LUND UNIVERSITY

Skeletal Development During the First Three Decades in Life

Lindgren, Erik

2021

Document Version:
Publisher's PDF, also known as Version of record

Link to publication

Total number of authors:
1

Skeletal Development During the First Three Decades in Life

ERIK LINDGREN
DEPARTMENT OF CLINICAL SCIENCES AND ORTHOPEDICS | LUND UNIVERSITY

Skeletal Development During the First Three Decades in Life

Author: Erik Lindgren, MD

Organization	Document name
LUND UNIVERSITY	DOCTORAL DISSERTATION
Clinical and Molecular Osteoporosis Research Unit Department of clinical sciences in Malmö	**Date of issue** May 26, 2021
Author: Erik Lindgren	Sponsoring organization

Title and subtitle
Skeletal Development During the First Three Decades in Life

Abstract

Background: Areal bone mineral density (aBMD) measured by dual-energy X-ray absorptiometry (DXA) is a surrogate estimate of bone strength. The highest value in life is referred to "peak bone mass" (PBM). In the femoral neck (FN), PBM is reached late in the second decade in life, after which there is an age-related decline in aBMD during adulthood. However, a decrease in aBMD may follow a decline in bone mass and/or an increase in bone size, and since the bending strength of tubular structures is related to the width of the structure, a decrease in aBMD could not automatically be translated to reduced bone strength. Since it is counterintuitive in an evolutionary perspective that FN bone strength should start to decline in such early ages, we hypothesized that peak FN.aBMD and peak bone strength do not correlate.

A lifestyle factor with great influence on the skeleton is physical activity (PA), where increased PA in childhood is associated with high PBM. PA in childhood could thus hypothetically counteract age-related bone mass attenuation in adult life, possibly postponing the onset of osteoporosis and reducing the number of fractures. It is debated, however, whether long-term interventions during puberty are effective, as children, especially girls, are known to reduce the level of PA in this period. Previous studies have also shown incongruent results as to whether PA-induced musculoskeletal gains are retained after reduction of PA levels.

The aims of the studies were to present normative data in young men of DXA and peripheral quantitative computed tomography (pQCT) estimated BMC, BMD, and bone structure, and in cross-sectional analyses, to evaluate whether there are associations between these traits and age. Another aim was to investigate whether a school-based PA intervention program from before to after puberty is associated with musculoskeletal benefits, and if these possible benefits are attenuated after termination of the intervention.

Methods: Bone mass and bone structure data were gathered from the cross-sectional MRPEAK study which included 1083 population-based men aged 18–28 years, scanned in the radius and tibia with pQCT and in FN by DXA. We also gathered data from the POP study, which at baseline included 349 children aged 7-9 years in four schools. Children in one school followed a 9-year intervention-program that included 40 minutes' daily physical education (PE), while children in the three control schools continued with 60 minutes of weekly PE. We assessed the children at baseline, at the end of the intervention, and mean 4 years after the last measurement during the intervention. Assessments included lifestyle evaluation and Tanner staging from questionnaires, measurements of anthropometrics by standard equipment, and musculoskeletal traits by DXA and a Biodex dynamometer.

Results: Estimates from pQCT of bone mass and bone density were higher with higher ages in the cortical diaphyseal regions of the radius and tibia, while we found no such associations in the trabecular bone in the ultra-distal regions. After age 19 there was a negative correlation between age and FN aBMD, and a positive correlation between age and bone size. When children were followed from Tanner stage 1 to 5, the intervention program was associated with musculoskeletal benefits in both sexes. After termination of the program, we found attenuation in muscle benefits but not in bone mass benefits. In spite of this, when evaluating the entire period from baseline to several years after program termination, benefits in both muscle strength and bone mass gain were evident in the intervention group.

Conclusions: Caution is advised when assessing bone strength and fracture risk using aBMD from DXA, since a decline in aBMD may, at least in men in young adulthood, in part be due to increased bone size that actually increases bone strength. pQCT measurements also indicate that bone strength may increase between ages 18 and 28. In children followed over puberty, daily school PA during the 9 compulsory school years is associated with beneficial gains in musculoskeletal traits that remain in young adulthood, several years after program termination.

Key words: Peak bone mass, DXA, pQCT, physical activity, musculoskeletal development

Classification system and/or index terms (if any)

Signature _____ Date 2021-04-19

Skeletal Development During the First Three Decades in Life

Author Erik Lindgren, MD

LUND
UNIVERSITY

To my loving family

Table of Contents

List of Original Papers

The thesis is based on the following papers, henceforth referred to in text by their respective Roman numerals:

I.   Lindgren E, Karlsson M, Lorentzon M, Rosengren BE. Bone Traits Seem to Develop Also During the Third Decade in Life–Normative Cross-Sectional Data on 1083 Men Aged 18-28 Years. J Clin Densitom. 2017;20(1):32–43.

II.   Lindgren E, Rosengren B, Karlsson M. Does Peak Bone Mass Corroborate with Peak Bone Strength? Normative Data in 1052 Men Aged 18–28 Years. BMC Musculoskelet Disord. 2019; 20: 404.

III.   Cronholm F, Lindgren E, Rosengren BE, Dencker M, Karlsson C, Karlsson MK. Daily School Physical Activity from Before to After Puberty Improves Bone Mass and a Musculoskeletal Composite Risk Score for Fracture. Sports (Basel). 2020 Mar 28;8(4).

IV.   Rosengren BE, Lindgren E, Jehpsson L, Dencker M, Karlsson MK. Musculoskeletal Benefits from a Physical Activity Program in Primary School are Retained 4 Years after the Program is Terminated. (Accepted for publication in Calc Tissue Int)

Abstract

Background: Areal bone mineral density (aBMD) measured by dual-energy X-ray absorptiometry (DXA) is a surrogate estimate of bone strength. The highest value in life is referred to "peak bone mass" (PBM). In the femoral neck (FN), PBM is reached late in the second decade in life, after which there is an age-related decline in aBMD during adulthood. However, a decrease in aBMD may follow a decline in bone mass and/or an increase in bone size, and since the bending strength of tubular structures is related to the width of the structure, a decrease in aBMD could not automatically be translated to reduced bone strength. Since it is counterintuitive in an evolutionary perspective that FN bone strength should start to decline in such early ages, we hypothesized that peak FN.aBMD and peak bone strength do not correlate.

A lifestyle factor with great influence on the skeleton is physical activity (PA), where increased PA in childhood is associated with high PBM. PA in childhood could thus hypothetically counteract age-related bone mass attenuation in adult life, possibly postponing the onset of osteoporosis and reducing the number of fractures. It is debated, however, whether long-term interventions during puberty are effective, as children, especially girls, are known to reduce the level of PA in this period. Previous studies have also shown incongruent results as to whether PA-induced musculoskeletal gains are retained after reduction of PA levels.

The aims of the studies were to present normative data in young men of DXA and peripheral quantitative computed tomography (pQCT) estimated BMC, BMD, and bone structure, and in cross-sectional analyses, to evaluate whether there are associations between these traits and age. Another aim was to investigate whether a school-based PA intervention program from before to after puberty is associated with musculoskeletal benefits, and if these possible benefits are attenuated after termination of the intervention.

Methods: Bone mass and bone structure data were gathered from the cross-sectional MRPEAK study which included 1083 population-based men aged 18–28 years, scanned in the radius and tibia with pQCT and in FN by DXA. We also gathered data from the POP study, which at baseline included 349 children aged 7-9 years in four schools. Children in one school followed a 9-year intervention-program that included 40 minutes' daily physical education (PE), while children in the three control schools continued with 60 minutes of weekly PE. We assessed the children at baseline, at the end of the intervention, and mean 4 years after the last measurement during the intervention. Assessments included lifestyle evaluation and Tanner staging from questionnaires, measurements of anthropometrics by standard equipment, and musculoskeletal traits by DXA and a Biodex dynamometer.

Results: Estimates from pQCT of bone mass and bone density were higher with higher ages in the cortical diaphyseal regions of the radius and tibia, while we found

no such associations in the trabecular bone in the ultra-distal regions. After age 19 there was a negative correlation between age and FN aBMD, and a positive correlation between age and bone size. When children were followed from Tanner stage 1 to 5, the intervention program was associated with musculoskeletal benefits in both sexes. After termination of the program, we found attenuation in muscle benefits but not in bone mass benefits. In spite of this, when evaluating the entire period from baseline to several years after program termination, benefits in both muscle strength and bone mass gain were evident in the intervention group.

Conclusions: Caution is advised when assessing bone strength and fracture risk using aBMD from DXA, since a decline in aBMD may, at least in men in young adulthood, in part be due to increased bone size that actually increases bone strength. pQCT measurements also indicate that bone strength may increase between ages 18 and 28. In children followed over puberty, daily school PA during the 9 compulsory school years is associated with beneficial gains in musculoskeletal traits that remain in young adulthood, several years after program termination.

Abbreviations

aBMD	areal bone mineral density
ANCOVA	analysis of co-variance
ANOVA	analysis of variance
BMC	bone mineral content
BMI	body mass index
BMU	basic multicellular unit
CI	confidence interval
CSA	cross-sectional area
CSMI	cross-sectional moment of inertia
CV	coefficient of variation
DXA	dual-energy X-ray absorptiometry
FN	femoral neck
MET	metabolic equivalent of task(s)
MSC	mesenchymal stem cell
PA	physical activity
PBM	peak bone mass
PE	physical education
POP study	Pediatric Osteoporosis Prevention study
pQCT	peripheral quantitative computed tomography
PT	peak torque
QUS	qualitative ultrasound
RCT	randomized controlled trial
ROI	region of interest
SD	standard deviation
SSI	strength strain index
TB	total body
vBMD	volumetric bone mineral density

Introduction

Bone

Bone tissue

In contrast to what one might think, bone is a highly dynamic tissue which is both vascularized and innervated, in order to facilitate and implement adaptations to withstand the surrounding demands. The complex material that forms bone comprises a vast number of components including bone cells, proteins, and the crystalline form of calcium phosphate (hydroxyapatite).

There are three distinct bone cells, namely osteoblasts, osteoclasts, and osteocytes. These cells constitute functional components called the basic multicellular units (BMU), which directly modulate bone tissue. Osteoblasts and osteoclasts are cells responsible for bone matrix formation and bone resorption, respectively. The rate and location of these two cell types largely determine the modeling and remodeling of bone composition and architecture. As an osteoblast produces bone matrix which is later mineralized, it encloses itself in a small cavity in the bone matrix called lacuna. When an osteoblast has fully enclosed itself, it differentiates into an osteocyte which then operates through connections with other osteocytes and osteoblasts through microscopic tunnel-like connections called canaliculi (1). Osteocytes constitute vast majority of cells (90%) in the adult bone (2) and it has been suggested that they act as sensors reacting to mechanical stimuli, which seems to play an important role in bone modeling and remodeling (3).

Bone matrix can be divided into organic and non-organic matrix. Organic matrix, which is formed by the aforementioned osteoblasts, contains mainly collagen type I (90%), but also non-collagenous proteins such as proteoglycans and glycoproteins. The non-organic matrix (65–70% of the wet weight of bone), mainly comprising calcium hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) surrounds the collagen fibers and constitutes about 99% of the body's calcium storage. Together, the network of collagen rope-like fibers and the crystalline calcium hydroxyapatite contributes to the flexibility and stiffness of bone (4).

Osteocytes and bone matrix are organized in ring-like shapes resembling the layers of an onion, forming the osteon. In the middle of the osteon run the vertical

Haversian canals, which in turn are connected by horizontal Volkmann's channels, containing nerves, blood vessels, and lymphatic vessels (figure 1).
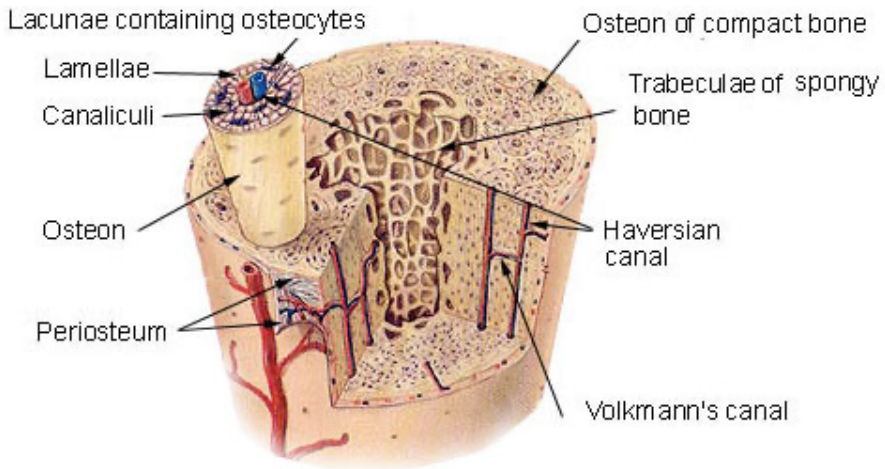
Figure 1: Macroscopic structure of bone (5)

Bone structure

The human skeleton comprises macroscopically different types of bones, generally named after their appearance or form. Long bones constitute the main part of the upper and lower limbs. As the name implies, these bones are longer than they are wide. In a long bone, the diaphysis, or shaft, is during growth surrounded on each side by the proximal and distal epiphyses (figure 2). In-between the epiphysis and diaphysis lies the epiphyseal line, or epiphyseal plate, where the longitudinal growth of a long bone occurs (see *Bone growth*). At the end of growth, the epiphyseal plate is replaced by bone.

Along the diaphysis, a cortical shell of compact bone encases the medullar cavity, which mainly consists of bone marrow. Outside the cortical bone is the periosteal membrane, and inside is the endosteal surface. At the epiphyses, a thinner cortical layer walls a network of spongy bone tissue called trabecular or cancellous bone (figure 2). The distinction between cortical and trabecular bone is usually defined by the bone porosity. The denser cortical bone has a porosity of 5%–15% whereas trabecular bone has between 40% and 95% (6). Apart from being macroscopically different, cortical and trabecular bone exhibit different biomechanical and biological characteristics. Bone turnover, for example, is higher in trabecular bone than in cortical bone due to the larger surrounding surface in relation to the volume

14

of bone tissue in the former. Furthermore, trabecular bone is primarily found in the vertebral bodies and cortical bone constitutes the majority of the total bone weight (7).
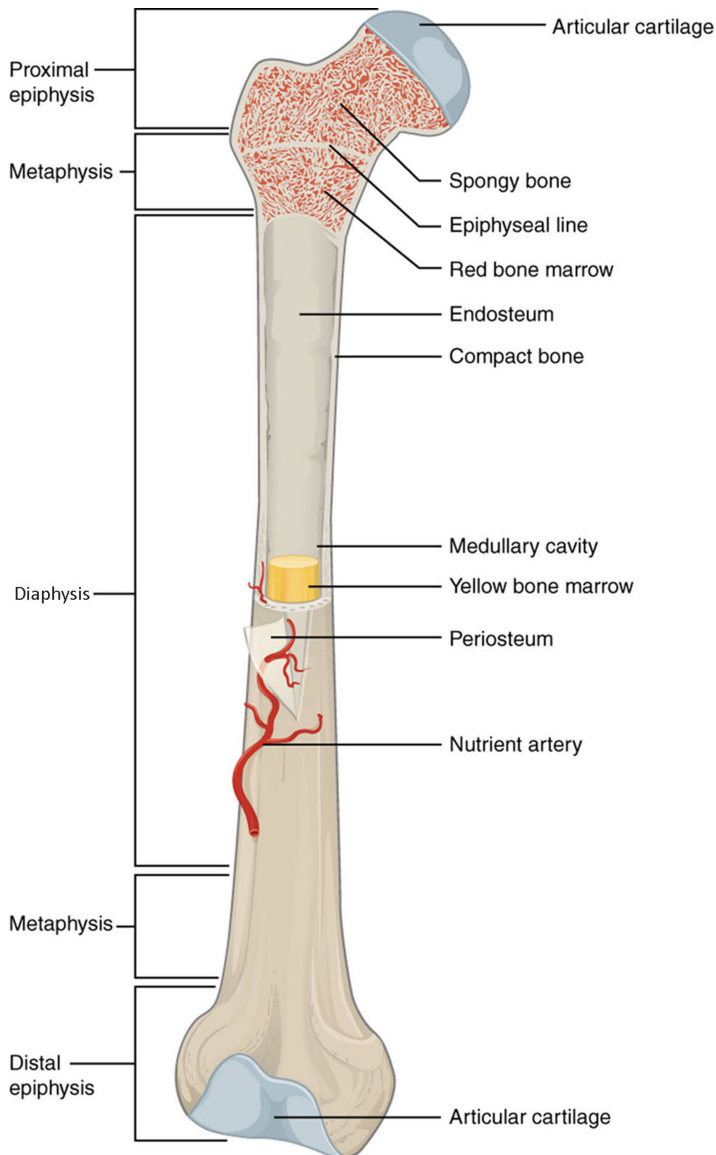
Figure 2: Gross anatomy of a long bone (femur) during growth (note the epiphyseal line). Source: OpenStax College (CC BY 3.0)

Bone remodeling and modeling

Bone remodeling refers to the renewal process of bone whereby, within the BMU, old bone gets resorbed by osteoclasts and is replaced with new bone from osteoblasts. It is said that the whole human adult skeleton is replaced every 10 years (8). The specific activity within the BMU varies with location on the skeleton, and with age, thus contributing to age-related bone loss. Regulation of bone remodeling occurs via numerous factors and processes such as nutritional status, endocrine factors, the autonomic nervous system, and biomechanical stress (8). Bone remodeling serves as a vital process for the structural strength of bone as well as for the mineral (mainly calcium) homeostasis of the body (9).

The form of bones is shaped and reshaped by the process called bone modeling (7). This is apparent during early life skeletal development but may actually continue throughout life. Bone modeling occurs through the independent activity of osteoblasts and osteoblasts, but in contrast to the remodeling process, not sequentially. This means that locally increased osteoblast activity (bone formation modeling), relative to osteoclast activity (bone resorption modeling), will increase the bone volume at this particular surface (periosteal, endosteal and trabecular) of the bone and vice versa. What determines whether the modeling process acts towards formation or resorption is essentially the amount of strain, in a given area surface. In low strain areas, bone resorption via osteoclasts dominates whereas if the amount of strain surpasses a given level, osteoblasts are activated and start to form bone (10).

Modeling plays a major role during longitudinal bone growth. As bone elongates through endochondral ossification, a coordinated reshaping process occurs at the metaphysis, where periosteal resorption and endochondral ossification enables preservation of the shape of the bone (figure 3 (10)).
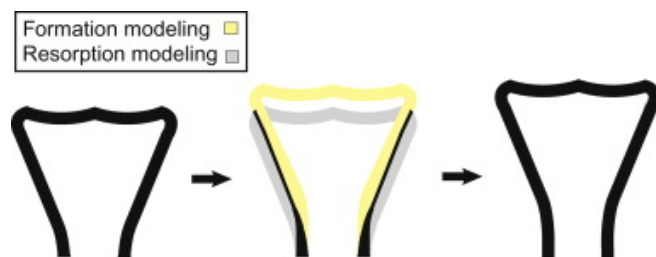
Figure 3: Bone modeling of the metaphysis following elongation through endochondral ossification (10)

During growth, an increase in width of the bone is orchestrated through modeling via periosteal expansion. This, in turn, occurs through increased periosteal apposition, coupled with endocortical resorption. This process allows for the cortical shell to maintain its thickness (10).

