 years	BMD of the LS	LS association significant
Slemada et al. [124]	90 twins 6-14 years	Correlation analysis	3 years	BMD of the LS, R, and PF	LS, R, and PF BMD were significantly correlated to physical activity
Bailey et al. [125]	60 boys, 53 girls 8-14 years	Inactive, average activity level, and active	6 years	BMC of the TB, PF, and LS	FN and TB BMC increased significantly more in active vs. inactive
Lehtonen-Veromaa et al. [126]	155 girls 9-15 years	Gymnasts, runners, CG	1 year	BMD and BA of the LS and PF	FN ,TR BMD increased significantly more in gymnasts than in runners vs. Cg
Forwood et al. [127]	109 males, 121 females 15-22 years	Inactive, average activity level, and highly active	7 years	TB and PF BMC HAS, and Z	PF and TB BMC, CSA, and Z increased significantly in highly active vs. inactive
Gustavsson et al. [128]	20 ice hockey players, 12 badminton players, 24 controls 16 years	Ice hockey Badminton	3 years	BMD of the TB, S, H, FN	TB, FN and H BMD increased significantly in athletes vs. the CG

BMD=bone mineral density, BMC=bone mineral content, BA=bone area, LS=lumbar spine, R=radius, FN=femoral neck, TB=total body, PF=proximal femur, TR=trochanter, HAS=hip structural analysis, BSI=bone strength index, CSA=cross sectional area, Z=section modulus of bone, H=humerus, S=spine, CG=Control Group.

Effects of physical activity on BMD in males

Cross-sectional studies have shown that male athletes involved in weight-bearing activities have higher BMDs than inactive controls [59, 60, 117, 118, 130] or athletes involved in non-weight-bearing sports, such as swimming [131, 132] or cycling [31-33].

Only one randomized controlled study has investigated the effects of physical activity on bone mass in young men [133] (**Table 5**). This four-month-long study used weight lifting as the intervention, and failed to demonstrate any significant differences in BMD between weight lifters and controls. The reason for these results might be the non-dynamic type of activity in which study participants participated or duration of follow-up, as it may be that four months is too short of a period of time to detect any changes in BMD by DXA. Data from two longitudinal observational studies, [134, 135] as well as retrospective studies with wide age span indicate that weight-bearing physical activity and an active lifestyle appear to be associated with higher BMD and less bone loss at weight-bearing sites in men [136-138]. However, it must be kept in mind that there is always a risk of recall bias in retrospective studies.

Table 5. The bone mass response to physical activity observed in studies in men.

Author	Participants /Age	Intervention /Exercise	Study period	Measurements	Results
Randomized interventional study					
Fujimura et al. [133]	17 males 23-31 years	Weight training 3 times a week	4 months	BMD of the LS, TB, FN, and R	NS
Longitudinal observational studies					
Delvaux et al. [135]	126 men 13 years at the start of the study	Self reported questionnaire evaluating exercise levels	27 years	BMC and BMD of the TB and LS	TB BMC and LS BMD were association significant
Daly et al. [139]	152 males 61.8±9 years at the start of the study	Interview-administered questionnaire	10 years	BMD of the R	Significantly less bone loss observed in active vs. inactive men
Retrospective studies					
Nguyen et al.[136]	690 men 60 years and older	Interview evaluating exercise levels		BMD of the LS and FN	FN BMD was significantly associated with exercise levels overall, but not after adjusting for age and BMI
Neville et al. [137]	242 20-25 years	Self-reported questionnaire evaluating exercise levels		BMD and BMC of the FN and LS	BMC and BMD of the FN and LS were significantly associated with exercise levels
Lynch et al. [138]	16 former professional football player, controls 66±6 years	Self-reported questionnaire evaluating exercise levels		BMC and BMD of the TB, LS, and PF	TB BMC and BMD, as well as LS and FN BMD were significantly increased in FP

BMD=bone mineral density, BMC=bone mineral content, LS=lumbar spine, PF=proximal femur, FN=femoral neck, TB=total body, R=radius, BMI=body mass index, FP=football players, NS= non-significant.

Effects of physical activity on BMD in premenopausal women

Interventional studies of premenopausal women have shown that high-impact physical activity has positive effects on bone mass (**Table 6a and b**) [140-145]. These effects seem to be site-specific. In most of the studies, exercise interventions have included exercise three times a week. In general, studies that have used weight training as an intervention has not shown any effects on bone mass [142, 146]. However, one study demonstrated small but significant increases in bone mass after 18 months of resistance training [140]. The inconsistency in results may be explained by the dissimilar ages of the persons included in the different studies and differences in the activities that were performed. In addition, the study groups have been rather small and the dropout rates have been relatively high in some of the studies.

Table 6a. The bone mass response to physical activity observed in interventional studies in premenopausal women.

Author	Participants /Age	Intervention /Exercise	Study period	Measurements	Results
Randomized controlled studies					
Lohman et al. [140]	56 women 28-39 years	Resistance training Calcium supplementation	18 months	BMD of the TB, LS, and PF	LS, TR BMD increased significantly in the IG as compared to the CG
Heinonen et al. [141]	98 women 35-45 years	High-impact 3 times a week	18 months	BMD of the FN, LS, R, C, and DT	FN and LS BMD increased significantly in the IG as compared to the CG
Sinaki et al.[142]	96 women 30-40 years	Weight lifting 3 times a week Calcium supplementation	3 years	BMD of the PF, LS, and R	NS
Bassey et al. [143]	55 women 37.5 years	High-impact 3 times a week	5 months	BMD of the PF and LS	TR BMD increased significantly more in the IG compared to the CG
Vainionpää et al. [144]	120 women 35-40 years	High-impact 3 times a week + home program	1 year	BMD of the PF, LS, DF and C as measured by QUS	FN, TR, and L1 BMD as well as C BUA increased significantly more in the IG as compared to the CG

BMD=bone mineral density, LS=lumbar spine, FN=femoral neck, TB=total body, PF=proximal femur, TR=trochanter, R=radius, DF=distal forearm, C=calcaneus, DT=distal tibia, L1=first lumbar vertebrae, QUS=quantitative ultrasound, BUA=broadband ultrasound attenuation, IG=Intervention group, CG=Control Group, UP= upper body, LB= lower body, NS=non-significant .

Table 6b. The bone mass response to physical activity observed in interventional studies in premenopausal women.

Author	Participants /Age	Intervention /Exercise	Study period	Measurements	Results
Randomized controlled study					
Winter-Stone et al. [145]	59 women mean 40 years	Resistance and jump LB, resistance + jump LB+ UP, controls 3 times a week	1 year	BMD of the TB, TR, FN, and LS	TR BMD increased significantly in both IGs; LS BMD increased significantly more in the UP+ LB group as compared to the CG
Non-randomized interventional studies					
Gleeson et al. [146]	68 women 24-46 years	Weight lifting Calcium supplementation	12 months	BMD of the LS	NS
Winters et al. [147]	65 women 30-45 years	High impact	12 months	BMD of the TB, TR, FN, and LS	TR BMD increased significantly in the IG as compared to the CG
Follow-up studies					
Heinonen et al. [148]	49 women 35-45 years	High impact 2 times a week, continued training	8 months	BMD of the PF, LS, and R	FN BMD increased significantly in the IG compared to the CG
Uusi-Rasi et al. [149]	133 women, 28 years at baseline	High and low activity group	10 years	BMC of the FN and TR	TR BMC was significantly higher in the physically active group as compared to the inactive group

BMD=bone mineral density, BMC=bone mineral content, LS=lumbar spine, FN=femoral neck, TB=total body, PF=proximal femur, R=radius, TR=trochanter UP=upper body, LB= lower body, IG=Intervention group, CG=Control Group, NS=non-significant.

Effects of physical activity on BMD in postmenopausal women

Results from randomized studies investigating the effects of physical activity on BMD in postmenopausal women have yielded varying results [150-157] (**Table 7a and b**). The effect of physical activity on BMD in post-menopausal women seems to be modest, although it seems that in some cases, physical activity might diminish BMD loss at sites that are exposed to mechanical loading. However, it is difficult to draw conclusions from these studies due to the different intervention times used, the limited and varying number of study subjects enrolled in the various studies, and the different types of physical activity evaluated. In addition, in some studies, calcium supplementation was used as an additional intervention along with physical exercise [153, 157].

Table 7a. The bone mass response to physical activity observed in randomized interventional studies in postmenopausal women.

Author	Participants /Age	Intervention /Exercise	Study period	Measurements	Results
Sandler et al. [150]	255 women 49-65 years	Walking	3 years	BMD of the R measured with CT	NS
Grove et al. [151]	15 women 49-64 years	High impact, low impact, or CG 3 times a week	1 year	BMD of the LS	LS BMD decreased significantly only in Cg
Nelson et al. [152]	40 women 50-70 years	Weight lifting 2 times a week	1 year	BMD and BMC of the TB, FN, and LS	FN and LS BMD increased significantly in the IG compared to the CG
Prince et al. [153]	168 women 50-70 years	Weight lifting and calcium supplementation or calcium supplementationn	2 years	BMD of the LS and PF	FN BMD decreased significantly less in the exercise + calcium group compared to the calcium group

BMD= bone mineral density, BMC=bone mineral content, LS=lumbar spine, FN=femoral neck, PF=proximal femur, R=radius, TB=total body, CT=computerized tomography, IG=Intervention group, CG=Control group, NS=non-significant.

Table 7b. The bone mass response to physical activity observed in randomized interventional studies in postmenopausal women.

Author	Participants/Age	Intervention /Exercise	Study period	Measurements	Results
Bassey et al. [154]	44 women 50-60 years	Weight-bearing or CG	1 year	BMD of the PF, LS, and R	NS
Kerr et al. [155]	56 women 40-70 years	Endurance resistance or high load resistance, one side of the body used as CG	1 year	BMD of the PF and R	PF and R BMD increased significantly in the high load resistance group compared to the CG and only R BMD in the endurance resistance group
Brooke-Wavell et al. [156]	84 women 60-70 years	Weight bearing or CG	1 year	BMD of the PF, LS, and C	C BMD increased significantly in the IG compared to the CG
Holm et al. [157]	38 women 55 years	Strength training + nutrient supplementation (IG) or strength training alone (CG)	6 months	BMD of the TB, LS, and TH	LS BMD increased significantly in the IG and the CG, and FN BMD increased significantly in the IG

BMD= bone mineral density, LS=lumbar spine, FN=femoral neck, TH=total hip, TB=total body, PF=proximal femur, IG=Intervention group, CG=Control group, NS=non-significant, R=radius, C=calcaneus.

Effects of physical activity on BMD in the elderly

The focus on studying the possible effects of physical activity on bone mass and BMD in the elderly has largely been placed on the association between fracture risk and physical activity levels in this age group. However, 3 randomized interventional studies have investigated the effects of combined weight-bearing activity on bone mass in women aged 66-87 years [158-160] (**Table 8a and b**). The studies showed improvements in bone density and structure as well as in function capacity and muscle strength after a physical activity intervention [158, 159]. It was also shown that these changes in bone structure and dynamic balance were partially maintained a year after cessation of supervised training [161].

One randomized 30-month-long interventional study assessed effects of home-based weight bearing exercise on BMD and BMC of the femoral neck, trochanter, and total hip in elderly women. This study failed to demonstrate any effects on BMD, but found a positive correlation between high-impact exercise and trochanteric BMC in the intervention group [160].

Observational studies focusing on physical activity and fracture risk reduction have shown that physical activity is associated with a reduced risk of fractures, especially hip fractures, in both men and women [162-164]. Even individuals who got only moderate levels of exercise and/or only participated in low-impact activities were shown to have a lower risk of hip fracture as compared to their less active peers. The authors of these studies have speculated that the reduced fracture risk they observed was not associated with enhanced bone mass alone, but could also have been affected by factors such as improved neuromuscular function as well as enhanced muscle strength, balance, and mobility that can results from low-impact activities.

Table 8a. The bone mass response to physical activity observed in interventional studies in the elderly.

Author	Participants /Age	Intervention /Exercise	Study period	Measurements	Results
Randomized controlled studies					
Karinkanta et al. [159]	149 women 70-78 years	Resistance training and balance-jumping combination (COMB), at least 2 times a week, or CG	1 year	BMD and BMC of the PF, HSA and R; T BSI measured with pQCT	T shaft BSI decreased 2% less in the COMB group compared to the CG
Englund et al. [158]	48 women 66-87 years	Weight bearing 2 times a week	1 year	BMD of the TB, head, arms, FN, TR, Ward´s, and LS	Ward´s BMD significantly increased in the IG as compared to the CG
Korpelainen et al. [160]	160 Women 73 years	Supervised + home-based weightbearingtraining Mean 3 times a week	30 months	BMD and BMC of the FN, TR, and TH	Positive effects on TR BMC in the IG
Follow-up study					
Karinkanta et al. [161]	120 women 70-78 years	Resistance training and balance-jumping combination (COMB) or CG at least 2 times a week	1 year	BMD and BMC of the PF, HSA and R; T shaft BSI measured with pQCT	T shaft BSI partially maintained in COMB group

BMD= bone mineral density, BMC=bone mineral content, LS=lumbar spine, FN=femoral neck, TB=total body, TH=total hip, TR=trochanter, T=tibia, R=radius, PF=proximal femur, HSA=hip structural analysis, IG=Intervention group, CG=Control Group, pQCT= peripheral quantitative computerized tomography, BSI=bone strength index, Ward´s= Ward's triangle .