Apart from the aforementioned mechanical loading, parathyroid hormone, sclerostin, and the influence of sex hormones (see *Bone during ageing and peak bone mass*) are factors that affect the timing and rate of radial modeling (10)).

Bone growth

The process of bone formation starts already in the womb. The process then continues through life as the bone grows in early life, accommodates changes in mechanical loading, and heals fractures.

There are in principle two different ways in which bones grow. In long bones this occurs through endochondral ossification, a process which starts during embryonical stages in life, where chondrocytes migrate from blood vessels to the middle of the long bone templates in primary ossification centers (figure 4). After producing cartilage around themselves the chondrocytes perish through apoptosis, leaving a cavity in which blood vessels invade. Through these vessels, osteoprogenitor cells migrate and are later turned into osteoblasts which then start to produce bone matrix which is later mineralized. Osteoclasts also migrate and start to remodel the bone so that a medullar cavity is formed (11, 12).

Secondary ossification centers with proliferating chondrocytes are formed around birth in the epiphyses of the bone. The hyaline cartilage formed is then mineralized throughout the epiphyses with the exclusion of a region called epiphyseal plate, or growth plate. Proliferation of chondrocytes in the growth plate contributes to lengthening the bone through a series of steps. The proliferated chondrocytes are stacked in a longitudinal orientation and subsequently undergo continuous maturation towards the diaphysis. First, the chondrocytes increase in size (hypertrophy). The hypertrophic chondrocytes later undergo apoptosis, and the contents of these cells contribute to calcification of the extracellular matrix. This immature calcified matrix is later replaced by more mature bone by osteoblasts and osteoclasts through the aforementioned remodeling process (10). This process allows the epiphyseal plate to maintain its thickness throughout skeletal growth. Elongation of long bones through endochondral ossification stops near the end of puberty, when the epiphyseal plate fuses (13).

A bone's growth in width occurs when osteoblasts migrate from the periosteal membrane to the cortical surface in a process called periosteal apposition. On the endosteal surfaces, both inside the diaphyseal cortical shell and towards the center from the growth plate, osteoclast activity dominates progressively, forming the medullar cavity, which in turn helps keep down the overall weight of the bone (11, 12).
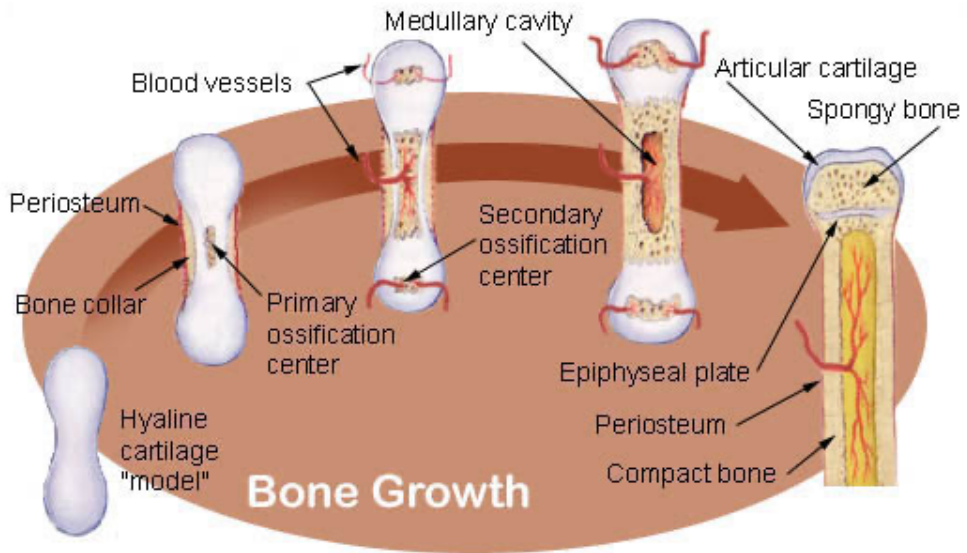
Figure 4: Endochondral ossification (5)

In flat bones such as the skull, bone forms through intramembranous ossification. In contrast to endochondral ossification this process occurs without the mineralization of cartilage but through proliferation of mesenchymal stem cells (MSC) within a membrane. The MSCs later differentiate to osteoblasts which initiate bone formation (11, 12).

Bone mineralization measurement techniques

When quantifying bone traits such as density and mass, the precision and accuracy of the specific method are two important factors. Precision equals the ability to reproduce the same numeric results when measuring the same subject repeatedly over a period of time, independent of the accuracy (figure 5) of the method (14). The short-term precision is usually presented as coefficient of variation (CV%), where lower CV% equals higher precision. A factor that is highly relevant for accomplishing low CV% in measurements of bone traits is standardized positioning of the subject.

The accuracy, on the other hand, reflects the ability of the method to conform to a "true" or known value, or how close the measurement mean is to that value (presented in %, figure 5).
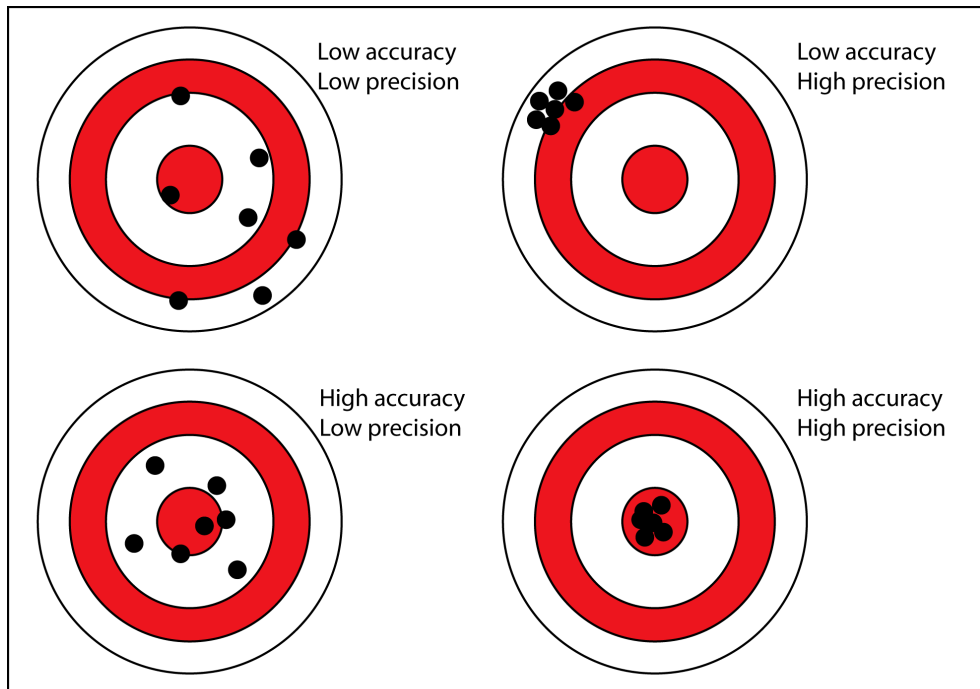
18

There are in principle two different groups of bone measurement techniques: ionizing and non-ionizing methods. Ionizing methods utilize a radiation source to produce images, from which data are obtained. The most common methods used in bone research are described below.

Dual energy X-ray absorptiometry (DXA)

The gold standard method in diagnosing osteoporosis, DXA was introduced in the late 1980's (15), nowadays frequently used in bone research as well as in clinical settings. As the name implies, DXA uses X-ray beams of two distinct energies that are absorbed by bone and surrounding soft tissue, thus enabling the built-in software to specifically analyze bone mineralization, also when covered by soft tissues (16). The technique is based on two-dimensional imaging and produces quantitative traits such as bone mineral content (BMC; g), bone size (cm^2) and areal BMD (aBMD; g/cm^2). Since aBMD is an area-based trait, it serves as a surrogate measure of volumetric bone mineral density (vBMD; mass per volume unit). This leads to the fact that larger bones will have higher aBMD, even when the vBMD is equal (figure 6). Due to the low amount of radiation, 1–8 µSv roughly equaling the 1/1000 of the yearly background radiation (17), the technique is not only used when assessing

19

adults but also a preferred densitometric tool for younger subjects. Virtually the whole skeleton can be assessed using DXA, with lumbar spine, hip, and total body as the most common regions of interest (ROI). In children, total body is measured excluding the head due to the discrepancy in proportion of the head size in different ages during childhood. Apart from measuring the skeleton, DXA has the ability to evaluate body composition with both lean mass and fat mass. The technique has in general an aBMD CV% of 0.5-3% and an accuracy of 3–9% depending on ROI (17). In vivo CV% of lean mass and fat mass show approximately 1% and 3% respectively (18).
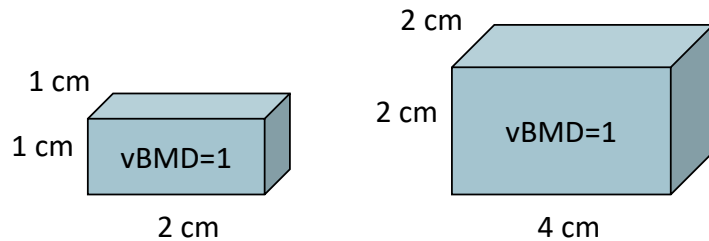
vBMD (g/cm³)	1	1
Volume (cm³)	2	16
BMC (g)	2	16
Projected area (cm²)	2	8
aBMD (g/cm²)	1	2

Figure 6: The relationship between areal bone mineral density (aBMD) relative to volumetric bone mineral density (vBMD) and the influence of size. In this example, an object with similar vBMD but twice the volume has twice the aBMD (BMC, bone mineral content)

Figure 7: Dual energy X-ray absorptiometry (DXA) scanning device. Photography by Nick Smith (CC BY-SA 3.0)

Peripheral quantitative computed tomography (pQCT)

Utilizing the ability to rotate the radiation beam and detector, peripheral quantitative computed tomography (pQCT) produces three-dimensional (3D) images of the ROI. Peripheral denotes that the technique is used on the radius and tibia, rendering lower radiation doses (<10 µSv) than central QCT (up to 1.5 mSv on spine and 3 mSv on hip protocols (19)).

Since 3D images are produced, pQCT has the ability to estimate volume-based BMD, in contrast to DXA. The technique also has the ability to separate cortical bone from trabecular bone, producing traits such as cortical cross-sectional area, cortical thickness, along with cortical and trabecular BMC and BMD.

With technical advances, high resolution pQCT (HR-pQCT) was recently introduced, having the ability to produce images of greatly increased resolution when compared to conventional pQCT (82 µm and 0.5 mm respectively) (20, 21).

Higher resolution enables assessment of even more specific traits such as trabecular thickness and cortical porosity including the amount (%), and diameter of the pores.

The general precision of pQCT (0.3-2.2%) and HR-pQCT (0.3–3.9%) is similar to that of DXA (22). The accuracy, however, is worse at about 5–15% (17).
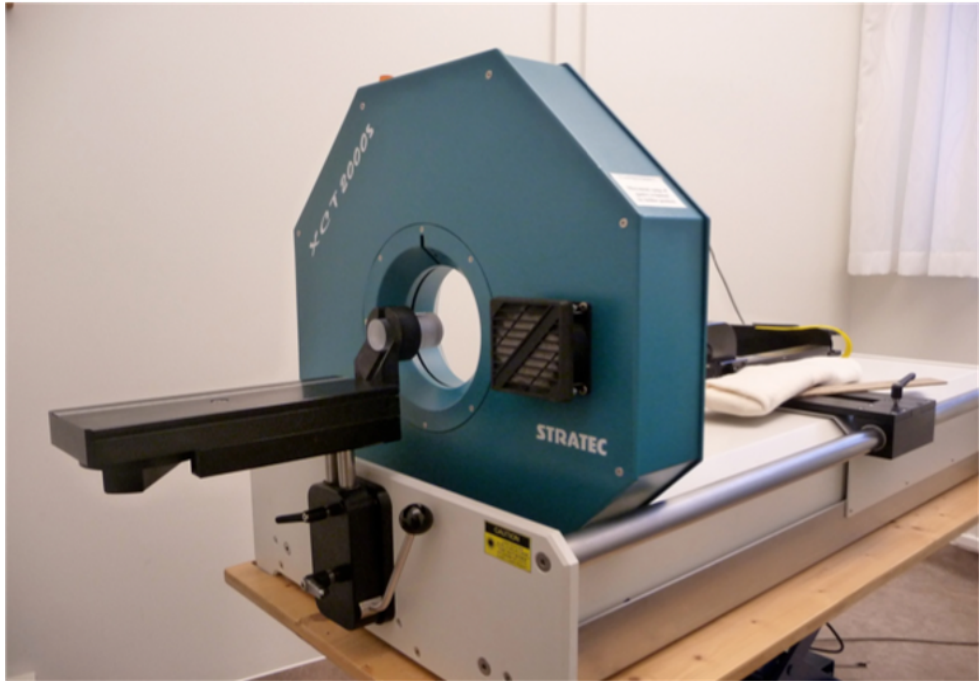
Figure 8: Peripheral quantitative computed tomography (pQCT (XCT 2000, Stratec®, Pforzheim)) scanning device

Quantitative ultra-sound (QUS)

A measurement technique that has none of the potential harm that radiation poses for the subjects is quantitative ultra-sound (QUS). The architecture and elasticity of bone is reflected in part by analyzing the speed of sound (SOS, m/s) and bone density by analyzing broadband attenuation (BUA, dB/MHz (17)). Although limited by the fact that it can only measure the bones with a thin enough layer of surrounding tissue around the bone, QUS has much the same ability as DXA when predicting fractures and the precision is estimated at 1.5–6% (17). Albeit having the ability to predict fractures, it should be noted that there is, relative to the widely used DXA, minute research based on QUS that evaluates the effect of anti-resorptive osteoporosis treatment, physical activity, etc.

Other techniques

Single photon absorptiometry (SPA) is one of the early bone measurement techniques, developed in the 1960's (23), utilizing the absorption of a single-energy gamma photon beam through the tissue. This technique was later developed into dual photon absorptiometry (DPA), thus gaining the ability to separately assess bone and surrounding soft tissue, without having to submerge the scanned limb as was necessary using SPA. Furthermore, this added the possibility to evaluate the mineralization of lumbar spine and proximal femur (24). The arrival of DXA, however, has in principle replaced the use of DPA.

Magnetic resonance imaging (MRI) is a relatively novel non-ionizing method that has the ability to produce high-resolution images. Apart from bone porosity and micro-architecture, marrow fat levels and composition, and vascularity are examples of what can be evaluated (25). Studies have shown that fat levels in the bone marrow are, independent of BMD, increased in osteoporotic patients (26).

Bone during ageing and peak bone mass

Bone formation dominates through the first two decades in life during the skeletal development, during which bone greatly accumulates in both size and mass. During the prepubertal period, the rate of the accrual of bone is influenced by the hormones growth hormone (GH) and insulin-like growth factor-1 (IGF-1), leading to similar mineralization between the sexes. At the beginning of puberty, with an increase of sex hormones, bone accrual greatly accelerates in both sexes, reaching peak mineralization at 11–13 years for girls and 13–15 years in boys. From two years before to two years after this peak, 39% of total body bone mass is attained. Furthermore, four years after the peak, 95% of adult total body bone mass has been achieved (27). During puberty boys' long bones grow faster in both width (through periosteal apposition) and length (through endochondral ossification) than in girls, in part due to the different levels of the respective sex hormones (28). Early research proposed that androgens were stimulatory and estrogens inhibitory with regard to bone mineralization (29, 30). However, recent research consensus indicates that mechanisms behind sexual dimorphisms in bone accrual during puberty follow more complex pathways (31).

Research has found that peripubertal chondrocytes contain androgen receptors (32, 33). However, androgens do not seem to influence longitudinal growth directly given the fact that individuals with androgen insensitivity syndrome reach taller statures when compared to females (32, 33). Estrogens, on the other hand, contribute greatly to longitudinal growth in early puberty in addition to determining the growth plate fusion (34).

During puberty, bone mass increments tend to rely on periosteal apposition in males when compared to females, where endocortical apposition dominates (35). This

leads to males having thicker cortex and larger bone. The increased bone accrual during puberty together with the fact that boys reach – and also finish – puberty a mean 2 years after girls (giving two more years of prepubertal bone accrual (36, 37)) lead to boys having larger and heavier bones at the end of puberty (38). The bone mineral density (BMD; mass per volume) within the periosteal envelope of bone, however, remains without differences between the sexes during the skeletal development period (39).

Around the age of 20 years bone mineralization decreases and by the end of the third decade it reaches a plateau (figure 9). This is the point of highest bone mass of the skeleton and is referred to as the "peak bone mass" (PBM). Ever since the discovery of PBM, however, there have been some discrepancies in how the term is used and interpreted. Even though the name implies mass, the most commonly used unit is DXA-based aBMD. Furthermore, PBM has been equated with peak bone strength (40) due to the fact that aBMD and BMC are considered valid measures of the ability of bones to withstand outer forces. The concept of PBM is further complicated by the fact that skeletal sites reach peak density levels at different ages. For example, lumbar spine and total hip aBMD in women peaks at 33-40 years and 16-19 years and in males at 19-33 years and 19-21 years respectively (41).
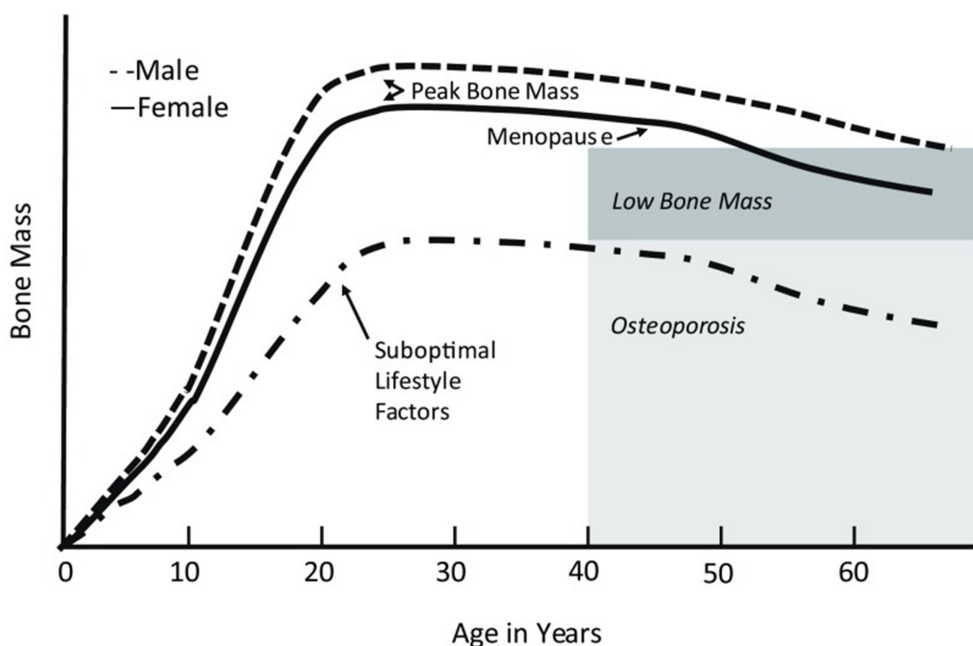
Figure 9: Bone mass throughout ageing in males and females. Source: Weaver et al (CC BY-NC 4.0 (40))

After reaching PBM, bone mass starts attenuating in similar patterns for both sexes. However, at the onset of menopause, decreasing estrogen levels lead to increased

24

loss of bone in females compared to males (figure 9). As bone loss continues, individuals risk reaching pathologically low levels, ultimately developing osteoporosis with greatly increased risk of sustaining fragility fractures.