Table 8b. The bone mass response to physical activity observed in interventional studies in the elderly.

Author	Participants /Age	Intervention /Exercise	Study period	Measurements	Results
Observational studies					
Cummings et al. [162]	9516 women 65 years or older	Questionnaire and interview to evaluate exercise levels	4.1 years	Potential risk factors for hip fracture	Weight-bearing training was associated with significant reduction of fracture risk
Gregg et al. [163]	9704 women 65 years or older	Questionnaire to evaluate exercise levels	7.6 years	Risk of hip, wrist, and vertebral fracture	Physical activity was associated with a reduction in hip fracture risk
Kujala et al. [164]	3262 men 44 years or older	Questionnaire to evaluate exercise levels	21 years	Risk of hip fracture	Physical activity was associated with a reduction in hip fracture risk

Detraining and bone mass

The cellular and molecular mechanisms of bone loss due to detraining are poorly understood. The mechanisms underlying the effects of unloading on bone have been studied in tail-suspended mice that were found to have increased numbers of osteoblasts and osteocytes that were undergoing apoptosis in both trabecular and cortical bone [165]. This was subsequently followed by increased numbers of osteoclasts, an increase in cortical porosity, and reduced trabecular and cortical widths. In other words, a reduction in mechanical stimulation eliminated the delivery of survival signals to the osteocytes, leading to cell apoptosis, the recruitment of osteoclasts, and further increases in bone resorption and bone loss [165].

Bed rest studies and space flights clearly show that long-term unloading leads to a negative calcium balance and demineralization of skeleton [166-168]. Weightlessness during space flight has been demonstrated to increase bone resorption and to diminish bone formation. In the proximal femur, astronauts typically lose as much bone mass in one month as postmenopausal women lose in one year [168]. The loss of BMD seems to be most marked at weight-bearing trabecular bone sites, such as the spine, femoral neck, trochanter, and pelvis with as much as 1.0-1.6% of BMD lost per month. In contrast, BMD losses in cortical bone sites, such as long bones, seems to occur at a rate of only 0.3-0.4% per month [169]. Additionally, 17 weeks of bed rest leads to similar losses in trabecular and cortical bone sites compared to the same time spent in space flights. Interestingly, no losses in BMD of the arms were detected in the bed rest study [170]. In addition, the recovery of the bone lost after bed rest or spaceflights seem to be only partial [170, 171].

Bone loss following limb disuse after fractures or spinal cord injuries has also been evaluated [172, 173]. After a fracture, there are an increased number of BMUs that demonstrate increased activity. These changes may be related to the traumatic process of the fracture and to fracture-related immobility. For example, after a tibial shaft fracture, bone loss occurs both proximal and distal to the fracture, with larger losses observed in the most distal region, which consists predominantly of trabecular bone [173]. In patients with quadriplegia after a spinal cord injury, a significant BMD loss was observed at trabecular bone sites in radius and tibia. In subjects with paraplegia due to spinal cord injury, bone loss occurred only in the tibia. In contrast, loss of cortical bone began later and occurred to a lesser extent [172].

The effects of detraining on bone mass have also been studied in athletes after the end of their active athletic career. Two Finnish studies have compared changes in BMC in the playing-to-non-playing arm in male and female racket sport players after four and five years, respectively, of decreased training [174, 175]. Both studies showed that exercise-induced bone gain did not disappear with reduced activity levels. Moreover, three studies have investigated changes in BMD in former gymnasts [176-178]. Two of these studies showed residual benefits in BMD from previous training at all measured sites. However, there were several limitations to these studies: the study designs in both studies were cross-sectional in nature; the study groups were rather small; and the time elapsed since retirement varied from 3 to 20 years [177, 178]. The third study that evaluated BMD in former gymnasts had a longitudinal study design [176]. It showed that the total body BMD (as well as BMD in several sites of the hip) of former gymnasts remained significantly higher than that of controls after four years of retirement, but that gymnasts had significantly higher declines in lumbar spine BMD compared to controls [176]. One study with eight years of follow-up evaluated the effects of reduced training on BMD in Swedish female soccer players [179]. The results indicate that decreased activity was associated with higher BMD loss of the femoral neck in the soccer players than in the controls. However, the former soccer players showed evidence of benefits in BMD of the legs even 20 years after retirement [179]. In summary, given the limited number of studies, the varying study designs, skeletal sites investigated, variations in time elapsed since retirement, and residual activity levels, no certain conclusions can be drawn.

Thesis aims and hypotheses

The overall aim of this thesis was to investigate the influence of weight-bearing physical activity and subsequent reduced physical activity on bone mass in young men. The specific aims of the thesis were the following:

- To investigate the effects of both sustained and reduced physical activity on BMD at various sites during a 12-year study period in athletes and controls.

  We hypothesized that the former athletes would have higher BMDs compared to controls, despite the intervening years of reduced activity levels.

- To determine if training and detraining have different effects on bone sites rich in trabecular and cortical bone, respectively, in athletes and controls during a 12-year study period.

  We hypothesized that bone sites consisting of predominantly cortical bone would show higher residual benefits in BMD compared to bone sites consisting of predominantly trabecular bone after detraining.

- To determine the effects of different types of weight-bearing loading on bone accrual in badminton and ice hockey players.

  We hypothesized that badminton would result in a greater osteogenic response due to the relatively higher resulting strains created in the bone.

- To investigate the associations between self-perceived health, various lifestyle factors, and BMD in a large cohort of men and women of varying ages.

  We hypothesized that high levels of self-perceived health and physical activity would be associated with a higher BMD.

Material and methods

Subjects

This longitudinal study began in 1994, when a group of healthy young Caucasian men were recruited through advertisements as well as through information distributed in two high schools, badminton, and ice hockey clubs in Umeå, a city in Northern Sweden. Of the volunteers, 116 qualified for inclusion in the study, and of these, 66 were ice hockey players, 22 were badminton players, and 28 were control subjects (**Figure 7**). At baseline, the subjects had a mean age of 17 years. At baseline and subsequent follow-up appointments, a questionnaire was used to record smoking habits, intake of dairy products, illnesses, or medication known to affect bone metabolism. None of the subjects had any disease known to affect bone metabolism and none admitted to smoking at baseline. The subjects pubertal stage was classified according to Tanner's definition by self-examination [180]. At baseline, all participants had reached at least Tanner stage 4. Information was also gathered about the type and amount of physical activity and age at which the young men began playing their respective sport via a questionnaire and through interviews with their coaches. Subjects in the control group did not participate in any organized physical training except for physical education classes at school. Control subjects were included in the study if they averaged three hours or less of physical activity per week during their sparetime. At baseline, the control subjects' average weekly total amount of physical activity was 2.6 ± 2.8 h/week. This training consisted mostly of playing soccer and floorball, distance running, and weight lifting. At baseline, athletes were training on a regular basis with their teams. They averaged 9.0± 2.8 h of training per week. This activity consisted mostly of matches as well as additional weight and aerobic training. Body weight was measured with an electronic scale and height was measured with a height meter. The groups were matched for age, weight, height, and pubertal stage. BMD measurements were also performed on the subjects' parents to evaluate the influence of heritable factors. The majority of athletes' parents had their BMD measured in 1996 and the majority of controls' parents had theirs measured in 2005. In studies I-II, BMD measurements of the cohorts' fathers were used, and in study III, BMD measurements of both parents were used.

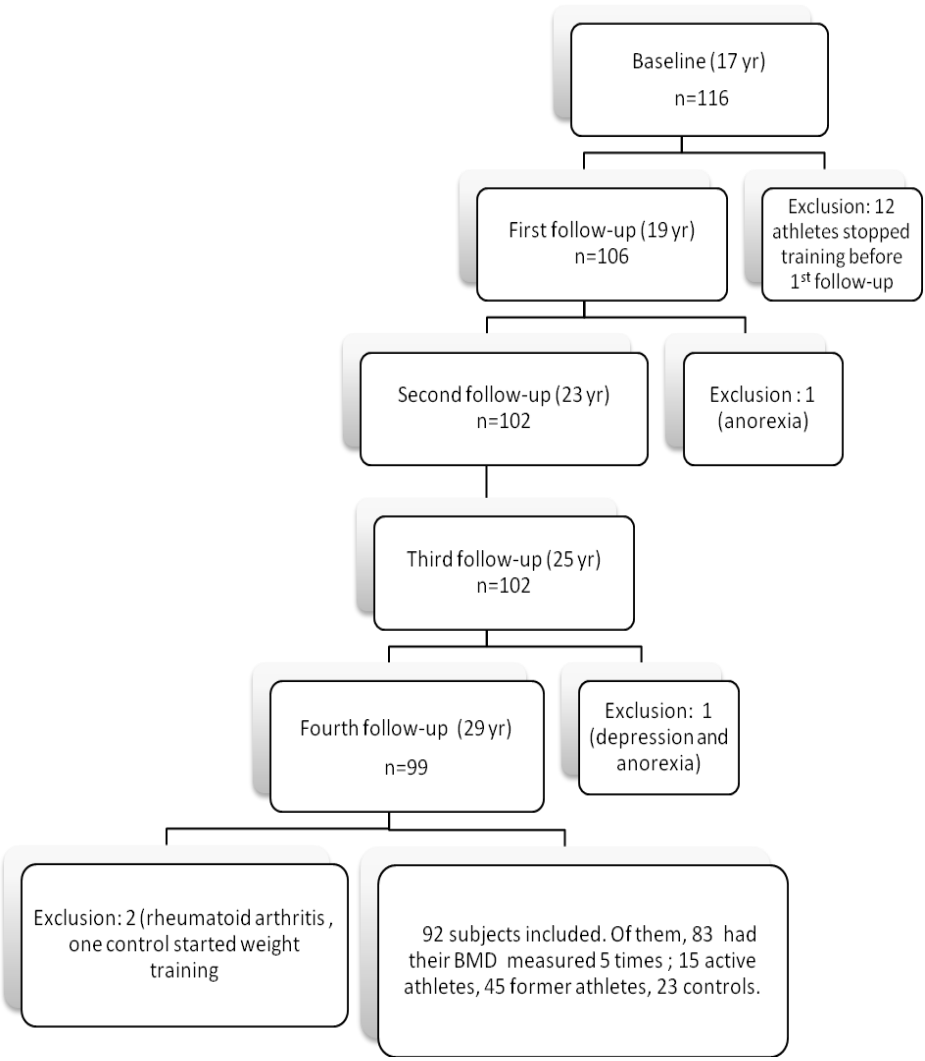


Figure 7. Flow charts of participants in studies I-III.

The first follow-up evaluation was conducted a mean of two years and three months after the baseline evaluation. At this time, one subject in the control group was diagnosed with an eating disorder and was therefore excluded from the study. Between the baseline evaluation and the first follow-up, 12 athletes had stopped training and were therefore excluded from the study. The second follow-up evaluation was conducted a mean of five years and seven months after the baseline evaluation, and a third follow-up evaluation was conducted a mean of seven years and eight months after the baseline evaluation. During the third follow-up evaluation, one subject in the control group was diagnosed with depression and an eating disorder and was excluded from the study. The fourth follow-up evaluation was conducted a mean of 11 years and 11 months after the baseline evaluation. At that time, one subject in the control group was found to be engaging in vigorous weight training and was therefore excluded. One of the athletes was also excluded due to a new diagnosis of rheumatoid arthritis.

Studies I, II and III included the same cohort.

The first and second studies (Study I and II) included of 51 athletes who had stopped their active career during the follow-up period (former athletes), 16 athletes who were active throughout the whole study period (active athletes), and 25 control subjects.

The third study (Study III) included 48 ice hockey players, 19 badminton players, and 25 control subjects. Differences between groups were assessed during the period of the study during which the athletes were active. During the study period, 11 badminton players and 39 ice hockey players stopped their active careers. Differences in BMD between the badminton and ice hockey players who stopped training during the study period were evaluated and the results were also compared with the BMD measurements of the control subjects (**Figure 8**).

Figure 8. The athletic groups consisted of badminton and ice hockey players in study I-III. (Photos by Marcus Lindberg)

The fourth study (Study IV) included a total of 1595 subjects (1389 women and 206 men) who had enrolled in the Västerbotten Intervention Program (VIP) and later had their BMD measured at the Sports Medicine Unit in Umeå. The VIP is a community-based project focusing on cardiovascular disease and diabetes that began in 1985 in Västerbotten county. The project entails inviting all residents of the county who are 40, 50, and 60 years of age to complete a standardized health survey at the primary care center in Västerbotten. All participants are asked to answer a questionnaire that includes questions about lifestyle and various psychosocial factors as well as to donate blood for use in future research. BMD has been measured by DXA since 1991 at the Sports Medicine Unit. Most of the subjects who have had their BMD measured have been referred for evaluation due to previous fractures or clinical suspicion of osteoporosis.