Osteoporosis is described as a condition of decreased bone strength and increased risk of fractures due to quantitative and qualitative deterioration of bone, and in post-menopausal women has been defined by the WHO since 1994 using aBMD-based T-scores (42). A T-score equals the number of standard deviations (SD) an individual's aBMD differs when compared to young adult mean aBMD. A T-score of –1 to –2.5 defines a pre-osteoporotic stage called osteopenia, whereas a score of –2.5 SD and below leads to the osteoporosis diagnosis (table 1 (42)). If an individual meets the criterium of osteoporosis and has had at least one fragility fracture, i.e. fracture of the femoral neck and/or vertebral compression fracture, the term "severe" or "established" osteoporosis is used (43).

Table 1: Illustrating DXA-based definition of normal bone, osteopenia, osteoporosis, and severe osteoporis. Adapted from Kanis et al. **(43)**.

Defined condition	DXA-based T-scores
Normal	> –1.0 SD
Osteopenia	< –1.0 and ≥ –2.5 SD
Osteoporosis	≤ –2.5 SD
Severe osteoporosis	≤ –2.5 SD and 1 or more osteoporotic fractures

In order to evaluate the risk of sustaining future fragility fractures, one of the most common instruments used is the web-based Fracture Risk Assessment Tool (FRAX (44)). The tool predicts the absolute 10-year probability of fracture and utilizes several risk factors including age, sex, weight, height, previous fractures, smoking, use of glucocorticoids, heredity of hip fractures, use of alcohol, prevalence of secondary osteoporosis and prevalence of rheumatoid arthritis, and with or without femoral neck aBMD (44).

The timing of developing osteoporosis is suggested to depend on the PBM and rate of bone loss. Thus, low PBM paired with increased bone attenuation will increase an individual's risk of developing osteopenia and osteoporosis (45). The importance of PBM was illustrated by Hernandez et al., showing in a theoretical model that a 10% increase in PBM would postpone the osteoporosis diagnosis with 13 years, and also suggesting that a 10% higher age of reaching menopause, or a 10% reduced age-related bone loss would postpone the same diagnosis 2 years (46).

Due to the apparent importance of, and the time prior to PBM, extensive research has focused on factors determining the site-specific timing and amplitude of PBM. This may aid in identifying possible interventions increasing PBM, thus possibly postponing -–or even avoiding – the onset of osteoporosis (45).

Parallel to the diminishing bone mass, cortical thickness attenuation and an increase in cortical bone porosity are apparent in the aging individual (47-49) As in the case for loss of bone mass, these changes occur in both sexes, but predominantly in females (49, 50). These age-related changes are in part attributed to a dysfunction in the remodeling process in the cortex, whose primary function is to maintain the mechanical function of bone. Matching young and old men and women for aBMD, Nicks et al. showed that the amount of porosity was significantly higher in the older subjects, thus stressing the importance of microstructural changes, independent of aBMD, in the development of fragile bone (48).

Along with the microstructural deterioration of cortical bone, trabecular age-related changes are thought to play important roles in the development of osteoporosis and include trabecular perforation along with thinning, and loss of connectivity of trabeculae (47).

"Tracking" of bone mass

One of the reasons why PBM has been so widely researched is the potential "tracking" of bone mineralization. It has been suggested that an individual with low BMD in younger years continues having low BMD throughout life, when compared to same-aged subjects in the respective gender category.

Since aBMD is widely used in the clinical setting for diagnosing osteoporosis and thus correlates well with the risk of sustaining fragility fractures in the elderly, "tracking" may facilitate finding individuals having low BMD in young age with risk of developing osteoporosis later in life. If these young "individuals at risk" were found, interventions could then be initiated, in order to counteract age-related bone attenuation and thereby postpone or even avoid future fragility fractures.

Studies have shown that aBMD "tracks" from childhood to adolescence (51, 52), from adolescence to young adulthood (53), as well as during 10 years in adult subjects aged 25–44 (54) and 45–84 years (55). Furthermore, in 1995 Melton suggested in a longitudinal study that bone loss "tracks" well within an individual with relatively constant attenuation in bone during up to 22 adult years in 20- to 94-year-old females with high correlations (r=0.8) between baseline and follow-up values (56). No longitudinal life-long studies, however, have yet shown that aBMD tracks from the time of PBM until old age.

Factors influencing skeletal development and bone health

The importance of genetics and bone mass is apparent when studying identical twins, with heritability explaining 60–80% of the variance in adult bone mineralization (57). However, genetic importance seems to be dependent on skeletal site, with less effect on the femoral neck than on lumbar spine bone mass (57).

Although playing the leading role, non-modifiable genetic factors do not paint the whole picture in bone mineralization, and environmental modifiable factors thus explain 20–40% of the variance.

Nutrition and physical activity are two of the most important modifiable factors for bone growth. Among nutritional factors, calcium, vitamin D, and proteins have been focal points of research (45).

Being the main mineral component in bone, calcium and calcium intake are well-investigated topics in skeletal research. The need for adequate calcium intake in relation to skeletal development and PBM is long since established (58). Low intake of milk, a dairy product rich in calcium, in childhood and adolescence have been shown to increase the risk of sustaining a future fracture (59). Whether this effect is due to calcium or other important milk constituents, such as magnesium, proteins, and vitamins, is not clear, however. In a RCT in 1997, the authors linked milk intake to greater bone mineral gain during puberty and attributed this to an increase in serum IGF-1 (60).

A meta-analysis in 2008 found that, in children, supplementary calcium intake has been demonstrated to increase BMC statistically significantly, albeit showing small effects (61). However, children with low intake levels at baseline exhibited greater gains in bone mass (61). Later studies have also indicated that calcium supplementation positively impacts bone accrual in both children and adolescents (62-64). In contrast, in a meta-analysis from 2015, increasing dietary calcium in adults showed small increases (1–2%) in aBMD and the authors concluded that this increase does not suggest clinically relevant effects on bone strength and fractures (65).

Vitamin D serves as an important factor in skeletal development mainly through calcium absorption and utilization. The vitamin is mainly produced by transforming a precursor to vitamin D in the skin through sunlight exposure (66). A high prevalence of vitamin D deficiency has been measured in hospitalized adult patients with hip fractures (67, 68). To compensate for low sunlight exposure in individuals, adequate vitamin D levels can be maintained through dietary supplementation.

There is good scientific evidence that regular physical activity promotes bone health in children and adolescents (see *Physical activity and bone*).

Several factors with a negative influence on bone health have been identified. These include medical conditions such as hyperthyroidism and medications using corticosteroids, but also lifestyle and environmental factors such as alcoholism or smoking.

Cigarette smoke is known to harm the skeleton directly by nicotine and cadmium exerting a toxic effect on the bone cells (69), and indirectly by decreasing intestinal absorption of calcium (70). The harmful effect on bone is further acknowledged by

the fact that smoking increases the risk of hip fractures and other fractures in both men women (71).

Bone strength

Perhaps the most vital endpoint in skeletal research, bone strength has been defined as the ability of bones to respond to mechanical demands (72). Another definition is that bone strength equals the maximum load that acts on the bone when it fractures (73). Despite being a most important trait, it has proven difficult to measure in a clinically relevant manner. This is mainly due to the multifaceted and complex components of bones' mechanical capability to withstand forces without fracturing. Bone material composition and quality, the geometry and internal architecture of bone, and the velocity, amount, and direction of mechanical force applied are some of the factors that must be taken into account when evaluating bone strength (6).

Bone exhibits anisotropic mechanical properties, meaning that due to non-uniform geometry, it reacts differently depending on the direction of the force acting upon it (74). The viscoelastic collagen fibers in the bone allow forces (stress) to conform (strain) the bone, and when the force ceases, the bone returns to a similar form as before. The stiffness of bone (described as Young's modulus or elastic modulus) is determined by the amount of stress (MPa) required to strain (%) the bone during the elastic phase (figure 10, first part of the curve). Higher modulus equals higher stiffness. If load increases, the yield point is passed, meaning that further strain will cause material degradation, and thus damage the bone tissue. If the load surpasses the ultimate point, the bone fractures. After passing the yield point, stiffness is decreased if load is reapplied. This material degradation has been defined as microdamage, first described by Frost in 1960 (75), is today considered a physiological response to physical loading of bone and is considered a most important trait in bone modeling through the "mechanostat theory". In contrast to the positive impact in strengthening bone, microdamage might also play a part in fragility fractures, and studies have shown that microcracks increase exponentially with age, and at a higher rate in women than in men (76, 77).
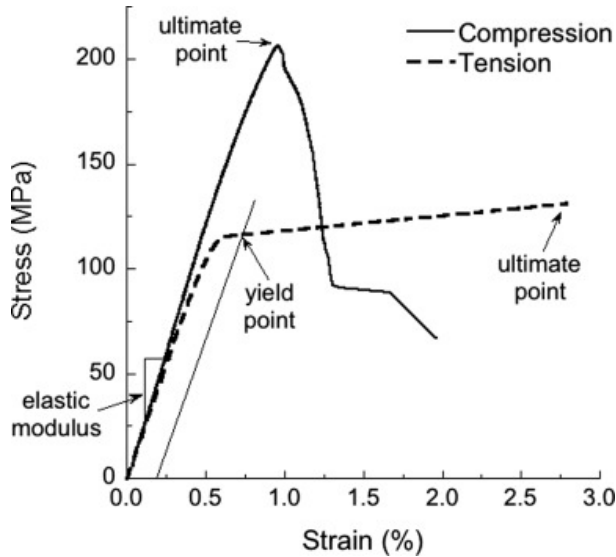
Figure 10: Stress/strain curve from compression and tension test of cortical bone along the longitudinal axis (78)

Whole bone measurement techniques that are commonly used to evaluate bone strength are 3- and 4-point bending and torsional tests, where forces are applied perpendicular to the bone, leading to compression of bone on the same side as the applied point of force and tension on the opposite side, until the bone fractures. This test is mainly utilized at diaphyseal sites, thus mainly establishing cortical bending resilience. The mechanical load is measured using a transducer and is later processed by built-in software (74). Another example includes compression test (figure 10), where force is along the longitudinal axis of a long bone i.e., the femur.

For obvious reasons, bending and compression tests are not performed in vivo on human subjects. Instead, surrogate measures must be used when aiming to estimate bone strength. As described earlier, imaging techniques such as DXA, QUS or pQCT all have the ability, to different extents, to evaluate bone mineralization, bone structure, and geometry. Of these, the most commonly used surrogate measure of bone strength is DXA-based aBMD, in part due to its ability to predict future fractures (79) and the correlation between aBMD and fracture load from in-vitro bending tests (80). Furthermore, studies have shown that an increase in aBMD of 1 SD is associated with a halved fracture risk (81-83).

A lower T-score is associated to a higher fracture risk (see *Bone during ageing and peak bone mass*). However, 50-year-old individuals have lower absolute yearly risk of sustaining a fracture than 60-year-old peers with similar T-score, and this age-related risk increase continues in older ages (84). Thus, increasing age additionally leads to changes in other parameters contributing to increased fracture risk.

Several different factors contributing to bone strength have been established, with bone mineralization being only one of them. As mentioned in *Bone during ageing and peak bone mass*, they include cortical and trabecular microstructure (85), along with other factors such as bone geometry and size, cortical thickness, and porosity (86).

As aBMD attenuates in post-menopausal women, mainly due to endosteal resorption (87, 88), bone size along with medullar cavity expands in the distal radius (89). Being a tubular structure, mechanical calculations state that the bending strength of a long bone increases by the fourth power of the distance from the neutral axis (90). This is underlined by other studies where bone size has been identified, along with aBMD, as an independent factor contributing to the structural strength, and fracture risk in tubular bones (91, 92). The aforementioned postmenopausal increase in bone size is suggested to serve as a "built-in" counteracting measure to prevent decreasing bone strength following lower aBMD by increasing the periosteal apposition.

Following the possibility of assessing separate cortical bone traits, further analyses reflecting bone strength have been developed from pQCT. These include cross-sectional moment of inertia (CSMI, mm^4), strength strain index (SSI, mm^3), and section modulus (SM, mm^3). Both CSMI and SM come from mechanical calculations on a tubular object, independent of material properties of bone, such as BMC or BMD and are based on the cortical cross-sectional area and the distance from the central axis to the cortex. In addition to these models, SSI takes bone material properties into account, producing a surrogate measure of bone strength (figure 11). Both CSMI and SSI have been shown to correlate well with bone strength through three- and four-point bending tests in animal models (93-96) along with the strong association with fracture risk in human subjects (97).
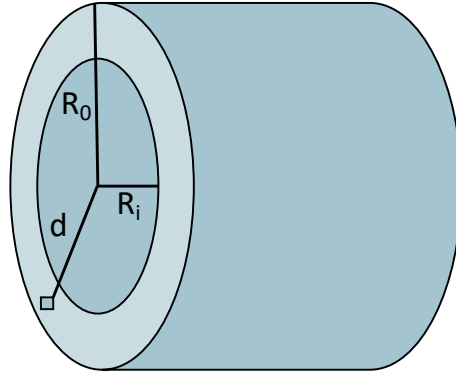
Cross-sectional moment of inertia = $(\pi/4)(R_0^4 - R_i^4)$

Section modulus = $((\pi/4)(R_0^4 - R_i^4) / R_0)$

Strength strain index = $\dfrac{\Sigma(d^{2*}A*vBMD_{vox}/vBMD_{max})}{R_0}$

Figure 11: Illustrating a schematic model and mathematical equations in a tubular object from which CSMI, SM and SSI derive. R_0 and R_i are the distance from the center of the axis to the outer and inner limitation of the cortical rim respectively. A is the cross-sectional area of a voxel, d is the distance of a voxel to the central axis. $vBMD_{vox}$ and $vBMD_{max}$ is the voxel's volumetric bone mineral density and maximum cortical volumetric bone mineral density under physiological conditions in a human bone

Muscle

Muscle tissue

There are in principle three different types of muscle in the human body: skeletal muscle, smooth muscle, and cardiac muscle. In this thesis, the focus will be on skeletal muscle.

Skeletal muscles stand under the influence of voluntary control and are attached to bones via tendons in order to allow for movement of body parts in relation to each other. Consisting of mostly proteins and water, skeletal muscle is the most abundant of muscle types and constitutes about 40% of total body weight, and about 50–75% of total body proteins (98). The amount, or mass, of muscle is under the influence of several different factors such as genetical predisposition, physical activity, diet, hormonal status, and several medical conditions.

Skeletal muscle operates through contraction and relaxation via sarcomere-containing myofibrils. Several myofibrils are oriented in a parallel fashion inside a single muscle fiber, and in turn, several muscle fibers run parallel to each other, surrounded by a connective tissue layer called perimysium (figure 12). Contraction and relaxation in the sarcomeres occur through sliding of the proteins actin and myosin in an opposite manner, thus shortening or lengthening the muscle tissue (99).

There are three main types of skeletal muscle fibers, type I (slow), type IIa (fast), and type IIx (very fast), based on their shortening speed and biochemical properties (100). Initially an individual muscle fiber was thought to contain only one muscle fiber type but later research, based on the respective contractile element called myosin heavy chain (MHC), has presented hybrid subtypes called type I/IIa, IIa/IIx (101). The earlier notion that the distribution of muscle types is fixed has now been altered, stating that muscle fiber types exhibit plastic properties and that physical activity seems to be the main regulatory element (102-105).
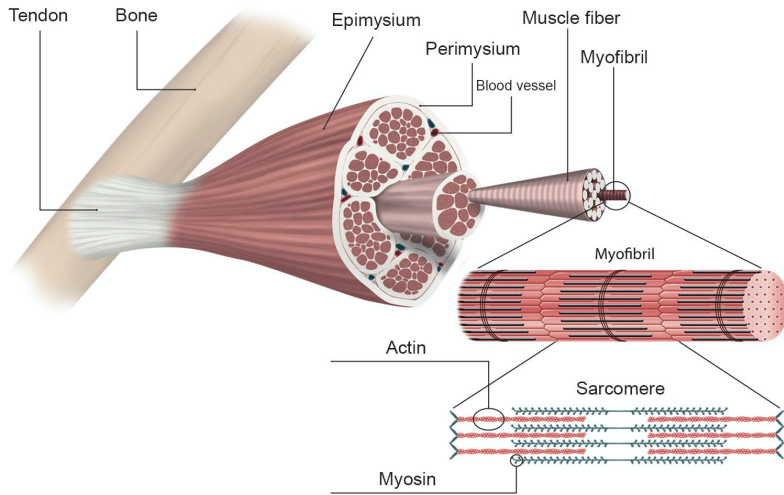
Figure 12: Illustration of the human skeletal muscle (106)

Muscle strength and neuromuscular function

The activation of skeletal muscle can occur in two distinct ways, namely static and dynamic. During static activation, the muscle length is constant, thus not leading to movement in a limb or joint. Dynamic muscle activation leads to lengthening (eccentric) or shortening (concentric) of the muscle, thus moving the attached body part (98).

Muscle strength is often defined as the maximal force a muscle or muscle group generates during pre-defined conditions, and dynamic muscle strength reflects this force at a specific velocity (107).

Neuromuscular function reflects the complex interaction between the central nervous system (CNS) and the use of nerve signals leading to muscle activation, utilizing voluntary movement of the body. These nerve signals convert to a release of neurotransmitter (acetylcholine) at the junction between a single nerve end and muscle called motor end plate which in turn activate the muscle. A nerve and its connected muscle fibers are called a motor unit. Motor units vary in size and function in different locations of the body depending on what movement is required by the respective body part. Small motor units are often called "slow" motor units, are often connected to type I muscle fibers and are connected to fewer muscle fibers than larger so-called "fast" motor units (108). Small motor units are often responsible for e.g. fine motor skills and postural control whereas large motor units are highly represented in muscles used to produce movement with great force such as jumping (108). Neuromuscular control reflects the complex coordination in the

33

CNS of muscle activity through feedback and feed-forward systems during which sensory information translates into coordinated movement (109).