Methods

Bone mass measurements, DXA

Areal bone mineral density (g/cm²) (BMD) was analyzed at the baseline and follow-up evaluations with a Lunar DPX-L dual energy x-ray absorptiometer using operating software version 4.6e (Lunar Co, Waukesha, WI)(study I-IV). In study I, measurements of the right femoral neck, total body, and lumbar spine (L2-L4) BMD were obtained. In study II, leg, pelvis, total spine, head, total hip, right femur, and dominant humerus BMD was measured. The BMD measurements of the legs, pelvis, total spine, and head were derived from the total body scan. Dominant humerus and right femur BMD were estimated by a single operator (T.T) using the region of interest program. In study III, BMD measurements of the head, total body, dominant humerus, femoral neck, legs, and lumbar spine (L2-L4) were made. In study IV, BMD measurements of the lumbar spine and femoral neck were obtained. In all of the measurements obtained in all of the studies, the DXA equipment was calibrated each day using a standardized phantom to detect drifts in BMD measurements. The coefficient of variation (SD/mean) was verified by scanning the same person seven times on the same day, with repositioning occurring between each scan. The coefficient of variation (CV) for the total body scan was 0.7%. The CV was approximately 1% for the femoral neck, 0.6% for the lumbar spine, and 2-3% for the humerus and femur (**Figure 9**).

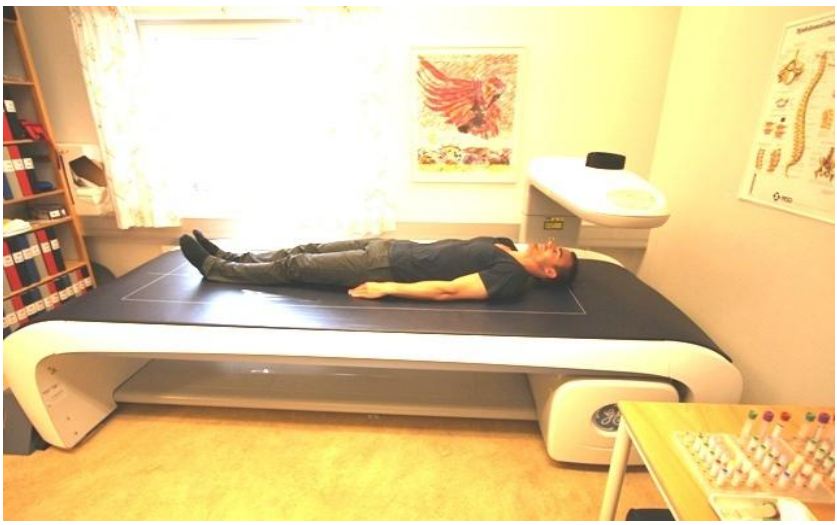


Figure 9. BMD values were measured by DXA in a studies I-IV. (Photo by Fredrik Eklund)

Measurements of vitamin D, fatty acids, and bone metabolism markers

In study III, serum levels of vitamin D, fatty acids (Pufa, Mufa, n-3, n-6) and bone metabolism markers [carboxy terminal telopeptide of type 1 collagen (CTX), parathyroid hormone PTH, and osteocalcin (OC)] were measured in controls and ice hockey players at the second follow-up evaluation. Serum samples were obtained under non-fasting conditions. Total plasma lipids were extracted according to the method described by Folch et al. [181] and phospholipids were isolated on 400 mg aminopropyl solid-phase extraction columns according to the method described by Helland et al. [182]. The results are expressed as grams of fatty acids per grams of serum phospholipids.

Serum vitamin D concentrations were measured using a high performance liquid chromatography (HPLC) system at Vitas AS in Norway. HPLC was performed with an HP 1100 liquid chromatography system (Agilent Technologies, Palo Alto, CA). The lower limit of detection was 1-4 nmol/L. The CVs were 5.8% (29.4 nmol/L) and 5.2% (73.6 nmol/L).

OC, PTH, and CTX levels were analyzed using a direct chemoiluminescence technique based on the sandwich technique at the Department of Clinical Chemistry of Uppsala University Hospital, Sweden. The CV was 1-7-5.2% depending on the concentration and molecule measured.

VIP Questionnaire

Subjects' responses to questions about self-perceived health, smoking status, diabetes, physical activity level, bicycling, snow shoveling, dancing, and berry/mushroom picking were obtained from their VIP questionnaires. Self-perceived health was coded as 0 (worse than other subjects of the same age), 1 (equal to other subjects of the same age), and 2 (better than other subjects of the same age). Current smoking status was coded as 0 (no) or 1 (yes). The number of cigarettes smoked per day was coded as 0 (smoking less than 15 cigarettes per day) or 1 (smoking more than 14 cigarettes per day) among the smokers. Diabetes was coded as 0 (no) or 1 (yes). Participation in physical training in training clothes during the preceding three months was coded as 0 (not regularly), 1 (1-2 times a week), and 2 (at least 2 times per week). Walking and bicycling producing at least some degree of sweating was coded 0 (2 times a month or less), 1 (3-4 times a month), and 2 (at least 2 times a week). Snow shoveling, dancing, and berry/mushroom picking were coded as 0 (never), 1 (at most once a week), and 2 (at least once a week).

Statistical methods

In studies I-III, differences in age, body weight, height, physical activity level, and BMD between the athlete groups and the controls and between the subjects and their parents were analyzed using Analysis of Variance (ANOVA) with Bonferroni's post hoc test for multiple comparisons or Analysis of Covariance (ANCOVA) using age, weight, and height as covariates.

In study I, the association between physical activity level and BMD measurements during the follow-up evaluations was investigated using Pearson's correlation coefficient. In study I and II, a multivariate mixed effects model was used to investigate the independent associations between BMD at each site measured and physical activity level, age, weight, and height. This mixed piecewise linear regression model with a random intercept was fitted with PROC MIXED. This method uses all of the repeated measurements and takes into account unevenly spaced follow-up measurements and missing data points. Since each individual acts as his own control, the method makes it possible to estimate intra-subject changes in BMD with good precision. The analysis was performed to estimate changes in each person's BMD measurements throughout the study period, and evaluate independent predictors of BMD. At each time point, physical activity level, age, weight, and height were used as covariates. A knot was inserted at 20 yr of age because the association between age and BMD was

not linear. In all models, the sandwich covariance estimator was used [183]. In study II, BMD changes between groups that occurred between the first and last follow-up evaluation were investigated with a multiple regression model in which comparisons were made with dummy variables for active athletes, former athletes, and controls. The model was adjusted for changes in age, weight, and height over time. Likewise, in study III, changes in BMD measurements from baseline to end of subjects' athletic career and from cessation of the career to final follow-up evaluation were compared using linear regression analysis. In both cases, the models were adjusted for changes in age, weight, and height.

In study IV, independent associations between lifestyle factors and BMD were analyzed using linear regression analysis with age, weight, sex, and time between VIP and BMD measurements as independent variables. The association between self-perceived health and several life style factors were evaluated using partial correlation analysis after adjusting for the influence of age.

In all studies, a p-value of less than 0.05 was considered to be significant. The SPSS software package (version 15 and 17, SPSS Inc. Chicago, IL) and SAS (version 9, SAS Institute Inc, Cary, NC) were used for the statistical analyses.

Ethics

All individuals provided informed consent for participation in each study. Additionally, the parents of the study participates provided informed consent for their participation. These studies (study I-IV) were approved by the Ethics Committee of the Medical Faculty of Umeå University in Umeå, Sweden.

Results

Study I

CONSTANT ADAPTATION OF BONE TO CURRENT ACTIVITY LEVEL: A 12-YEAR LONGITUDINAL STUDY IN MALES

We investigated BMD 5 times during a 12-year study period in 3 different groups of male subjects: 1) a group of 51 athletes who stopped their active careers during follow-up (former athletes); 2) a group of 16 athletes who stayed active throughout follow-up (active athletes); and 3) a group of 25 control subjects. The baseline mean age of the subjects was 17 years. After adjusting for age, weight, and height, the former athletes were found to have higher BMD than controls at the femoral neck, lumbar spine, and total body BMD than the controls at every follow-up visit except the last one ($p < 0.05$). When compared to controls, the active athletes had significantly higher BMD measurements at all measured sites from the second follow up and throughout the entire study period ($p < 0.05$). The relationship between physical activity and BMD appeared to be nearly linear at the femoral neck for all groups and during all follow-up evaluations when the data were presented graphically. Changes in BMD at the different sites measured were analyzed starting at the first follow-up evaluation because the former athletes reduced their activity levels from that point forward. During that time period (from the first follow-up evaluation forward), the former athletes showed smaller increases in total body BMD and lumbar spine BMD ($p < 0.05$) than active athletes, and former athletes lost more BMD than active athletes and controls at the femoral neck ($p < 0.05$). A multivariate mixed effects model showed that an additional hour of physical activity per week was associated with an increase in BMD at multiple sites. This increase was greatest at the femoral neck (0.009 g/cm^2), followed by the spine (0.007 g/cm^2), and total body BMD (0.002 g/cm^2) ($p<0.05$ for all sites). To evaluate the influence of hereditable factors on BMD, BMD measurements of the cohort's fathers were obtained. After adjusting for age, weight, and height, there were no significant differences in BMD observed between the three groups of fathers at any site measured, suggesting the absence of selection bias.

Study II

REDUCED PHYSICAL ACTIVITY CORRESPONDS WITH GREATER BONE LOSS AT TRABECULAR BONE SITES THAN CORTICAL BONE SITES IN MEN

A total of 3 groups with a baseline mean age of 17 years were evaluated 5 times during the study period. The 3 groups included: 1) a group of 51 athletes who ceased their career during the study period (former athletes); 2) a group of 16 athletes who were active throughout the study period (active athletes); and 3) a group of 25 control subjects. Former athletes discontinued their active sport career an average of 8.2 years before the end of the study. BMD loss at the hip, spine and pelvis (which mainly consist of trabecular bone) was compared to BMD loss at the femur, humerus, and legs (which mainly consist of cortical bone). After adjusting for age, weight, and height, the former athletes were found to have significantly higher BMDs than control subjects at humerus, legs, and right femur at all follow-up examinations ($p < 0.05$), higher spine BMD than control subjects from the baseline evaluation to the second follow-up evaluation ($p < 0.05$), and higher pelvis and hip BMD compared to the control group from the baseline evaluation and all follow-up evaluations except for the last one ($p < 0.05$). After adjusting for age, weight, and height, active athletes were found to have significantly higher BMDs than the controls at the pelvis and total hip throughout the entire study period ($p < 0.05$), they were found to have higher BMDs at the humerus and femur than the control subjects at all follow-up evaluations ($p < 0.05$), and they were found to have higher BMDs in the total spine and legs ($p < 0.05$) from the second to the final follow-up evaluations. BMD changes were studied from the first to last follow-up evaluation. Active athletes gain more BMD in the humerus ($p = 0.01$), total spine ($p = 0.001$), total hip ($p = 0.01$), and pelvis ($p = 0.004$) compared to former athletes. During the entire study period, changes in hours of physical activity per week were more strongly associated with changes at bone sites that are comprised mainly of trabecular bone, such as the hip, spine, and pelvis ($B = 0.005\text{-}0.008$ g/cm^2), than bone sites that consist mainly of cortical bone, such as the humerus, legs, and femur ($B = 0.002\text{-}0.003$ g/cm^2).

Study III

EFFECTS OF BADMINTON AND ICE HOCKEY ON BONE MASS IN YOUNG MALES : A 12-YEAR FOLLOW-UP

Total body BMD, as well as BMD at the humerus, lumbar spine, femoral neck, legs, and head were measured 5 times in 19 badminton players, 48 ice hockey players, and 25 controls over a 12–year period. Moreover, we evaluated serum osteocalcin, carboxy terminal telopeptide of type 1 collagen (CTX), vitamin D, and fatty acids levels in relation to BMD in a cross-sectional fashion during the second follow-up evaluation, and longitudinally from the second to final follow-up evaluation. We also evaluated BMD in the cohort's parents. During the time when badminton and ice hockey players were active in their sport, they gained significantly more BMD at the femoral neck, humerus, and lumbar spine than control subjects ($p < 0.05$). Moreover, badminton players gained significantly more BMD in the legs compared both to ice hockey players and control subjects. A total of 11 badminton players and 39 ice hockey players ended their career about 8 years before the final follow-up evaluation. During this time, the former ice hockey players lost more BMD at the femoral neck and lumbar spine than control subjects ($p < 0.01$). Additionally, badminton players had a tendency to lose more BMD at the femoral neck than control subjects over the same time period ($p = 0.05$). At the final follow-up evaluation, badminton players still had significantly higher BMD values than ice hockey players and control subjects at the humerus, femoral neck, lumbar spine, and legs, although the physical activity levels were found to be the same in all groups. Serum vitamin D and fatty acid levels were not found to be related to changes in BMD at any site from the second to the final follow-up evaluation ($p > 0.05$ for all sites). Changes in leg BMD was found to be negatively associated with CTX levels ($p < 0.001$), and changes in humerus BMD were found to be positively associated with levels of osteocalcin ($p < 0.05$). BMD measurements of the cohort's mothers and fathers revealed that there were no significant differences in BMD between the groups' parents after adjusting for age, weight, and height, suggesting that results of the study were not influenced by genetic factors regulating BMD.