With age, the amount and function of motor units deteriorate, leading to a progressive decrement in general muscle mass, muscle quality, and function, defined as sarcopenia (110). This primarily occurs through the apoptosis, or "programmed cell death", of the motor neurons in a motor unit and these changes gradually increase throughout life, with an annual muscle mass attenuation of 1–2% per year, and (111), accelerating after the age of 60 years (112, 113). These age-related changes lead to loss of muscle strength, contraction velocity, and steadiness which in turn increases the risk of fractures from falling (114, 115) and predicts increasing disability, loss of independence, and even risk of dying (116). However, optimizing factors such as regular physical activity may help in counter-acting these changes, thus having great impact not only on musculoskeletal but also on the overall health of the aging population (117).

Physical activity and muscle

Like other organ systems in the human body, muscles have a remarkable ability to adapt to different situations and demands. Studies have shown that physical activity increases muscle strength, throughout the entire life span (118, 119). The form of physical activity will lead to specific adaptations within the muscle tissue. Resistance training such as weight lifting and other anaerobic activities, for example, will lead to muscle hypertrophy and enhanced neuromuscular function, in turn leading to improved muscle strength (120, 121). Aerobic training including running, cycling, and cross-country skiing have a different effect on muscle with increased amount and size of muscular mitochondria thus improving the translation of energy to muscle activation in the presence of oxygen (122).

Muscle measurement techniques

There are several ways of measuring muscle tissue and function. It is important to note that, due to the activity-based adaptations, muscle strength and function to a high degree depend on where they are measured anatomically, making it hard to evaluate the "overall" muscle strength. Some techniques utilize dynamic measurements of strength, and an example of this is an isokinetic dynamometer, which has the ability to measure the maximum amount of torque, or peak torque (PT, newton meter (Nm)). The most commonly estimated muscle strength location is the knee joint, which measures the strength of the quadriceps femoris and hamstring muscles. The device is passive and resists extension or flexion force in the knee joint, keeping the angular velocity of the limb constant (isokinetic) throughout the range of motion, with commonly used speeds of 60 and 180 degrees per second (123). A drawback in using this method is that it depends on the

cooperation and motivation of the measured subject in order to produce the maximal effort.

A method with the ability to estimate muscle mass is DXA, where lean mass of the total body, which mostly comprises muscle but also skin and viscera, can be estimated (124).

Physical activity

Physical activity (PA) is defined as movement of any sort, produced by skeletal muscle activity, resulting in the expenditure of energy above resting levels (125). For many people, PA equals sport activities such as football, track and field, or ice-hockey. However, other parts of daily life such as manual transportation, e.g. by foot or by bike, also counts as PA. Exercise, a subgroup of PA, is a planned or organized PA of a repetitive nature with the aim of gaining health benefits such as improved endurance as well as stronger muscles and skeleton (126). It should be noted that isometric work, where muscles contract but do not generate movement, also increase energy expenditure above resting levels but do not count as physical activity according to the aforementioned definition (127).

Health aspects of physical activity, inactivity and sedentary behavior

There is a strong consensus that PA is one of the most important, and highly modifiable, lifestyle factors in maintaining health. Reducing depression symptoms, preserving cognitive function, and decreasing risk of cardiovascular disease are just a few examples of the many positive effects PA has on the human body (128). With improving technology and through the development of vehicles and machines, physical activity levels all across the world have been diminishing. Sadly, despite the apparent evidence regarding the positive effects of PA, worldwide physical inactivity levels have reached worrying heights, with physical inactivity being placed as the fourth leading cause of death in 2018 by the World Health Organization (WHO), only surpassed by hypertension, use of tobacco and diabetes (129). Put in economic terms, physical inactivity costs approximately 54 billion international dollars, (INT$, equivalent value of a US dollar in a given year) with additional costs of INT$ 14 billion in lost productivity in 2013 (130).

At the same time as PA levels are attenuating, the time spent on sedentary behavior seems to be going in the opposite direction. Sedentary behavior is defined as ≤1.5 metabolic equivalent of tasks (METs), where 1 MET equals the expenditure of energy when sitting still (131). The use of video games, mobile phones, tablets and laptops are some examples of sedentary activities that have increased during the last few decades. Sedentary behavior significantly increases the risk of all-cause

mortality independent of PA levels, thus stressing the need to address this problem parallel to PA (132).

Figure 13: The author demonstrating an example of physical activity

Physical activity in children and adolescents

Even though many of the conditions that PA helps to treat and prevent usually affect the adult population, there is growing evidence of the importance of PA early in life, with evidence suggesting that PA levels "track" from childhood to adulthood (133-136). The importance of addressing PA is further stressed as PA levels remained

unchanged between 2001 and 2016 in children and adolescents, with 80% of 11–17-year-olds globally not meeting the recommended daily amount of PA (137). In a study from southern Sweden, the trend seems to be similar, with lower levels of PA in boys and girls aged 11–14 in 2000 compared to 2017 (138).

Well-known examples of positive and health-promoting effects of PA in children include cardiovascular (139, 140), psychiatric (141), and academic performance (142-144). Furthermore, the importance of physical activity in children and adolescents is acknowledged by WHO, stating that 5–17-year-olds should incorporate at least 60 minutes daily of moderate to vigorous physical activity, (mainly aerobic PA) and vigorous-intensity muscle and bone-strengthening activities minimum three times per week (145).

Physical activity and bone

The positive effect of PA on bone is well established. In the late 1980's Frost proposed the "mechanostat theory", stating that bone modeling and remodeling are affected by the mechanical strain exerted on the bone (146). As previously described (see *Bone modeling and remodeling*), mechanical loading enhances osteoblast and inhibits osteoclast activity, thus enhancing bone formation, in part through the act of the mechanical sensor function of the osteocytes through fluid movement in the canaliculi (147).

From a clinical standpoint, several studies have shown that regular PA increases bone strength and lowers the risk of fragility fracture in older adults (148-152). The fracture-preventing effect of PA is further underlined by the negative effect of physical inactivity on bone (153, 154). Even though osteoporosis is a disease mainly affecting the older individuals of a population, skeletal development during the first two decades is of great importance in order to reach the maximum potential of bone accrual (see *Bone during ageing and peak bone mass* (40)).

In a meta-review in 2016 based on 36 randomized controlled trials (RCTs) and 20 observational studies, the authors concluded that PA increases BMC and BMD in children (40). Independent of density and mass, PA may also affect structural changes in bone, such as size and cortical area, leading to increased bone strength (155-158).

Research has found that the most efficient PA, with regard to skeletal response, is dynamic, with a short load duration, non-repetitive in load direction, applied with high velocity, of moderate to high amplitude, and with periods of rest between the activities (159). Examples of such activities are jumping, racquet sports, and weightlifting. Studies have shown that in contrast, low-impact activities of a repetitive nature desensitize the osteocytes, thus not leading to any major gain in bone mass (160, 161). Given that the strain of bone must exceed a given threshold in order to initiate the osteocyte-driven osteogenesis, it must be considered that

children with insufficient levels of PA may reach the osteogenic response already in activities with lower levels of impact as compared to peers with high PA levels (159).

Despite the positive effects of PA on bone, it must be noted that there is an increased risk of fracture, independent of bone size and BMD, in children participating in vigorous physical activities once a day or more (162). Another study showed that this risk is evident when comparing individuals having low levels of PA with the most physically active ones (163). In the POP study, where children had 40 minutes of daily physical education, the fracture risk was higher after one year. However, fracture risk was halved after seven years of intervention, pointing towards to the conclusion that the possibly initially increased fracture risk of PA is well compensated by its long-term beneficial effects, at least from a daily PA school program (164).

Measuring physical activity

Determining the level of PA in an individual has proven to be a somewhat difficult task. Earlier research often relied on the use of questionnaires and self-reported measures, which do have advantages of low cost and being easy to use (165). In children, however, there are some drawbacks with these methods including the difficulty for both children and parents to recall and accurately report the duration and intensity of the specific PA. Newer techniques include accelerometers and pedometers which convert bodily movements into electric signals, thus enabling objective measures of PA.

Physical education in Sweden

Throughout nine mandatory school years in Sweden, physical education (PE) is a compulsory subject. During these years, the municipalities currently distribute a minimum of 600 hours of PE in the curriculum over the nine different school years (166). In fact, the PE hours were increased from 500 to 600 in Sweden by the Swedish National Agency for Education in 2019, as a result of research showing the benefits of increased PE (166). Given the importance of PA, and in order to meet the WHO recommendations for school children (see *Physical activity in children and adolescents*), this could and perhaps should be increased even further. This was acknowledged by the Swedish parliament, which in late 2020 filed a motion to the government, suggesting further increasing PE, a suggestion based on the results from the POP study (see *Material and methods* (136))

Physical activity interventions

Because children and adolescents are less physically active, a part of the solution might be PA interventions. Together with the obvious positive health effects in children and the fact that PA levels seem to "track" (see *Physical activity in children and adolescents*), these interventions should focus on children of young ages. From a skeletal point of view, it is important to note that interventions should start before puberty, as effects are shown, at least in girls, to be twice as effective on the gain in bone mass (167). Another study showed that increased school PE in 12–16-year-old children improved bone mass and density in boys but not in girls, thus suggesting that interventions would have been more effective if started earlier (168). The lack of effect of PA interventions after puberty has been found also by others (169-171)

An effective way of incorporating the larger part of the population, regardless of socioeconomic situation and geographic position, is by school interventions. Since school is compulsory, children will then be able to attend organized and highly modifiable PA during PE. A counter-argument against implementing increased PE in school is that it comes at a cost. The hours of increased PE must come at the expense of either redistributing the curriculum with fewer hours spent on theoretical subjects, or by prolonging the school day. The concern that decreased theoretical hours would reduce knowledge in the affected subjects, however, should perhaps be mitigated by the fact that increased PE correlates well with improved academic performance (142, 143). This association is also underlined by Cöster et al, in 6–15-year-old boys in Sweden following an increase in PE (144).

In 1998, Rowland introduced the "activity-stat theory", suggesting that the amount of PA an individual child has is constant, and therefore, increasing PA during schooltime would only result in less PA outside school, thus rendering PE interventions without effect (172). A review by Gomersall et al. in 2013, however, based on studies supporting as well as studies refuting the theory, did not find evidence for either side (173). Since then, several RCTs have presented evidence refuting the "activity-stat theory", thus supporting the ability to increase PA levels through interventions (174-176).

Aims of thesis

Papers I and II

- To increase the general knowledge of skeletal development in young men by presenting normative data and identifying possible age-related differences in bone traits in 18–28-year-old men using pQCT on the forearm and lower leg (**paper I**) and DXA on the femoral neck (**paper II**).

- To estimate what effects these possible age-related differences have on bone strength, and if peak bone mass and peak bone strength correspond.

Papers III and IV

- To evaluate whether a daily 40-minute physical activity intervention program during nine primary-school years, compared to having physical activity 1–2 times per week:

  - is associated with beneficial changes in musculoskeletal health from before to after puberty during ongoing intervention (**paper III**)

  - is associated with attenuation of the musculoskeletal gains from intervention after the intervention is terminated (**paper IV**)

Material and methods

The MRPEAK study (papers I and II)

The MRPEAK study is a population-based cross-sectional study aimed at assessing the skeletal health of young adult men. The subjects were randomly selected and invited using a 1-year stratified sampling procedure from the Swedish official population registry provided by the Swedish Tax Agency (Skatteverket) and included 18–28-year-old men residing in the greater area of Malmö (population in 2014: 318,107).

Subjects

In total, 4503 subjects were invited and of these, 2280 did not respond, 883 declined, and 1340 consented to participate and were subsequently called for assessment. Of the 1340 accepting subjects, 51 were excluded as they did not meet predefined inclusion criteria, 26 because they exceeded the age maximum, 23 due to exceeding the number of subjects in each age group, one due to language difficulties, and one due to impaired movement. Of the remaining 1289 subjects, 1101 attended the assessment after 78 cancelations and 110 subjects failing to show without notice. Subjects were assessed from August 2006 to May 2012. Of the 1101 attending subjects, pQCT could not be performed at all in 18 subjects (figure 14). In addition, 12 radius scans and 82 tibia scans were not performed or were excluded, rendering 1071 radius scans and 1001 tibia scans (figure 14). Regarding DXA, of the 1101 eligible and attending subjects, 49 scans were excluded, mainly due to the use of another DXA-device (n=35), rendering 1052 DXA scans (figure 15). Prior to study start, written consent was obtained from all participants.

Subject recruitment
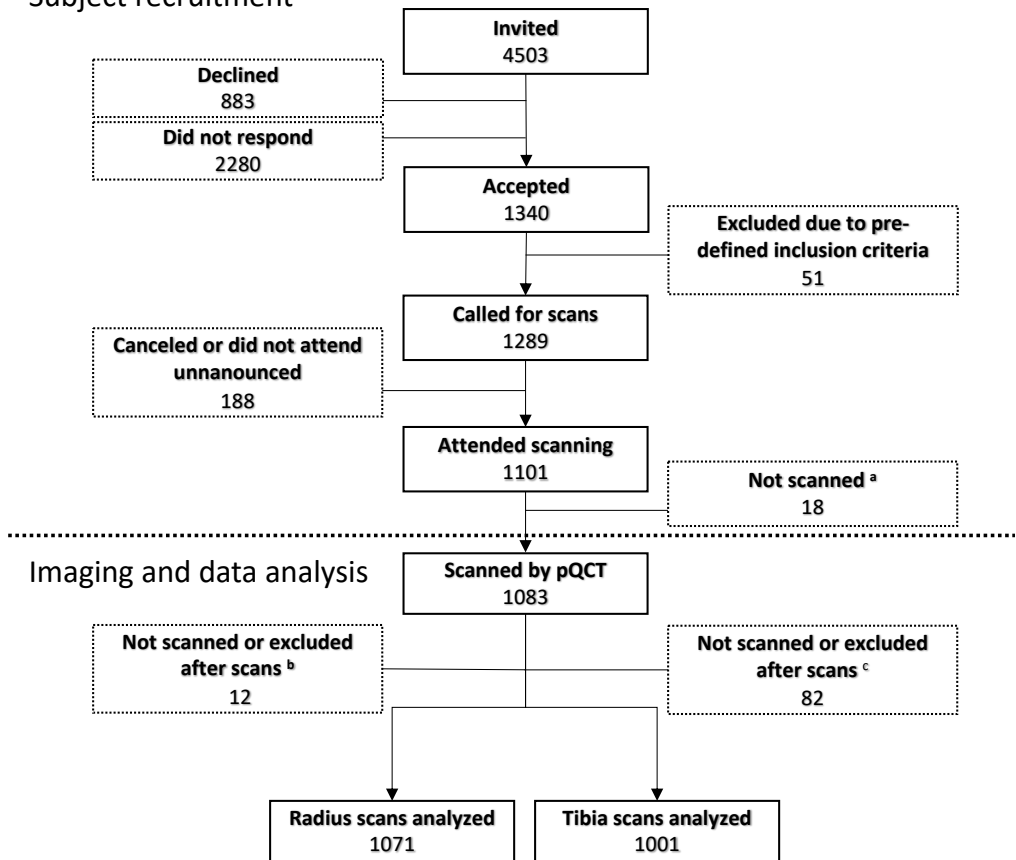
Figure 14: Flow chart illustrating subject recruitment, exclusion, scanning and data analyses in paper I

From original article (177):
a) Difficulty for subject to remain still during scans (n=2), pQCT device malfunction (n=12), subject leaving due to lack of time (n=1), overall disability for subject to move extremities (n=1), or for unspecified reason (n=2)
b) Inability to define correct baseline placement (n=4), metal object implanted in subject's wrist (n=1), difficulty for subject to remain still during scans (n=2), subject having nonremovable bracelet (n=1), pQCT device malfunction (n=3), or subject having arm pain (n=1)
c) Inability to define correct baseline placement (n=7), metal object implanted in subject's ankle (n=3), subject fainting (n=1), inability to correctly place lower leg in the computed tomography device due to size of subject's lower leg (n=19) or foot (n=40), due to insufficient range of motion in subject's knee joint (n=1) or talocrural joint (n=1), or for unspecified reason (n=10)

Subject recruitment
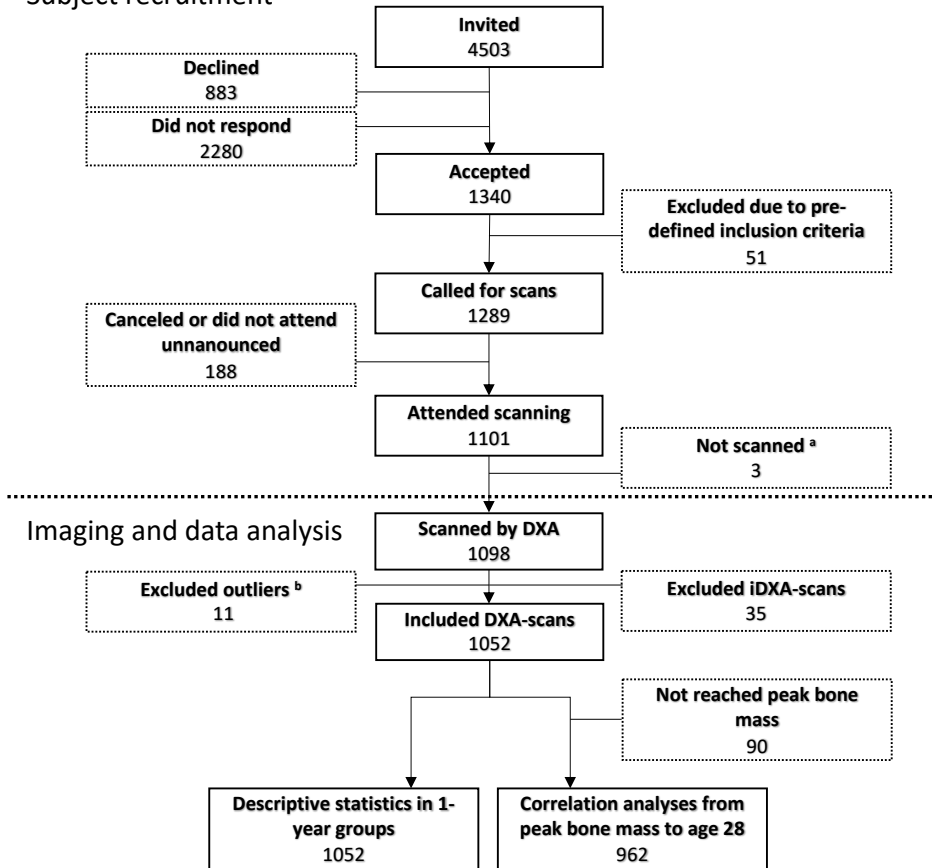
Figure 15: Flow chart illustrating subject recruitment, exclusion, scanning and analyses in paper II

a) Too large for DXA-machine (n=1), No hip-scan done (1), or hip prosthesis (n=1)
b) By outlier labeling rule and a g-value of 2.2, described by Hoaglin et al. (178)

Measurements

Paper I

Subjects were scanned using pQCT (model XCT 2000s, by Stratec Medizintechnik GmbH, Pforzheim, Germany) with software versions 5.5 and 6.0. The scanned sites were the tibia, a high load site, and radius, a low load site, of the non-dominant side, where the dominant side was defined by the preferred hand of writing. Before performing the main scans, using built in standardized protocol provided by the manufacturer, the extremities were measured in length. Via a scout view (coronary slice), ROI locations, in % relative to baseline, were determined. Within each

extremity we chose to include a distal region (4%) and mid-diaphyseal regions (38% and 66%), containing predominantly trabecular and cortical bone respectively. The thin cortex at the 4% ROI makes it difficult to produce accurate cortical measures, thus we included trabecular volumetric bone mineral density (Tr.vBMD; mg/cm^3) and total bone cross-sectional area (TB.CSA; mm^2). At mid-diaphyseal ROIs we included cortical vBMD (mg/cm^3), cortical CSA (mm^2), cortical thickness (Ct.Th; mm), periosteal circumference (PC; mm), endosteal circumference (EC; mm), along with polar cross-sectional moment of inertia (CSMI; mm^4) and polar strength strain index (SSI; mm^3). To ensure reliability, the pQCT machine was calibrated daily and monthly using manufacturer-provided standard and cone phantom respectively.

Paper II

Subjects were scanned using DXA (Lunar Prodigy, by GE Medical Systems, Madison, WI, USA) with software versions 9.20.122–9.30.044. The site scanned was the hip and the ROIs were femoral neck (FN), trochanter (Troch), and total hip (TH). These ROIs were chosen due to the difference in bone composition where FN has relatively thick cortex, Troch consisting mainly of trabecular bone, and TH which has a combination of the two. Apart from bone traits, total body fat mass (kg) and total body lean mass (kg) were also assessed by DXA. Manual measurements included height (cm) and weight (kg), from which body mass index (BMI; kg/m^2) was calculated.

Statistical analyses

In **papers I** and **II** we used IBM SPSS (version 22.0, IBM Corp., Armonk, NY, USA). We created 1-year age groups where subjects aged 18.00 to 18.99-years represented age group 18 and so forth. Descriptive data are presented in tables including mean values and standard deviations (SD) of the measured traits in each age group. In graphs, mean values with 95% confidence intervals (CI) are presented. To evaluate possible age group differences, we performed analyses of variation (ANOVA). In **paper II** we added Pearson's correlation analysis of the DXA bone traits with increasing age from PBM of the hip (19 years in men). In the ANOVA and correlation analyses, p-values below 0.05 were considered statistically significant.

The Pediatric Osteoporosis Prevention (POP) Study (papers III and IV)

The Pediatric Osteoporosis Prevention (POP) Study, initiated in 1999, is a population-based, prospective, and controlled intervention study with the aim of investigating the potential effect of daily physical activity (PA) by increasing physical education (PE) in the nine years of primary school. Four government-funded schools in Malmö, Sweden, within the same geographical area and with similar area-level socioeconomic status were asked to participate in the study. Ängslättsskolan accepted participation first and thus became the intervention school, with Fridhemsskolan, Mellanhedsskolan, and Ribersborgsskolan being the control schools. The home address of the individual child determined what school he or she went to. The level of PE in each of the schools, before the study start, was 60 minutes per week in accordance with the national curriculum set by the Swedish National Agency for Education (Skolverket). This amount of PE, divided into 1–2 sessions a week, continued in the control schools after the study started, whereas the intervention school introduced 40 minutes of daily PE totaling 200 minutes per week. In both the intervention school and the control schools, the specific content of the PE remained unchanged, was provided by the regular teachers, was held both indoors and outdoors, and contained varying activities such as running, jumping, dancing, and ball sports. The principal of the intervention school increased the school day duration slightly along with decreasing the amount of non-compulsory subjects, in order to accommodate the increase in PE. However, these alterations were in accordance with the national curriculum.

Figure 16: Children in the intervention school (Ängslättsskolan) during a physical education (PE) class

Subject invitation, inclusion, dropout, and exclusion

In the intervention group, children who started school in the intervention school 1998–1999, a total of 89 boys and 61 girls, were invited to participate in the study. In the control group, children who started school in the three control schools 1999–2000, 170 boys and 157 girls, were invited. Eighty-four boys and 56 girls in the intervention group, and 68 boys and 64 girls in the control group accepted participation. An additional 43 boys and 44 girls in the intervention group were invited a year later into the study (year 2000), of which 39 boys and 38 girls accepted inclusion. In total, 123 boys and 94 girls accepted participation. Written consent was

obtained from the child and/or a parent/guardian prior to study start. Four boys and 2 girls in the intervention group and 1 girl in the control group were excluded due to chronic disease or medication that possibly interfered with bone growth (figures 17 and 18).

In **paper III**, we excluded 30 boys and 31 girls in the intervention group, and 11 boys and 22 girls in the control group who, at the follow-up (mean age 15 years; mean follow-up time 7.5 years) had not ended puberty (reached Tanner 5). In addition, between baseline assessment and follow-up in the 9th grade, 26 boys and 27 girls in the intervention group, and 31 boys and 24 girls in the control group left the study (figure 17).
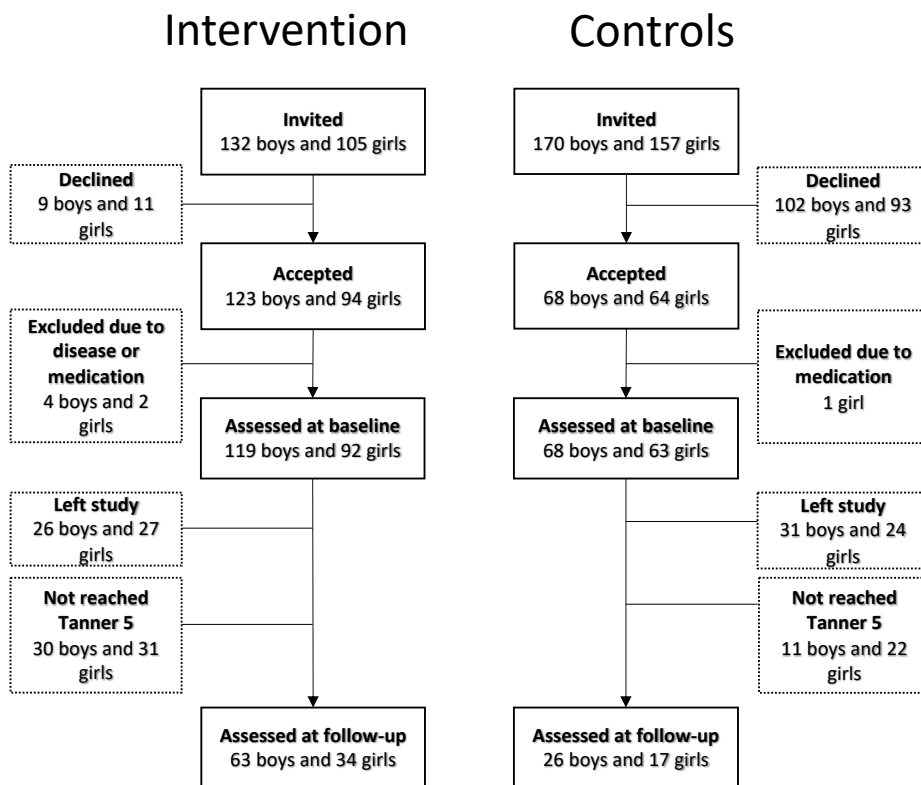
Figure 17: Participant flow chart in paper III.

In **paper IV**, between baseline assessment and follow-up (mean age 19 years; mean 4 years post-intervention) 74 boys and 56 girls in the intervention group, and 47 boys and 41 girls in the control group left the study (figure 18). Two boys and 1 girl in the intervention group, and 2 boys and 1 girl in the control group did not

participate at the end of the intervention (9th grade) but did participate at follow-up (figure 18).
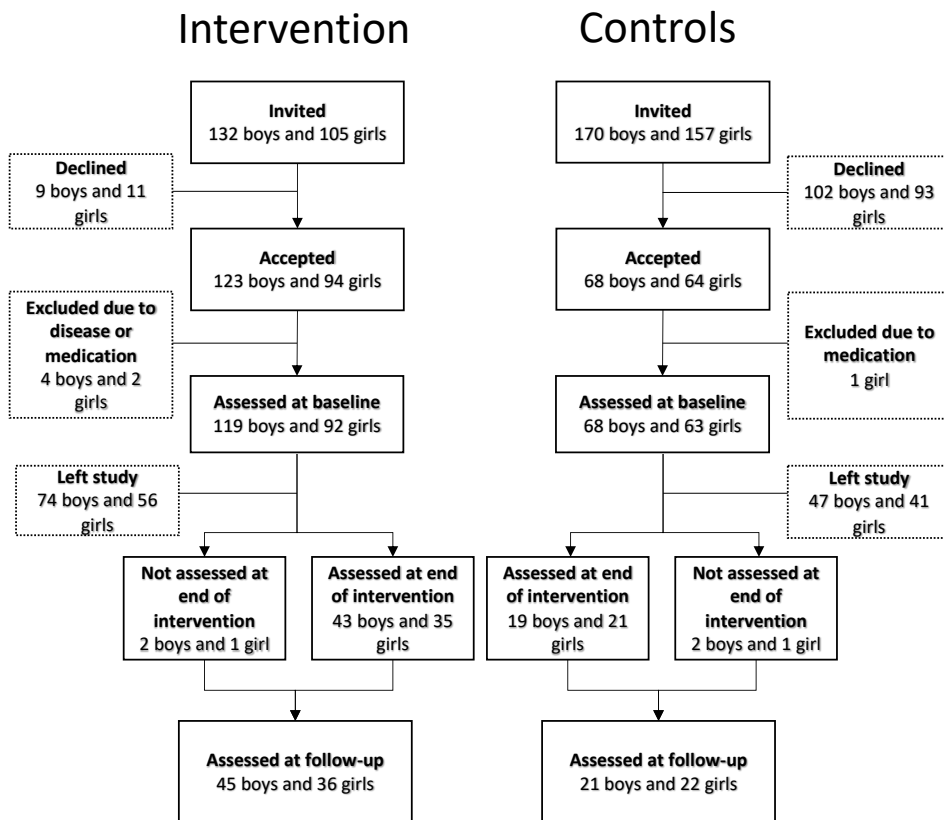
Figure 18: Participant flow chart in paper IV

Measurements

Subjects were assessed using the same methods at baseline and at follow-up in respective paper. In **paper III**, subjects were assessed before intervention start (baseline) with follow-up in the 9th grade, last year of compulsory school in Sweden. In **paper III**, assessments included a questionnaire, anthropometric data, pubertal status, bone densitometry by DXA and QUS, body composition by DXA, muscle strength by computerized dynamometer, and fracture incidence.

In **paper IV**, subjects were assessed at baseline with follow-ups (i) at the end of the intervention, and (ii) a mean 4 (range 3–5) years after the last measurement during the intervention. Measurements in **paper IV** include the questionnaire,

anthropometric data, pubertal status, bone densitometry by DXA, and muscle strength by computerized dynamometer.

Questionnaire

We assessed the children by a non-validated questionnaire, used in several earlier studies (168, 179, 180), that contained questions regarding lifestyle (dietary intake, smoking, and alcohol use), medical conditions (asthma, achondroplasia, diabetes, epilepsy, kidney disease, and thyroid disease) use of medication (levaxine, liothyronine, cortisone, insuline, antiepileptic drugs, anti-depressants, and oral contraception pills), along with specific questions regarding the amount of physical activity and sedentary activity. We asked for the specific amount of organized leisure-time physical activity, defined by us as activities in sport clubs or sports association. This question had two parts, one regarding summer and one regarding winter season as children may participate to a higher extent in activities in either of the seasons. The two seasons' values were then divided by two in order to get an annual mean. We calculated the total physical activity by adding the sum of organized leisure-time physical activity to school-based physical education, data provided by the principal of the specific school. We did not include questions regarding non-organized leisure-time activities, such as transportation or playing with friends. However, earlier studies have shown that the amount of organized physical activity correlates well with the total amount of physical activity (181). We have also performed post-hoc analyses using accelerometers on study subjects, finding associations between the amount of questionnaire based total physical activity and objective accelerometer-based levels. The questionnaires were sent to each of the subjects' home addresses and in younger years they were completed with the help of parents/guardians.

Anthropometric data

In order to assess body weight (kg) and height (cm), we used a digital scale (Avery Berkel HL120) and a Holtain Stadiometer respectively. We calculated body mass index (BMI) by dividing the weight in kg by height in meters squared.

Pubertal status

At baseline, a research nurse estimated the pubertal maturation using a Tanner scale (182, 183). At follow-ups, this was self-assessed by the subject, which has been found by others to correspond to the pubertal assessment by a doctor or a nurse (184).

Bone densitometry

We used dual-energy X-ray absorptiometry (DXA; DPX-L®, version 1.3z, Lunar Corporation, Madison, WI, USA) to evaluate different bone traits at both baseline and follow-ups. Assessed regions by DXA in **paper III** include total body less head,

left femoral neck (FN) and first to fourth lumbar vertebra (L1L4). Measured traits in these regions include bone mineral content (BMC; g), areal bone mineral density (aBMD; g/cm^2). In addition, bone area (BA; cm^2) was measured in FN and L1L4, and body composition (BC) as lean mass (kg). We also used quantitative ultrasound (QUS; Lunar Achilles model 1061®, Lunar Corporation, Madison, WI, USA) and assessed speed of sound (SOS; m/s).

In **paper IV**, we used the same DXA scanner as in **paper III** at baseline and follow-up at end of intervention. At the second follow-up (mean 4 years after end of intervention), we used iDXA® (version encore 13.60, Lunar Corporation, Madison, WI, USA) in 33 subjects from the intervention and 22 from control group, and DXA-Prodigy® (version encore 9.30, Lunar Corporation, Madison, WI, USA) in 48 subjects from intervention group and 21 from the control group. Regarding the bone traits by DXA in **paper IV**, we included BMC (g) and aBMD (g/cm^2) in total body less head, arms, legs, spine (total spine from total body scans), and FN.

Muscle strength

Muscle strength was measured using a Biodex System III Pro® (Biodex Medical systems Inc, Shirley, NY, USA) computerized dynamometer. Concentric isokinetic peak torque (PT; Nm) from right knee flexion ($_{flex}$) was assessed at 60 and 180 degrees/second (highest value from five attempts). In **paper IV** we calculated PT in relation to total body weight [PTTBW; ((PT/total body weight) * 100); Nm/kg].

Figure 19: A child participating in the POP study being assessed in a Biodex computerized dynamometer

Fracture composition score

In **paper III**, we aimed to present a musculoskeletal composite risk score for fracture, as the mean Z-score (the number of standard deviations differing from age- and gender-matched peers) of five factors that all have earlier have been associated to fracture risk, including L1L4 (BMC and BA), lean mass, SOS, and $PT_{flex180}$.

Statistical analyses

In **paper III**, we used IBM SPSS® (version 23, IBM Corp., Armonk, NY, USA) in all statistical analyses. Descriptive data are presented as absolute numbers (n) and proportions (%) or means with standard deviations (SD), and inferential statistics as mean differences with 95% confidence intervals (95% CI). Changes between baseline assessment and follow-ups were calculated by subtracting baseline values from follow-up values for each trait. Regarding the differences in changes in the respective groups, we used analysis of covariance (ANCOVA), adjusted for baseline trait value and age at follow-up. We calculated the fracture composite score correlation with the average amount of total physical activity (PA) during the study period through estimation with Spearman's test. The average PA was calculated as

the sum of school-based PA and leisure time PA, measured at baseline, halfway into the study, and at follow-up, divided by three.

In **paper IV**, we used Statistica® (version 12.0, Statsoft Inc®) in all statistical analyses. We present descriptive data as numbers (n), proportions (%), means with SD, along with inferential statistics as mean differences with 95% CI. We calculated study period changes by subtracting (i) intervention values and (ii) baseline values from follow-up values (mean 4 years after end of intervention). We calculated group differences in trait changes by using ANCOVA, adjusted for the length of the follow-up period and the proportion of boys and girls. In both **paper III** and **IV**, p-values of <0.05 were considered statistically significant.

Dropout analyses

In **paper III**, we compared all traits in subjects who were assessed at baseline and at follow-up with subjects having only baseline values (dropouts). Using this drop-out analysis we found no clinically or statistically significant differences (table 2).

Table 2: Dropout analysis regarding **paper III**. Data are presented as means ± standard deviations (SD). Participants refers to subjects assessed at baseline and follow-up, and dropouts refers to subjects with only baseline assessments. Bone mineral content (BMC; g), areal bone mineral density (aBMD; g/cm^2), and bone area (BA) were assessed using dual-energy X-ray absorptiometry (DXA). Calcaneal speed of sound (SOS; m/s) and peak torque (PT; Nm) were assessed using quantitative ultra-sound (QUS) and computerized dynamometer respectively.

	Participants (n=140)	Dropouts (n=108)	p-value
Age (years)	7.7 ± 0.6	7.8 ± 0.6	0.33
Height (cm)	129.3 ± 6.1	129.5 ± 6.5	0.79
Weight (kg)	28.4 ± 5.3	27.9 ± 5.9	0.51
BMI (kg/m^2)	16.9 ± 2.4	16.5 ± 2.4	0.23
Lean mass (kg)	21.3 ± 2.8	21.2 ± 3.0	0.62
Bone mineral content (BMC; g)			
Total body less head	993.4 ± 172.6	969.6 ± 182.8	0.31
Lumbar spine	20.0 ± 4.4	19.2 ± 4.1	0.19
Femoral neck	2.8 ± 0.5	2.8 ± 0.7	0.77
Areal bone mineral density (aBMD; g/cm^2)			
Total body less head	0.85 ± 0.05	0.84 ± 0.05	0.12
Lumbar spine	0.68 ± 0.10	0.66 ± 0.08	0.19
Femoral neck	0.77 ± 0.11	0.76 ± 0.11	0.89
Bone area (BA; cm^2)			
Lumbar spine	29.2 ± 3.5	28.9 ± 3.9	0.51
Femoral neck	3.6 ± 0.3	3.7 ± 0.6	0.61
Speed of sound (SOS; m/s), peak torque (PT; Nm)			
Calcaneal SOS	1530.2 ± 20.9	1528.7 ± 19.6	0.62
PTflex60°/s	23.6 ± 6.4	23.4 ± 6.5	0.85
PTflex180°/s	21.5 ± 6.0	21.0 ± 5.8	0.49

In **paper IV**, we conducted two dropout analyses. Firstly, we found no statistically significant differences when comparing baseline values including height, weight, and BMI from subjects that accepted participation with the declining individuals through the compulsory first grade school health examinations (179). Secondly, we compared height, weight, BMI, BMC, aBMD, and PT between subjects assessed at baseline and at the follow-up, mean 4 years after end of intervention. No statistically significant differences were found (table 3).