Study IV

ASSOCIATION BETWEEN SELF-PERCEIVED HEALTH, PHYSICAL ACTIVITY AND BMD IN MIDDLE AGED MEN AND WOMEN

The Västerbotten Intervention Program (VIP) is a community based project focusing on the risk of cardiovascular disease and diabetes. All participants are asked to answer a comprehensive questionnaire including questions about psychosocial conditions and lifestyle. Overall, 1595 subjects who had been enrolled in the VIP study and who had subsequently had their BMD evaluated were included in the study. The mean age of this cohort was 57 years (range: 30-74) at baseline. After adjustment for weight, age, sex and time between the VIP examination and the BMD measurements, BMD of the femoral neck was positively associated with self-perceived health, training, and snow shoveling. In contrast, smoking more than 15 cigarettes per day was negatively associated with BMD at the femoral neck. Only self-perceived health, age, and weight were found to be related to BMD at the spine. In summary, self-perceived health was found to be related to several lifestyle factors, such as training and snow shoveling, factors that were also found to be related to BMD.

Discussion

Osteoporosis is a multi-factorial, insidious disease that progresses silently before a fragility-induced fracture occurs. It is a growing public health dilemma for the world's continuously aging population [8], leading to suffering and handicap for those afflicted as well as placing a heavy burden on society [4, 184, 185]. The lifetime risk of osteoporotic fracture is about 50% for women and 25% for men [1, 2]. It seems that incidence of osteoporosis-related fractures have increased more in men than in women [186], and the mortality rate during the year after hip fracture is higher among men than women [187]. It has also been suggested that osteoporosis is an under-recognized and undertreated disease in men [188]. Consequently, it is no longer considered only a disease of women, and therefore it is important to include men as well as women in studies of osteoporosis.

Approximately 50-70% of PBM is dictated by genetic factors [15, 16]. Lifestyle factors, such as physical activity level and nutrition, are important factors that contribute to PBM [39, 189, 190]. It has been suggested that a high BMD due to training during younger years may be preserved in former athletes after they retire, and it has also been proposed that this could be protective against the development of osteoporosis later in life [6, 114]. However, this is a theory based mostly on retrospective studies of former athletes [138, 177, 178, 191, 192] or longitudinal studies of relatively short durations [174-176, 193]. The main aim of this thesis was to investigate the effects of physical activity on bone gain in young Swedish males as well as to determine if the bone gain acquired from previous physical activity was maintained after retirement from an athletic career.

In study I and II, we investigated the effects of sustained and reduced physical activity on BMD in a group of athletes and control subjects. The total follow-up time in this study was 12 years and it included males with an average baseline age of 17 years. At the beginning of the study, there were no significant differences observed between groups with regard to age, anthropometric measures, or pubertal stage. After adjusting for age, weight, and height, the athletes were found to have a higher bone mass than controls at several sites at the baseline evaluation and during the period when the athletes were active. It has previously been suggested that physical activity in the pre- and peripubertal years is a crucial factor in the attainment of PBM [55, 115, 194]. In addition, the results from both study I and II revealed that physical activity has an important effect on PBM in the post-pubertal period as well. The results of both studies suggest that the positive influence of training is

site-specific. For example, we could not detect any significant differences in head BMD between the groups.

A hypothesis tested in study I was that former athletes would have a higher BMD than controls, despite years of reduced activity following the completion of their athletic careers. The results revealed that former athletes (who had reduced their activity levels at a mean of eight years before the study ended) lost BMD during the course of the study, especially at the femoral neck. Thus, former athletes lost more BMD at the femoral neck than both active athletes and controls. Hence, our results were in line with Wolff's Law, which suggests that bone adapts constantly to an individual's present activity levels [27].

Our result from study I was not in line with the data presented by Kontulainen et al., [174, 175] who showed that the increased arm BMD attained by racket players during their growth periods might be maintained in adulthood, despite a subsequent reduction in activity levels. The different results might be related to the different effects that detraining has on trabecular vs. cortical bone. This hypothesis was tested in study II, using the same cohort as in study I. The study showed that former athletes lost more BMD at bone sites comprised largely of trabecular bone (the hip, spine, and pelvis) than at bone sites comprised largely of cortical bone (the femur, humerus, and legs) after eight years of reduced activity. Moreover, at the final follow-up evaluation, former athletes still had a significantly higher BMD than controls at the bone sites comprised mainly of cortical bone, such as humerus and legs.

It has previously been proposed that a high PBM from previous training could be protective against the development of osteoporosis later in life [6, 114]. Our studies (study I and II) indicate that this is not the case at trabecular bone sites if the athletic activity is not maintained. This finding was strongly supported by the results from study I. There was an almost linear relationship observed between the amount of physical activity at each of the five follow-up evaluations and BMD, especially at the femoral neck. This was true for all three groups studied. In the total cohort, we also found that every hour of decrease in physical activity was independently associated with a decrease in neck BMD of 0.009 g/cm^2. This association was also evident for the spine (0.007 g/cm^2 per hour of reduced activity) and total body (0.002 g/cm^2 per hour of reduced physical activity), but the correlations were weaker. Thus, the femoral neck seems to be a site that is highly sensitive to changes in levels of physical activity. This may relate both to the fact that weight-bearing physical activity likely results in high strain at the femoral

neck and to that the femoral neck is composed of a rather high amount of trabecular bone.