Table 3: Dropout analysis regarding **paper IV**. Data are based on baseline assessment values and are presented as means ± SD. Participants refers to subjects assessed at baseline and follow-up (mean 4 years after end of intervention), and dropouts refers to subjects that were assessed only at baseline. Bone mineral content (BMC; g), areal bone mineral density (aBMD; g/cm^2), and bone area (BA) were assessed using dual-energy X-ray absorptiometry (DXA). Peak torque (PT; Nm) was assessed using computerized dynamometer.

	Boys (n=187)			Girls (n=155)		
	Participants (n=66)	Dropouts (n=121)	p-value	Participants (n=58)	Dropouts (n=97)	p-value
Age (years)	7.7 ± 0.6	7.7 ± 0.6	0.55	7.6 ± 0.6	7.7 ± 0.6	0.74
Height (cm)	129.0 ± 7.2	128.7 ± 6.2	0.78	127.2 ± 6.9	128.5 ± 7.0	0.25
Weight (kg)	27.6 ± 5.1	27.8 ± 5.9	0.84	26.7 ± 5.5	27.6 ± 5.2	0.31
BMI (kg/m^2)	16.5 ± 2.2	16.6 ± 2.4	0.70	16.4 ± 2.3	16.7 ± 2.5	0.47
Bone mineral content (BMC; g)						
Total body less head	655.0 ± 154.8	654.2 ± 153.4	0.97	607.4 ± 139.9	626.3 ± 136.7	0.42
Arms	88.7 ± 20.5	87.2 ± 19.7	0.62	79.1 ± 17.7	81.3 ± 17.4	0.47
Legs	282.3 ± 72.7	284.1 ± 72.2	0.87	268.3 ± 64.5	280.4 ± 67.0	0.28
Spine	85.1 ± 20.3	84.9 ± 20.8	0.95	80.0 ± 18.7	81.0 ± 17.6	0.74
Femoral neck	2.8 ± 0.6	2.9 ± 0.7	0.38	2.6 ± 0.7	2.6 ± 0.5	0.76
Areal bone mineral density (aBMD; g/cm^2)						
Total body less head	0.69 ± 0.06	0.69 ± 0.06	0.71	0.68 ± 0.05	0.69 ± 0.05	0.82
Arms	0.62 ± 0.05	0.61 ± 0.04	0.47	0.60 ± 0.05	0.60 ± 0.04	0.55
Legs	0.76 ± 0.08	0.75 ± 0.07	0.69	0.75 ± 0.07	0.75 ± 0.07	0.68
Spine	0.69 ± 0.06	0.68 ± 0.06	0.50	0.69 ± 0.07	0.68 ± 0.06	0.78
Femoral neck	0.77 ± 0.12	0.78 ± 0.10	0.57	0.71 ± 0.11	0.72 ± 0.08	0.61
Peak torque (PT; Nm)						
PTflex60°/s	23.0 ± 6.6	23.2 ± 7.3	0.85	21.9 ± 5.6	21.8 ± 5.6	0.93
PTflex180°/s	21.0 ± 6.0	21.1 ± 6.2	0.85	19.6 ± 5.6	19.6 ± 5.1	0.96

Ethics

In **papers I–IV**, prior to study start, a regional ethical review board approved the MRPEAK study (LU 205-75) and the POP study (LU 453-98, LU 368-99). Furthermore, the POP study was registered as a clinical trial (ClinicalTrials.gov. NCT 00633828). Both studies were completely voluntary and every participating individual had the possibility to withdraw at any point in time. Data were presented as group means, were anonymized, and no individual data were presented. If any pathological results were found, the individual would be referred to a physician for further medical assessment and treatment. No assessments caused any pain or discomfort to any of the subjects. As stated earlier, the radiation dose of DXA (and pQCT in **paper I**) is very low. Before inclusion in respective study, individuals consented in written form. In the POP study, each subject's parent/guardian also received information regarding the study.

In the POP study (**papers III and IV**), children in the intervention school were obliged to attend the daily compulsory physical education (PE). However, they were not obliged to participate in the study or the study assessments. Some subjects may have found the increase in PE to cause discomfort, i.e., if the subject disliked physical activity (PA). However, at baseline, 98% of the children who replied answered "*yes*" to the question "*Do you enjoy physical education classes?*". It should also be stressed that PA improves several health aspects in children, and children are allowed to participate in PE activities at their own level.

Summary of papers

Paper I

Introduction: Finding individuals with low peak bone mass (PBM) may allow for early interventions aiming to reduce fracture risk. A relatively new technique, with less available data compared to DXA, for assessing bone mass and geometry is peripheral quantitative computed tomography (pQCT), which has the ability to discriminate between cortical and trabecular bone from three-dimensional images. This may be important since cortical and trabecular bone have independent effects on bone strength. In this study, we aimed (i) to identify possible ages of peak values with reference to PBM and bone strength, and (ii) to provide normative data from pQCT.

Materials and methods: In this cross-sectional study, we assessed 1083 randomly invited 18–28-year-old Swedish males, divided into 1-year age groups, using pQCT on radius and tibia. Regions of interest (ROI) included ultra-distal (4%), and diaphyseal (38% (tibia only) and 66%) sites. We assessed trabecular volumetric bone mineral density (vBMD; mg/cm^3) and total bone cross-sectional area (TB.CSA; mm^2) at the ultra-distal sites, and cortical volumetric bone mineral density (Ct.vBMD; mg/cm^3), cortical cross-sectional area (Ct.CSA; mm^2), cortical bone mineral content (Ct.BMC; mg/mm slice), cortical thickness (Ct.Th; mm), TB.CSA (mm^2), periosteal circumference (PC; mm), endosteal circumference (EC), polar cross-sectional moment of inertia (CSMI; mm^4) and polar strength strain index (SSI; mm^3) at the diaphyseal sites. Analysis of variance (ANOVA) was used to determine statistically significant differences between the age groups. Normative descriptive statistics were calculated and presented as means with standard deviations, whereas inferential statistics were calculated and presented graphically as means with 95% CI.

Results: We found, in all of the traits at diaphyseal sites (except CSMI in radius) statistically significant ($p<0.001$–0.036) differences between age groups. No apparent peak values were found. In tibia, most of the diaphyseal traits exhibited higher values with higher ages. At the 38% site in tibia, compared with the 18-year-olds, the 28-year-olds had higher values in Ct.vBMD (2.5%), Ct.BMC (14.1%), Ct.CSA (11.3%), and Ct.Th (9%). No differences were found in bone traits at ultra-distal sites.

Conclusion: Important factors independently beneficial to bone strength such as bone mass and density, together with structural benefits such as cortical thickness and bone size, displayed highest values in the 28-year-olds, and with trait-specific indications of incremental patterns. A larger bone would lead to increased bone strength but also lowered aBMD (by DXA), thus illustrating a discrepancy between the age of PBM (estimated by DXA) and peak bone strength (estimated by pQCT).

Paper II

Introduction: Areal bone mineral density (aBMD) by dual-energy X-ray absorptiometry (DXA) is the gold standard for diagnosing osteoporosis, and has been shown to predict fracture. Highest value of aBMD in life, often referred to as peak bone mass (PBM), in the femoral neck in the hip occurs at age 18-19 years in men and the subsequent attenuation in aBMD suggests a decrease in bone strength. This attenuation in aBMD could be a product of decreased bone mass, increased bone size, or both. However, an increase in bone size would also, independent of bone mass and density, contribute to an increase bone strength. In this study, we set out to evaluate possible changes in bone size following the age of PBM in the hip in men, and how they may influence bone strength. We also aimed to provide normative DXA data for young men.

Materials and methods: We assessed 1052 randomly selected 18–28-year-old men once using DXA on the left femoral neck (FN), trochanter area (Troch), and total hip (TH). At these sites, we estimated bone mineral content (BMC; g), bone area (cm^2), and aBMD (g; cm^2). After dividing the subjects into 1-year age groups, we used analysis of variance (ANOVA) to assess possible differences between age groups. We also performed Pearson's correlation analysis between each of the traits and age after the age of PBM (19 years, from literature). Normative descriptive data were presented as means with standard deviations, and inferential data were presented graphically with means and 95% CI.

Results: We found the highest mean of FN.aBMD in the 19-year-olds. When assessing the 19–28-year-olds, we found negative correlations between age and FN.BMC (r= –0.07; p=0.02), FN.aBMD (r= –0.12, p<0.001), Troch.aBMD (r= –0.095, p<0.001), and TH.aBMD (r= –0.087; p<0.01). There was a positive correlation between age and FN bone area (r=0.064, p=0.048), and TH bone area (r=0.094; p<0.01).

Conclusion: After PBM in the hip, an increase in size during the third decade in life may counteract the decrease in aBMD with regard to bone strength. Thus, the direct translation between PBM and peak bone strength is questionable.

Paper III

Introduction: Physical activity (PA) improves bone mass and muscle function, and also decreases fracture risk. In children, interventions with regular PA have also shown these beneficial effects. During puberty, however, the amount of PA often decreases, which in turn may lead to an attenuation of these effects. Therefore, we set out to investigate whether a PA intervention in a school setting would render beneficial changes in bones and muscles from before (Tanner 1) until after (Tanner 5) puberty.

Materials and methods: Sixty-three boys and 34 girls were exposed to a school-based PA intervention program with 40 minutes of daily physical education (PE). In the control group, twenty-six boys and 17 girls maintained a regular amount of PE (60 minutes per week). Subjects were assessed at baseline (Tanner stage 1, mean age 8 years) and at follow-up (Tanner stage 5, mean age 15 years). At both baseline and follow-up, using DXA, we assessed total body less head, lumbar spine (L1L4) and left femoral neck (FN) including bone mineral content (BMC; g), bone area (BA; cm^2, not in total body), and areal bone mineral density (aBMD; g/cm^2). We also used quantitative ultra-sound (QUS) measuring speed of sound (SOS; m/s)) on the calcaneal bone, and computerized dynamometer measuring concentric isokinetic peak torque from right knee flexion (PT; Nm). We also created a musculoskeletal composite score for fracture, a mean Z-score of the five assessed traits, in order to estimate the overall musculoskeletal effect of the intervention. Spearman's test was the used to determine changes in the composite score between baseline and follow-up.

Results: In boys, we found improvement of BMC (p=0.02), aBMD (p=0.03), and BA (p=0.03) in the lumbar spine, along with PT (p=0.008) in the intervention group compared to the controls. In girls, we found improvement of BMC (p=0.003) in the lumbar spine and calcaneal SOS (p=0.003). Composite score gains in both boys and girls were higher (both p=0.02) in the intervention group compared to the control group.

Conclusion: In boys and girls, from before (Tanner 1) to after (Tanner 5) puberty, a school-based intervention program with increased PA is associated with significant beneficial gains in bone mass, bone structure, and a composite score for fracture.

Paper IV

Introduction: Increased physical activity (PA) from a school-based intervention during 9 primary-school years in Sweden leads to beneficial changes in musculoskeletal traits and decreasing fracture incidence. We set out to investigate whether these changes remain after the termination of the program and into young adulthood.

Materials and methods: Forty-five boys and 36 girls increased their level of physical education (PE) during primary school through an intervention program with 40 minutes of PE per day. In the control group, 21 boys and 22 girls had 60 minutes of PE per week throughout the study period. Subjects were assessed at baseline (mean age 8 years), at the termination of the study (mean age 15 years), and at follow-up a mean 4 years (range 3-5) after the last measurement during the intervention (mean age 18 years). Each assessment comprised DXA measurements of total body less head, arms, legs, spine, and femoral neck including bone mineral content (BMC; g), areal bone mineral density (aBMD; g/cm^2), and peak torque from right knee flexion (PT; Nm) with both absolute amount and amount per body weight times 100 (PTTBW; Nm/kg*100). We calculated group comparisons (i) for changes between termination of the intervention and follow-up, and (ii) for changes between baseline and follow-up. When comparing the differences, we used analysis of co-variance (ANCOVA), adjusted for the proportion of boys and girls in the compared groups as well as duration of the follow-up period.

Results: Changes in both BMC and aBMD were similar in the two groups between end of intervention and follow-up, whereas muscle strength (PT) diminished (–5.6 Nm; p=0.02) more in the intervention group. From baseline to follow-up, the intervention group exhibited higher gains in spine BMC (32 g; p<0.001) and arms aBMD (0.06 g/cm^2; p<0.001). Muscle strength per total body weight (PTTBW) also increased more in the intervention group (12.1 Nm/kg*100; p=0.02).

Conclusion: Following the termination of a 9-year intervention program of daily PE, bone mass and density benefits seem not to diminish, whereas those acquired regarding muscle strength do. Over the entire study period, however, gains in bone mass and muscle strength were superior in intervention subjects. This indicates that daily PE in primary school may lead to improved peak bone mass and peak bone muscle strength, thus possibly counteracting the age-related loss in bone and muscle strength.

General discussion

Peak bone mass and bone strength

In **papers I** and **II** we present normative data including bone mineralization and structure in young adult men, thus helping both researchers and clinicians, by adding updated reference values based on a large cohort, to be used for comparison with other cohorts in future studies or in clinical settings when evaluating bone mineralization in patients. To this date and to our knowledge, the majority of papers with pQCT reference material include children and adolescents in their second decade in life (185-188), and the few studies in the young adult age-span in men have included small sample sizes (189). Our contribution in **papers I** and **II** may thus possibly aid in skeletal research in a wider sense than by just answering our research-specific questions in the two papers. We were also able to present data describing bone mineralization, and structural traits in bones exposed to different physiological loads, namely upper and lower extremities of the body. By analyzing data from two different bone-densitometric imaging techniques, pQCT and DXA, we were able to illustrate the discrepancies in how data may be interpreted when using different techniques and measured regions of interest. The two techniques may thus lead to some discrepancies in the inferences, when approximating the ability of bone to withstand mechanical forces acting upon them, also known as bone strength, when estimating bone strength through surrogate measures of bone mineralization and bone geometry. It should then also be noted that bone strength is also determined by qualitative properties of the skeleton, including bone turnover, micro-architecture, regional distribution of mineralization, collagen quality, along with mineral and matrix composition (190). Thus, we acknowledge that our methods and findings solely illustrate a quantitative aspect that is measurable, and that it would have been preferable if qualitative aspects had also been incorporated when investigating the complex entity of bone strength.

In **paper I** we found, in concordance with others (186, 191), that trabecular bone in ultra-distal radius and tibia has already reached maximal levels before the age of 18. Regarding cortical bone in diaphyseal radius and tibia, however, we found indications of higher values in the older age groups, and in our cohort we actually found the highest values in the oldest subjects (ages 27 and 28 years) for most of the traits, including bone mass, bone density, periosteal area, and cortical thickness. Notably, all of these traits are proven to be positively correlated to bone strength.

Given these indications of the positive age-related effects in the lower extremity (tibia), we speculated as to whether other parts of the lower extremities follow similar patterns.

Peak bone mass (PBM) from DXA-estimated aBMD at the femoral neck is attained by 19 years of age in men according to a longitudinal study including 527 men aged 16-40 years (41). The subsequent decrease in aBMD after PBM could potentially be the effect of loss of bone mass, or due to an increase in bone size, since aBMD, estimated by DXA, is calculated as BMC divided by bone area (a two-dimensional estimate). In **paper 2**, in accordance with aforementioned literature, we found indications suggesting, following PBM at the femoral neck in men, an attenuation in bone density and bone mass. Simultaneously, however, our results indicated an increase in bone size with higher ages. Given that the size of a tubular structure, independent of other factors, contributes to the resistance against bending, a possible increase in size with age could counteract the diminishing densitometric variables with regard to bone strength (90-92). We consider it unlikely that bone strength would decrease already during the third decade in life, from an evolutionary perspective. Reports indicate that the total fracture incidence in Swedish males decreases after 20 years of age, and stabilizes around the age of 30 years until the age of about 70 years, when it quite rapidly increases (192). More specifically, proximal femur fracture incidence in men is kept at low rates until the age of 50–60 years (192). We argue that, from the time of PBM by DXA in the femoral neck, age-related bone mass and density attenuation do not necessarily reflect a decrease in bone strength and should therefore be interpreted carefully.

Physical activity intervention and musculoskeletal development

During the peripubertal period the human skeleton undergoes remarkable changes. This is illustrated by the fact that during four years surrounding the highest rate of bone mineral accrual around the early teens, almost 40% of maximum adult bone mass is attained (27). In view of this, interventions with the aim of promoting bone health should focus on this period. The positive effects of regular physical activity on bone health in children, adolescents, and adults are well known facts in the research community. With this in mind, studies have shown that physical activity levels generally decrease during puberty (193, 194), with the addition of an unfortunate trend, in recent years, of diminishing levels due to secular changes (195). On a further note, physical activity interventions seem to have greater effects on bone mineralization if the intervention started before or in early puberty (167).

The vast majority of studies evaluating effects of regular physical activity on bone only include effects in prepubertal children, and have found that the effects, if any,

seem to be smaller during the pubertal years (196). It remains unclear whether the preponderance of studies including pre-pubertal subjects reflects that fewer studies including more mature adolescents were conducted or not reported (due to publication bias). Thus, there are different views of whether physical activity interventions have an effect during the peripubertal years.

School-based physical activity interventions in children and adolescents have shown to be effective in several studies (197-200). However, these studies focused on interventions including specific high-impact activities, and they were relatively short in duration (less than a year), and included pre- or early-pubertal children. Thus, we identified a need for studies encompassing the entire period of pubertal maturation.

Earlier findings from the POP study showed that the initial statistically significant positive effects of increased physical activity on bone diminished, mainly in boys, from ages 7 to 15 years (201) It should be noted that, at follow-up, boys of different pubertal stages were included, which may have confounded the possible effects, or lack thereof. This was the reason why we in **paper III** set out to conduct a study evaluating the effect of increased physical activity on children from before (Tanner 1) to after (Tanner 5) puberty. By doing so, we found higher musculoskeletal gains in children of both sexes in the intervention group compared to the controls. This underlines the importance of following individuals until the end of puberty, instead of only during early stages of maturation, when aiming to evaluate the effect of a physical activity intervention on the skeleton.

The musculoskeletal benefits of a daily physical activity intervention we found in **paper III**, including gains in bone mineralization and bone size, and also muscle strength, are all factors contributing to reduced fracture risk (82, 114, 202, 203). About 20% of the variance in peak bone mass is modifiable through external factors, among which physical activity is considered perhaps the most important (40). Thus, interventions that increase the level of physical activity may prove an effective way of reaching a higher peak bone mass, consequently counteracting the age-related attenuation in bone mass, and ultimately possibly postponing osteoporosis (46). This notion is further supported in **paper IV**.

A physical activity intervention during childhood and adolescence is associated with positive musculoskeletal effects and indicates a reduced risk of sustaining a fracture during the intervention (201). However, some studies have suggested that age-related bone loss is higher in subjects that have reduced the amount of PA from sports (204-206). Thus, we wanted to evaluate whether the beneficial musculoskeletal effects that we found in **paper III** remain after the termination of the daily physical activity intervention program. In **paper IV**, from the mean age of 7 to 18 years, we found that the intervention subjects exhibited higher gains in bone mass in the spine, aBMD in the arms, and muscle strength in the lower extremity, even after several years. It should also be noted that most of the other traits also had

higher mean values, albeit not statistically significant. We argue that this possibly could be due to lack of statistical power (see *Strengths and limitations*), illustrated by the greater confidence intervals in the control group, which increases the risk of ruling out possible true effects of the intervention (type II error). However, even with a lack of statistical power, we found statistically significant differences between the groups, corroborating that that a school-based daily physical activity intervention program has positive effects on bone and muscular health that stretches beyond the intervention period itself. When comparing end of intervention to end of study, mean 4 years later, we only found statistically significant lower gains in muscle strength in the intervention group. In all other assessed traits, we found similar development between former intervention and control subjects. We stress that these findings could be due to lack of statistical power but argue that this indicates persisting positive effects after the termination of the intervention.

The indication of these persisting positive effects could be the result of different factors and the causality between the intervention and these effects remains to be determined. One explanation could be that subjects in the intervention group have adapted a more physically active lifestyle (207). This is further supported by the fact that, in **paper IV**, mean 4 years after ending the intervention, the intervention group boys had a higher total amount of organized PA compared to controls.

Strengths and limitations

Papers I and II

Strengths

We consider the large number of participants per 1-year age group, and the use of few and experienced research technicians, as strengths of these papers.

Limitations

A major limitation is the use of cross-sectional data, which in contrast to longitudinal data make interpretations regarding true changes over time impossible. However, we believe that possible differences between age groups in our papers reflect at least indications of age-related changes in each trait. The fact that data were collected over 6 years is also a study limitation. However, secular changes during these years (2006–2012) should be minimal, supported by the National Swedish Public Health institute, which found no changes in sedentary time and prevalence of obesity in Swedish men between the years 2004 and 2011. It should also be stressed that we discuss the potential implications of changes in mineralization and size on bone strength from a hypothetical standpoint, since no mechanical tests were used.

Of the individuals who were asked to participate, only 30% accepted; we regard this as a study limitation since it potentially can lead to a selection bias. However, when comparing the anthropometrics of the participating subjects with men aged 20–29 years in the general population in Sweden 2008–2011 (from Statistics Sweden, not presented in papers), we found similar weight, height, and BMI. We thus argue that our sample reflects the general population.

As we did not find indications of peak bone mass in radius or tibia, it would been valuable to include both younger and older subjects. Another limitation is the inclusion of only male subjects, and it would have been advantageous to investigate the possible age-related changes in female bone around the time of peak bone mass, since male and female skeletal development exhibit different patterns i.e., during the peripubertal years.

Papers III and IV

Strengths

The population-based participant inclusion, the prospective and controlled study design along with long follow-up duration are strengths in these papers. The fact that PE is compulsory subject lessens the risk of selection bias, as participants in the intervention do not only consist of volunteers, as in many previous studies, even if participation in the annual measurements in the study was on a voluntary basis.

Limitations

A vital part of an interventional study, in order to decrease the amount of bias through confounding factors, is randomization of subject to either intervention or control groups. Even though this is ideal, for practical reasons, parents and teachers opposed the idea of randomization. Instead, children were assigned to the intervention or control group depending on which school they went to. With this in mind, causality between intervention and effects on physical activity levels and musculoskeletal traits should be avoided due to lack of control of other factors that may influence the assessed parameters. Instead, we use the term association when drawing conclusions from these studies.

Assessing the amount of organized leisure-time physical activity, and thus total amount of physical activity, using a non-validated questionnaire is a possible limitation due to recall bias. However as previously described (see *Materials and methods*), we performed a post-hoc analysis showing correlations between questionnaire-estimated and accelerometer-based amount of physical activity. Another limitation is that we did not include non-organized leisure-time physical activities such as transportation and playing activities. However, as also described earlier (see *Materials and methods*), the literature suggests an association between amount of organized leisure-time physical activity and total amount of physical activity (181).

A possible limitation in **papers III and IV** is selection bias. In the recruitment process for the study, there was a higher ratio of children who declined participation in the control group (60%) than in the intervention group (8%). However, to control for possible selection bias, we conducted dropout analyses where we compared the individuals accepting and declining baseline assessment in the compulsory 1st grade school health examination, finding no statistically (or clinically) significant differences. In **paper III**, there was a higher dropout rate between baseline and follow-up in the control group than in the intervention group, 42% and 25% respectively. In **paper IV** the dropout ratio between baseline and follow-up (mean 11 years later) was 67% in the control group and 62% in the intervention group. Any dropout could potentially lead to a selection bias. To control for this, in both **paper III and IV**, we conducted dropout analyses showing, at baseline, no

statistically significant differences between the later dropouts and individuals who also participated in the follow-ups (table 2 and 3). Furthermore, we compared anthropometrics and DXA traits for male subjects at the femoral neck at the last follow-up in **paper IV** with the youngest age group in the MRPEAK study (**papers I and II**). From this, we found no statistically significant differences (table 4). Knowing that we had a small sample, mainly in the control group (n=21), we think that this argues against a possible selection bias.

Table 4: Inferential anthropometric and DXA data on 18-year-old male subjects from the two cohorts in the MRPEAK study (paper I and II) and in the POP study (papers III and IV), presented as absolute mean values with 95% confidence intervals bracketed.

	MRPEAK (n=90)	POP$_{intervention}$ (n=45)	POP$_{control}$ (n=21)
Height (cm)	181.9 (180.4-183.4)	180.4 (178.3-182.5)	180.2 (176.9-183.5)
Weight (kg)	75.1 (72.4-77.8)	77.0 (72.3-81.7)	74.3 (69.3-79.3)
BMI (kg/m^2)	22.7 (22.0-23.4)	23.7 (22.3-25.1)	22.9 (21.4-24.4)
FN.BMC (g)	6.4 (6.17-6.63)	6.4 (6.05-6.75)	6.2 (5.86-6.54)
FN.aBMD (g/cm^2)	1.15 (1.12-1.18)	1.16 (1.11-1.21)	1.15 (1.09-1.21)

We defined sedentary activities as "screen-time activities", without including more modern devices such as laptops, smartphones, or tablets. Despite being a limitation, it should be noted that these contraptions were not at all as commonly used around the turn of the millennial shift as they are to today. Another limitation is that we did not assess the level of intensity during organized leisure-time physical activity or during physical education. Today, due to technical advances, we would be able to assess both the level of intensity as well as the amount of sedentary time more accurately given that accelerometers now work over long periods of time. When the study started, accelerometers could only record data from a few days, making it impossible to extrapolate the amount of physical activity and sedentary time to a whole year.

In **paper III**, we aimed to assess pubertal stage by Tanner evaluation which depends on evaluation of genitalia (boys), breasts (girls), and pubic hair growth (boys and girls). At baseline, the assessment was conducted by a research nurse. At later follow-ups, the subjects themselves assessed pubertal staging, with the help of standardized pictures of the different stages, but without any further guidelines on how to differentiate between pubertal stages, which is considered a study limitation. We pre-defined that subjects had reached the end of puberty in Tanner 5, which is a limitation, as some females only reach Tanner 4, even though no longer in puberty. In our opinion, the limitations of pubertal staging in this study should affect assessments similarly in both the intervention and the control group.

Since we presented a composite score with the aim of evaluating fracture risk in **paper III**, it would be of value to compare the score with actual fracture risk. However, this could not be assessed due to lack of statistical power. In a subsequent

paper, authors showed that the composite score was able to predict fractures as accurately as femoral neck aBMD by DXA in old men (208).

We used knee flexion as the trait reflecting muscular gains. This is a limitation as we only evaluate the lower extremity, and thus are not able to assess possible effects of the intervention program on other muscles, i.e., upper extremities.

The children in this study were of Caucasian ethnicity and were living in a socioeconomical middle-class area, hence limiting the ability to translate the effects found to the general population. Thus, it would have been ideal to include children of other ethnicities and from other socioeconomic areas.

Due to dropouts between baseline and follow-up in **paper IV**, mainly in the control group, the low number of participants is a limitation. This may lead to decreased power, which in turn increases the risk of type II errors, where we erroneously accept the null hypothesis. In an ideal situation, we of course would like to have fewer dropouts and/or include more subjects. This underlines one of the difficulties in assessing a long-term intervention study such as this. However, we stress that to date there are no other similar intervention studies of this duration published.

Conclusions

From this thesis, we conclude that:

- In young adult males aged 18–28:
    - pQCT-estimated radial and tibial cortical thickness, bone size, and cortical volumetric bone mineral density exhibit higher values with higher ages, suggesting an increase in bone strength during this period.
    - caution is advised when assessing bone strength and fracture risk using DXA in femoral neck, as the lower values in bone minerals with higher ages are counteracted by greater bone size, thus counteracting a decline in bone strength during this period.
- A daily 40-minute physical activity intervention program during the nine primary-school years, compared to having physical activity 1–2 times per week, is associated with:
    - benefits in musculoskeletal gains in both sexes when the participants are followed over puberty, during ongoing intervention.
    - attenuation of muscle strength after the intervention is terminated.
    - benefits in musculoskeletal gains, from baseline to several years after the intervention terminated.

Future perspectives

In future research, when aiming to improve evaluation of the timing and importance of PBM, there is a need for longitudinal data, use of different densitometric measuring techniques, sex-specific evaluations and evaluations of PBM in different anatomical regions. Furthermore, to better understand the age-related densitometric and structural changes during young adulthood and the possible impact the different traits may have on bone strength, mechanical testing would be preferable, albeit practically challenging. With technical advances, it would also be ideal to quantify and incorporate factors that today are considered of qualitative nature, thus adding more pieces to the complex bone strength puzzle.

In order to evaluate the residual effects after termination of increased PA during growth, study participants ought to be assessed repeatedly throughout life, preferably until ages when osteoporosis develops and fragility fractures usually occur in the population. Since fracture prevention is one of the major goals of our PA intervention, future studies should not only include surrogate measures of bone strength, but also fractures. However, from a shorter perspective, since fracture risk is low in young adulthood, it would to start with be interesting to continue to follow the study participants, and evaluate whether the beneficial musculoskeletal effects associated with the intervention are retained through the third decade in life.

In our studies, we have shown that a school-based intervention is associated with a positive effect on musculoskeletal development. We still need to decipher in what way an intervention leads to these positive effects, to be better able to tailor future interventions. Future studies aiming to assess the effect of a school-based physical activity intervention should now include schools from different socioeconomic areas, use validated and objective methods when assessing the total amount of physical activity and new measuring techniques such as high-resolution pQCT.

Finally, given the worrying secular trend of increasing sedentation along with attenuation of physical activity in children and adolescents of today and the beneficial effects found to be associated with increased PA at growth, there is an urgent need to transfer our research results to society and the general population, so that children, parents, and governments react and initiate changes with the aim of increasing the amount of physical activity, both in school through physical education and during spare time.

Summary in Swedish – Populärvetenskaplig sammanfattning

Allmän introduktion

Bentäthet och benmassa mäts vanligen med dual-energy X-ray absorptiometry (DXA), vilket är en tvådimensionell röntgenmetod och denna anses vara förstavalet vid diagnostik av benskörhet, eller osteoporos. I studier har det visats att den av DXA uppskattade bentätheten har en god förmåga hos äldre människor att förutse risken att drabbas av framtida frakturer. Denna metod har dock vissa inbyggda svagheter. Då tekniken återskapar en tvådimensionell bild av ett tredimensionellt objekt, dvs. ben, är den uppskattade densiteten area- och inte volymbaserad. Detta leder bl.a. till att den areabaserade bentätheten blir högre i större rörben jämfört med mindre, även om den verkliga bentätheten är samma. Vidare räknas bentätheten ut genom benmassan dividerat med benarean, vilket leder till att en minskad bentäthet potentiellt kan bero på antingen förlorad benmassa och/eller ökad benstorlek. Detta kan vara missvisande i relation till benets motståndskraft mot fraktur eftersom en ökad storlek på benet ger ökad hållbarhet. En nyare teknik som kan utvärdera bentäthet och benstorlek är peripheral computed tomography (pQCT), vilket är en tredimensionell metod som kan mäta perifera delar av kroppen, exempelvis underarm och underben. Dessvärre är mängden normalmaterial på unga personer relativt sällsynt med pQCT.

Människan genomgår stora kroppsliga förändringar under barn- och ungdomsåren. Inom loppet av denna tid växer skelettet med såväl ökad massa som storlek. Under puberteten når individer en punkt när skelettet växer som snabbast, vilket leder till att större delen av benmassan vid vuxen ålder är nådd vid pubertetens slut. I de sena tonåren nås vad som kallas "peak bone mass" (PBM), den högsta totala mängden benmassa en individ uppnår under sin livstid. Efter denna punkt hålls mängden ben relativt konstant under ett par decennier, varpå mängden benmineral sjunker kontinuerligt med tiden. Hos kvinnor sjunker benmassan i ökad hastighet efter klimakteriet, vilket i ökad utsträckning kan leda till osteoporos. Genom att öka mängden benmassa vid tiden för PBM skulle man kunna motverka den efterföljande förlusten av benmassa och därmed skjuta upp, eller rentav undgå att nå gränsen för osteoporos och därvid minska risken att drabbas av fragilitetsfrakturer.

I lårbenshalsen nås PBM, mätt med DXA, i sena tonåren. Därefter börjar bentätheten relativt omgående att minska, vilket borde innebära minskad hållfasthet i benet. Det kan tyckas märkligt ur ett evolutionärt perspektiv, att benet blir skörare, redan vid så ung ålder.

En faktor som visats ha mycket goda effekter på bentäthet, benmassa, muskelstyrka och även frakturrisk genom hela livet är fysisk aktivitet. Genom att öka mängden skolgymnastik under grundskoleperioden skulle man potentiellt kunna öka skelettets hållfasthet som i sin tur skulle kunna leda till ökad bentäthet vid tiden för PBM, och därmed ha en frakturförebyggande effekt på såväl kort som lång tid. Detta stödjs av litteraturen som visat det finns ett samband mellan mängden benmassa man har som ung och mängden benmassa man har som vuxen. Tidigare studier har visat att det är svårt att positivt påverka ben- och muskler genom ökad fysisk aktivitet under puberteten, framförallt hos flickor.

Övergripande syften med avhandlingen

Denna avhandling syftar till att: (i) Studera skelettutvecklingen hos unga män genom att presentera normativa data baserade på pQCT av underarmen/underbenet och på DXA av lårbenshalsen samt att identifiera eventuella åldersrelaterade skillnader i benmineralisering och benstruktur hos 18-28 år gamla män samt (ii) uppskatta vilken effekt dessa eventuella skillnader har på benets hållfasthet. (iii) Huruvida en intervention med daglig skolgymnastik har positiva muskuloskeletala effekter som sträcker sig över puberteten (under pågående intervention) samt (iv) ifall dessa effekter kvarstår efter avslutad intervention.

MR PEAK

Under åren 2006-2012 genomfördes en tvärsnittsstudie vid namn MR PEAK där totalt 1083 från folkbokföringsregistret slumpmässigt utvalda män, 18-28 år gamla och boende i Malmö, mättes med pQCT av underarmen och underbenet samt med DXA av lårbenshalsen.

Bunkefloprojektet

Fyra skolor i Malmö ingick år 1999 i det s.k. "Bunkefloprojektet" (eng. The Pediatric Osteoporosis Prevention (POP) study). På en av skolorna implementerades en intervention som innefattade 40 minuter daglig skolgymnastik (200 minuter per vecka) och de tre övriga kontrollskolorna fortsatte med 60 minuter skolgymnastik per vecka under hela den nioåriga grundskoletiden. Av de totalt 564 inbjudna barnen som börjat skolan åren 1998-2000, valde 349 att delta i studien. Barnen utvärderades upprepade gånger under studieperioden med sista mätningen under interventionen kring nionde klass samt i medeltal 4 år senare, efter avslutad intervention. Utvärderingarna innefattade självskattningsformulär samt mätningar av skelettmassa, skelettstruktur, muskelmassa och muskelstyrka via vårt forskningslaboratorium.

74

Resultat

Från pQCT-mätningarna fann vi i rörbenens skaft i underarmen och underbenet högre värden för benmassa, bentäthet och benstorlek hos de äldsta undersökta individerna. Från DXA-mätningarna fann vi att lårbenshalsens areabaserade bentäthet och benmassa korrelerade negativt medan storleken korrelerade positivt med högre ålder. Från före till efter puberteten fann vi positiva effekter på skelett (både pojkar och flickor) och muskler (enbart pojkar) hos barn som haft daglig skolgymnastik jämfört med barnen i kontrollskolorna. Efter avslutad intervention minskade muskelstyrkan i benen i högre utsträckning hos barnen i interventionsgruppen jämfört med kontrollgruppen. Dock, sett över den totala perioden, från första klass i grundskolan till efter avslutad intervention fann vi positiva effekter på såväl skelett som muskelstyrka.

Slutsatser

Det verkar som att PBM uppnås efter 28 års ålder i underarm och underben. Efter PBM i lårbenshalsen rekommenderas försiktighet när man vill dra slutsatser om huruvida minskad bentäthet uppskattat via DXA innebär att skelettet försvagas eftersom benets storlek ser ut att öka, vilket i själva verket bidrar till en ökad hållfasthet. Daglig 40 minuters skolgymnastik är associerat med en positiv muskuloskeletal utveckling såväl under pubertet som flertalet år efter avslutad intervention, jämfört med skolgymnastik 60 minuter per vecka.

Acknowledgements

I would like to extend my gratitude towards all of the people that have helped and supported me along the way in completing this thesis. I also want to thank the participants in MR PEAK and POP study who made this possible. Some people deserve a special mention and I sincerely thank you:

My supervisor, professor **Magnus Karlson** for never giving up on me, for pushing me towards the goal, and for always being available whenever I needed your guidance. You will always have my utter respect, both as a professional and as a person.

My co-supervisor, professor **Björn Rosengren**, for the delicate questions and your wise and thoughtful input along the way.

Lars Jehpsson and **Jan Åke Nilsson** for your advice in the intriguing world of statistics.

Åsa Almgren, **Susanne Jönsson** and **Marika Bergman** for all the help with my research project.

My high-school teacher **Agneta Öhrngren** for an inspiring introduction to biology and chemistry.

My sisters **Anna** and **Karin** for your support through good and bad times.

My parents **Kristina** and **Gunnar** for always believing in me and pushing me to do my very best. You have taught me what's important in life.

Finally, my wife **Johanna**, thank you for your love, affection, and encouragement throughout life. I love you deeply and stand by your side - then, now, and always.

References

1. Bullough PG. Orthopaedic pathology. Maryland Heights, Mo.: Mosby/Elsevier; 2010.

2. Knothe Tate ML, Adamson JR, Tami AE, Bauer TW. The osteocyte. Int J Biochem Cell Biol. 2004;36(1):1-8.

3. Kular J, Tickner J, Chim SM, Xu J. An overview of the regulation of bone remodelling at the cellular level. Clin Biochem. 2012;45(12):863-73.