To our knowledge, there are only a few longitudinal studies that have investigated the effects of reduced physical activity on bone mass [174-176, 179]. Although these studies have shorter follow-up times, their results are somewhat in line with ours. Kontulainen et al. [174, 175] showed that exercise-induced bone gain in racket sport players was maintained in the humerus, which consists mostly of cortical bone, in both women and men after four and five years of decreased training, respectively. As in our second study, Valdimarsson et al. found residual benefits in leg BMD after an average of eight years of detraining in female soccer players [179]. In addition, Valdimarsson et al. also showed that among former athletes, a more rapid loss in femoral neck BMD occurs compared to controls after reduced activity [179]. In summary, previously published longitudinal studies are to some extent in line with our results, suggesting that exercise-induced bone gain is lost at sites rich in trabecular bone. Therefore, because low-energy fractures predominantly affect sites rich in trabecular bone, a high peak bone mass from previous physical activity may not prevent these fractures later in life if an individual's activity levels are reduced.

The main aim of study III was to investigate the effects of different types of weight-bearing loading on bone gain in badminton and ice hockey players, as well as to evaluate the effects of reduced activity on BMD among athletes who played these sports. Badminton players were found to gain more BMD in the femoral neck, humerus, and lumbar spine than controls during the active period, which lasted a mean of five years. Furthermore, they gained more BMD in the legs compared to both the ice hockey players and controls. Although both activities are osteogenic, it seems that badminton results in a higher bone accumulation than ice hockey does. Presumably, the higher ground reaction forces and quick changes in direction that occur during badminton play cause higher strain rates in the skeleton than ice hockey does. A few other studies have also shown that athletes involved in sports with high ground reaction forces in combination with rapid versatile movements have a higher BMD compared to athletes participating in lower-impact sports [36-38, 58] . A study performed in a similar cohort of young men as was used in the present thesis, that had four-year follow-up period, had similar results to our study. This study suggested that badminton is related to higher gains both in bone mass and size of the clinically important hip compared to ice hockey [195] (**Figure 10**).

Figure 10. Badminton may generate a higher osteogenic response in the skeleton compared to ice hockey. (Photo by Marcus Lindberg)

In study III, we also noticed that the BMD benefits derived from badminton were partially sustained after a period of reduced activity. At the final follow-up evaluation, badminton players still had a significantly higher BMD at the femoral neck, lumbar spine, humerus, and legs compared to both ice hockey players and controls. Ice hockey players lost significantly more BMD at the femoral neck and lumbar spine than controls did over the time elapsed between the end of their career to the end of the study period. Moreover, badminton players showed also a tendency to lose more BMD in femoral neck than controls. Furthermore, there were no significant differences when BMD loss was compared among ice hockey and badminton players. Thus, it seems likely that the residual benefits in BMD derived from physical activity will also disappear in the former badminton players after a longer follow-up period, at least at sites composed predominately of trabecular bone. To our knowledge, there are no other longitudinal studies that have compared the effects of detraining in athletes taking part in different weight-bearing activities. Only a few studies have investigated the effects of detraining in highly trained athletes [174-176, 179]. These studies, including our study, indicate that high BMD is sustained after training ends in bone sites rich in cortical bone.

To our knowledge, this study, with its 12-year follow-up (I-III), is the longest prospective follow-up study that has been performed investigating the effects

of detraining on BMD. The strengths of the studies include their longitudinal study design, their relatively large study populations, and the low dropout rate. Furthermore, at baseline, there were no significant differences in age or pubertal stage observed between the groups that could have influenced either the baseline BMD or the changes in BMD that occurred during the study. Nevertheless, in observational studies, there is always the risk of selection bias, e.g., that subjects who have genetically stronger bones and muscles are more likely to participate in sports activities. In studies I-II, we therefore evaluated the BMD of 81 of the young men's fathers, and found no differences in BMD between the athletes' fathers and the controls fathers', suggesting the absence of a selection bias. In addition, we evaluated also 73 of the study participants' mothers in study III. In study III, we did not observe any differences in BMD between the paternal groups, a finding similar to our findings in studies I-II. Other factors that could influence BMD include different aspects of nutrition. We also found in previous studies that serum vitamin D and fatty acid levels were associated with BMD in similar cohorts as the one investigated in the first three studies of the present thesis [195-197]. These compounds might also affect BMD loss. Vitamin D and fatty acids were found to be related to BMD in a cross-sectional analysis. However, we did not detect any association between changes in BMD during the study period and serum levels of vitamin D or fatty acids. Consequently, our results in studies I-III do not seem to be influenced by genetic or nutritional factors, such as vitamin D or fatty acids. The major limitations of these studies include lack of other background data that might influence the changes in BMD that we observed, and the fact that physical activity was not measured by an objective method, but by a questionnaire, thereby increasing the risk of recall bias.

In study IV, we evaluated the associations between several lifestyle factors, self-perceived health, and BMD at the spine and femoral neck in a well-defined cohort of middle aged women and men. To our knowledge, this is the first study to investigate these associations. We found that higher BMD at the femoral neck was related to better self-perceived health as well as increased levels of physical activity and increased frequency of snow shoveling. BMD at the lumbar spine was only found to be related to self-perceived health. The survey question relating to physical activity did not give us information about the type of the activity individuals performed, and we can therefore only assume that it was weight-bearing because it was associated with BMD of the femoral neck. On the other hand, lifestyle factors such as cycling, walking, dancing, and berry/mushroom picking were not related to BMD at either the lumbar spine or the femoral neck. We suggest that snow shoveling is presumably more osteogenic than these activities because it was associated with increased BMD at the femoral neck. We also

found that self-perceived health was associated with physical activity, snow shoveling, cycling and berry/mushroom picking. Consequently, several lifestyle factors which were related to self-perceived health were also found to be related to BMD at the femoral neck.

Summary and conclusions

A high level of weight-bearing physical activity seems to be associated with significant increases in BMD at multiple sites after puberty in men. These benefits seem to be site-specific and related to the strains resulting from the type of physical activity.

High BMD from previous training seems to be lost at sites that are rich in trabecular bone, such as the hip and spine, as no sustained effects from previous physical activity were observed at these sites. In contrast, a higher BMD was still evident among former athletes a mean of eight years after end of an active sports career than among control subjects at bone sites predominantly comprised of cortical bone, such as the femur and humerus.

Badminton seems to be a more osteogenic activity than ice hockey in terms of maximizing peak BMD in young men. However, with reduced activity, these benefits in BMD seem to be lost at similar rates in both badminton players and ice hockey players.

In a large cohort of middle aged men and women, lifestyle factors, such as physical activity, were related to higher self-perceived health. Several of these lifestyle factors were also associated with higher BMD measurements, especially at the femoral neck.

In summary, the results of the present thesis indicate that consistent weight-bearing physical activity is likely important in maximizing peak bone density in young men, as well as for maintaining a high BMD, especially at the clinical important femoral neck, throughout life.

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