4. Fuchs RK, Warden SJ, Turner CH. Bone anatomy, physiology and adaptation to mechanical loading. 2009:25-68.

5. U.S. National Cancer Institute's Surveillance EaERSP. [Available from: https://training.seer.cancer.gov/anatomy/skeletal/tissue.html.

6. Morgan EF, Unnikrisnan GU, Hussein AI. Bone Mechanical Properties in Healthy and Diseased States. Annu Rev Biomed Eng. 2018;20:119-43.

7. Langdahl B, Ferrari S, Dempster DW. Bone modeling and remodeling: potential as therapeutic targets for the treatment of osteoporosis. Ther Adv Musculoskelet Dis. 2016;8(6):225-35.

8. Martin TJ, Rodan GA. Intercellular Communication during Bone Remodeling. 2008:547-60.

9. Orwoll ES, Bilezikian JP, Vanderschueren D. Osteoporosis in men the effects of gender on skeletal health. 2010.

10. Allen MR, Burr DB. Chapter 4 - Bone Modeling and Remodeling. In: Burr DB, Allen MR, editors. Basic and Applied Bone Biology. San Diego: Academic Press; 2014. p. 75-90.

11. McNamara L. 2.210 - Bone as a Material. In: Ducheyne P, editor. Comprehensive Biomaterials. Oxford: Elsevier; 2011. p. 169-86.

12. Maes C, Kronenberg HM. Chapter 60 - Bone Development and Remodeling. In: Jameson JL, De Groot LJ, de Kretser DM, Giudice LC, Grossman AB, Melmed S, et al., editors. Endocrinology: Adult and Pediatric (Seventh Edition). Philadelphia: W.B. Saunders; 2016. p. 1038-62.e8.

13. Crowder C, Austin D. Age ranges of epiphyseal fusion in the distal tibia and fibula of contemporary males and females. J Forensic Sci. 2005;50(5):1001-7.

14. Baim S, Wilson CR, Lewiecki EM, Luckey MM, Downs RW, Jr., Lentle BC. Precision assessment and radiation safety for dual-energy X-ray absorptiometry: position paper of the International Society for Clinical Densitometry. J Clin Densitom. 2005;8(4):371-8.

15. Cullum ID, Ell PJ, Ryder JP. X-ray dual-photon absorptiometry: a new method for the measurement of bone density. Br J Radiol. 1989;62(739):587-92.

16. Lorente Ramos RM, Azpeitia Arman J, Arevalo Galeano N, Munoz Hernandez A, Garcia Gomez JM, Gredilla Molinero J. Dual energy X-ray absorptimetry: fundamentals, methodology, and clinical applications. Radiologia. 2012;54(5):410-23.

17. Hagenfeldt K, Johansson C, Johnell O, Ljunggren Ö, Mørland B. Osteoporos – prevention, diagnostik och behandling. En systematisk litteraturöversikt. Statens beredning för medicinsk utvärdering (SBU). 2003.

18. Economos C, Nelson M, Fiatarone MA, Dallal G, Heymsfield S, Wang J, et al. A multi-center comparison of dual energy X-ray absorptiometers: In vivo and in vitro soft tissue measurement. European journal of clinical nutrition. 1997;51:312-7.

19. Engelke K, Adams JE, Armbrecht G, Augat P, Bogado CE, Bouxsein ML, et al. Clinical use of quantitative computed tomography and peripheral quantitative computed tomography in the management of osteoporosis in adults: the 2007 ISCD Official Positions. J Clin Densitom. 2008;11(1):123-62.

20. Lala D, Cheung AM, Gordon C, Giangregorio L. Comparison of Cortical Bone Measurements Between pQCT and HR-pQCT. Journal of Clinical Densitometry. 2012;15(3):275-81.

21. Boutroy S, Bouxsein ML, Munoz F, Delmas PD. In vivo assessment of trabecular bone microarchitecture by high-resolution peripheral quantitative computed tomography. J Clin Endocrinol Metab. 2005;90(12):6508-15.

22. Augat P, Gordon CL, Lang TF, Iida H, Genant HK. Accuracy of cortical and trabecular bone measurements with peripheral quantitative computed tomography (pQCT). Physics in Medicine and Biology. 1998;43(10):2873-83.

23. Cameron JR, Sorenson J. MEASUREMENT OF BONE MINERAL IN VIVO: AN IMPROVED METHOD. Science. 1963;142(3589):230-2.

24. Allen MR, Krohn K. Chapter 5 - Skeletal Imaging. In: Burr DB, Allen MR, editors. Basic and Applied Bone Biology. San Diego: Academic Press; 2014. p. 93-113.

25. Chang G, Boone S, Martel D, Rajapakse CS, Hallyburton RS, Valko M, et al. MRI assessment of bone structure and microarchitecture. J Magn Reson Imaging. 2017;46(2):323-37.

26. Schwartz AV. Marrow fat and bone: review of clinical findings. Front Endocrinol (Lausanne). 2015;6:40.

27. Baxter-Jones AD, Faulkner RA, Forwood MR, Mirwald RL, Bailey DA. Bone mineral accrual from 8 to 30 years of age: an estimation of peak bone mass. J Bone Miner Res. 2011;26(8):1729-39.

28. Seeman E. Clinical review 137: Sexual dimorphism in skeletal size, density, and strength. J Clin Endocrinol Metab. 2001;86(10):4576-84.

29. Turner RT, Wakley GK, Hannon KS. Differential effects of androgens on cortical bone histomorphometry in gonadectomized male and female rats. Journal of Orthopaedic Research. 1990;8(4):612-7.

30. Callewaert F, Venken K, Kopchick JJ, Torcasio A, van Lenthe GH, Boonen S, et al. Sexual dimorphism in cortical bone size and strength but not density is determined by independent and time-specific actions of sex steroids and IGF-1: Evidence from pubertal mouse models. Journal of Bone and Mineral Research. 2010;25(3):617-26.

31. Filip C, Mieke S, Evelien G, Steven B, Dirk V. Skeletal sexual dimorphism: relative contribution of sex steroids, GH–IGF1, and mechanical loading. Journal of Endocrinology. 2010;207(2):127-34.

32. Vanderschueren D, Vandenput L, Boonen S, Lindberg MK, Bouillon R, Ohlsson C. Androgens and bone. Endocr Rev. 2004;25(3):389-425.

33. Vanderschueren D, Vandenput L, Boonen S. Reversing sex steroid deficiency and optimizing skeletal development in the adolescent with gonadal failure. Endocr Dev. 2005;8:150-65.

34. Seeman E. Estrogen, androgen, and the pathogenesis of bone fragility in women and men. Curr Osteoporos Rep. 2004;2(3):90-6.

35. Ortner DJ. The earlier gain and the later loss of cortical bone. By Stanley M. Garn. 146 pp. and 34 illustrations. Charles C Thomas, Springfield. 1970. $12.00. American Journal of Physical Anthropology. 1972;36(2):304-5.

36. Seeman E. Periosteal bone formation--a neglected determinant of bone strength. N Engl J Med. 2003;349(4):320-3.

37. Bachrach BE, Smith EP. The Role of Sex Steroids in Bone Growth and Development: Evolving New Concepts. The Endocrinologist. 1996;6(5):362-8.

38. Nieves JW, Formica C, Ruffing J, Zion M, Garrett P, Lindsay R, et al. Males Have Larger Skeletal Size and Bone Mass Than Females, Despite Comparable Body Size. Journal of Bone and Mineral Research. 2005;20(3):529-35.

39. Lu PW, Cowell CT, SA LL-J, Briody JN, Howman-Giles R. Volumetric bone mineral density in normal subjects, aged 5-27 years. J Clin Endocrinol Metab. 1996;81(4):1586-90.

40. Weaver CM, Gordon CM, Janz KF, Kalkwarf HJ, Lappe JM, Lewis R, et al. The National Osteoporosis Foundation's position statement on peak bone mass development and lifestyle factors: a systematic review and implementation recommendations. Osteoporosis International. 2016;27(4):1281-386.

41. Berger C, Goltzman D, Langsetmo L, Joseph L, Jackson S, Kreiger N, et al. Peak bone mass from longitudinal data: implications for the prevalence, pathophysiology, and diagnosis of osteoporosis. J Bone Miner Res. 2010;25(9):1948-57.

42. World Health O. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis : report of a WHO study group [meeting held in Rome from 22 to 25 June 1992]. Geneva: World Health Organization; 1994.

43. Kanis JA, Melton LJ, 3rd, Christiansen C, Johnston CC, Khaltaev N. The diagnosis of osteoporosis. J Bone Miner Res. 1994;9(8):1137-41.

44. Black DM, Rosen CJ. Clinical Practice. Postmenopausal Osteoporosis. N Engl J Med. 2016;374(3):254-62.

45. Bonjour JP, Chevalley T, Ferrari S, Rizzoli R. The importance and relevance of peak bone mass in the prevalence of osteoporosis. Salud Publica Mex. 2009;51 Suppl 1:S5-17.

46. Hernandez CJ, Beaupré GS, Carter DR. A theoretical analysis of the relative influences of peak BMD, age-related bone loss and menopause on the development of osteoporosis. Osteoporosis International. 2003;14(10):843-7.

47. Zebaze RM, Ghasem-Zadeh A, Bohte A, Iuliano-Burns S, Mirams M, Price RI, et al. Intracortical remodelling and porosity in the distal radius and post-mortem femurs of women: a cross-sectional study. Lancet. 2010;375(9727):1729-36.

48. Nicks KM, Amin S, Atkinson EJ, Riggs BL, Melton LJ, 3rd, Khosla S. Relationship of age to bone microstructure independent of areal bone mineral density. J Bone Miner Res. 2012;27(3):637-44.

49. Nirody JA, Cheng KP, Parrish RM, Burghardt AJ, Majumdar S, Link TM, et al. Spatial distribution of intracortical porosity varies across age and sex. Bone. 2015;75:88-95.

50. Shanbhogue VV, Brixen K, Hansen S. Age- and Sex-Related Changes in Bone Microarchitecture and Estimated Strength: A Three-Year Prospective Study Using HRpQCT. J Bone Miner Res. 2016;31(8):1541-9.

51. Kalkwarf HJ, Gilsanz V, Lappe JM, Oberfield S, Shepherd JA, Hangartner TN, et al. Tracking of Bone Mass and Density during Childhood and Adolescence. The Journal of Clinical Endocrinology & Metabolism. 2010;95(4):1690-8.

52. Tveit M, Rosengren BE, Nilsson J, Ahlborg HG, Karlsson MK. Bone mass following physical activity in young years: a mean 39-year prospective controlled study in men. Osteoporos Int. 2013;24(4):1389-97.

53. Wren TA, Kalkwarf HJ, Zemel BS, Lappe JM, Oberfield S, Shepherd JA, et al. Longitudinal tracking of dual-energy X-ray absorptiometry bone measures over 6 years in children and adolescents: persistence of low bone mass to maturity. J Pediatr. 2014;164(6):1280-5 e2.

54. Emaus N, Berntsen GKR, Joakimsen RM, Fønnebø V. Longitudinal Changes in Forearm Bone Mineral Density in Women and Men Aged 25–44 Years: The Tromsø Study: A Population-based Study. American Journal of Epidemiology. 2005;162(7):633-43.

55. Emaus N, Berntsen GKR, Joakimsen R, Fonnebø V. Longitudinal Changes in Forearm Bone Mineral Density in Women and Men Aged 45–84 Years: The Tromsø Study, a Population-based Study. American Journal of Epidemiology. 2006;163(5):441-9.

56. Melton LJ, 3rd, Atkinson EJ, Khosla S, Oberg AL, Riggs BL. Evaluation of a prediction model for long-term fracture risk. J Bone Miner Res. 2005;20(4):551-6.

57. Eisman JA. Genetics of osteoporosis. Endocr Rev. 1999;20(6):788-804.

58. Matkovic V. Calcium and peak bone mass. J Intern Med. 1992;231(2):151-60.

59. Kalkwarf HJ, Khoury JC, Lanphear BP. Milk intake during childhood and adolescence, adult bone density, and osteoporotic fractures in US women. Am J Clin Nutr. 2003;77(1):257-65.

60. Cadogan J, Eastell R, Jones N, Barker ME. Milk intake and bone mineral acquisition in adolescent girls: randomised, controlled intervention trial. BMJ. 1997;315(7118):1255-60.

61. Huncharek M, Muscat J, Kupelnick B. Dairy products, dietary calcium and vitamin D intake as risk factors for prostate cancer: a meta-analysis of 26,769 cases from 45 observational studies. Nutr Cancer. 2008;60(4):421-41.

62. Ma XM, Huang ZW, Yang XG, Su YX. Calcium supplementation and bone mineral accretion in Chinese adolescents aged 12-14 years: a 12-month, dose-response, randomised intervention trial. Br J Nutr. 2014;112(9):1510-20.

63. Lambert HL, Eastell R, Karnik K, Russell JM, Barker ME. Calcium supplementation and bone mineral accretion in adolescent girls: an 18-mo randomized controlled trial with 2-y follow-up. Am J Clin Nutr. 2008;87(2):455-62.

64. Yin J, Zhang Q, Liu A, Du W, Wang X, Hu X, et al. Calcium supplementation for 2 years improves bone mineral accretion and lean body mass in Chinese adolescents. Asia Pac J Clin Nutr. 2010;19(2):152-60.

65. Tai V, Leung W, Grey A, Reid IR, Bolland MJ. Calcium intake and bone mineral density: systematic review and meta-analysis. BMJ. 2015;351:h4183-h.

66. Office of the Surgeon G. Reports of the Surgeon General. Bone Health and Osteoporosis: A Report of the Surgeon General. Rockville (MD): Office of the Surgeon General (US); 2004.

67. LeBoff MS, Kohlmeier L, Hurwitz S, Franklin J, Wright J, Glowacki J. Occult vitamin D deficiency in postmenopausal US women with acute hip fracture. JAMA. 1999;281(16):1505-11.

68. Thomas MK, Lloyd-Jones DM, Thadhani RI, Shaw AC, Deraska DJ, Kitch BT, et al. Hypovitaminosis D in medical inpatients. N Engl J Med. 1998;338(12):777-83.

69. Riebel GD, Boden SD, Whitesides TE, Hutton WC. The effect of nicotine on incorporation of cancellous bone graft in an animal model. Spine (Phila Pa 1976). 1995;20(20):2198-202.

70. Krall EA, Dawson-Hughes B. Smoking increases bone loss and decreases intestinal calcium absorption. J Bone Miner Res. 1999;14(2):215-20.

71. Kanis JA, Johnell O, Oden A, Johansson H, De Laet C, Eisman JA, et al. Smoking and fracture risk: a meta-analysis. Osteoporos Int. 2005;16(2):155-62.

72. Medina-Gomez C. Bone and the gut microbiome: a new dimension. Journal of Laboratory and Precision Medicine. 2018;3:96-.

73. Vahle JL, Ma YL, Burr DB. Chapter 32 - Skeletal Assessments in the Nonhuman Primate. In: Bluemel J, Korte S, Schenck E, Weinbauer GF, editors. The Nonhuman Primate in Nonclinical Drug Development and Safety Assessment. San Diego: Academic Press; 2015. p. 605-25.

74. Gunson D, Gropp KE, Varela A. Chapter 23 - Bone and Joints. In: Wallig MA, Haschek WM, Rousseaux CG, Bolon B, editors. Fundamentals of Toxicologic Pathology (Third Edition): Academic Press; 2018. p. 749-90.

75. Frost HM. Presence of microscopic cracks in vivo in bone. Henry Ford Hospital Medical Bulletin. 1960;8: No. 1:25-35.

76. Courtney AC, Hayes WC, Gibson LJ. Age-related differences in post-yield damage in human cortical bone. Experiment and model. J Biomech. 1996;29(11):1463-71.

77. Schaffler MB, Radin EL, Burr DB. Mechanical and morphological effects of strain rate on fatigue of compact bone. Bone. 1989;10(3):207-14.

78. Morgan EF, Barnes GL, Einhorn TA. Chapter 1 - The Bone Organ System: Form and Function. In: Marcus R, Feldman D, Dempster DW, Luckey M, Cauley JA, editors. Osteoporosis (Fourth Edition). San Diego: Academic Press; 2013. p. 3-20.

79. Hannan MT, Felson DT, Dawson-Hughes B, Tucker KL, Cupples LA, Wilson PWF, et al. Risk factors for longitudinal bone loss in elderly men and women: The Framingham Osteoporosis Study. Journal of Bone and Mineral Research. 2000;15(4):710-20.

80. Cheng XG, Lowet G, Boonen S, Nicholson PH, Brys P, Nijs J, et al. Assessment of the strength of proximal femur in vitro: relationship to femoral bone mineral density and femoral geometry. Bone. 1997;20(3):213-8.

81. Johnell O, Kanis JA, Oden A, Johansson H, De Laet C, Delmas P, et al. Predictive value of BMD for hip and other fractures. Journal of Bone and Mineral Research. 2005;20(7):1185-94.

82. Cummings SR, Black DM, Nevitt MC, Browner W, Cauley J, Ensrud K, et al. Bone density at various sites for prediction of hip fractures. The Study of Osteoporotic Fractures Research Group. Lancet. 1993;341(8837):72-5.

83. Marshall D, Johnell O, Wedel H. Meta-analysis of how well measures of bone mineral density predict occurrence of osteoporotic fractures. British Medical Journal. 1996;312(7041):1254-9.

84. Kanis JA, Johnell O, Oden A, Dawson A, De Laet C, Jonsson B. Ten year probabilities of osteoporotic fractures according to BMD and diagnostic thresholds. Osteoporos Int. 2001;12(12):989-95.

85. Sornay-Rendu E, Boutroy S, Munoz F, Bouxsein ML. Cortical and trabecular architecture are altered in postmenopausal women with fractures. Osteoporosis International. 2009;20(8):1291-7.

86. Patsch JM, Burghardt AJ, Kazakia G, Majumdar S. Noninvasive imaging of bone microarchitecture. Annals of the New York Academy of Sciences. 2011;1240(1):77-87.

87. Frost HM. On the Estrogen–Bone Relationship and Postmenopausal Bone Loss: A New Model. Journal of Bone and Mineral Research. 1999;14(9):1473-7.

88. Riggs BL, Khosla S, Melton Iii LJ. A Unitary Model for Involutional Osteoporosis: Estrogen Deficiency Causes Both Type I and Type II Osteoporosis in Postmenopausal Women and Contributes to Bone Loss in Aging Men. Journal of Bone and Mineral Research. 1998;13(5):763-73.

89. Ahlborg HG, Johnell O, Turner CH, Rannevik G, Karlsson MK. Bone Loss and Bone Size after Menopause. New England Journal of Medicine. 2003;349(4):327-34.

90. Ruff CB, Hayes WC. Sex differences in age-related remodeling of the femur and tibia. J Orthop Res. 1988;6(6):886-96.

91. Srinivasan B, Kopperdahl DL, Amin S, Atkinson EJ, Camp J, Robb RA, et al. Relationship of femoral neck areal bone mineral density to volumetric bone mineral density, bone size, and femoral strength in men and women. Osteoporos Int. 2012;23(1):155-62.

92. Szulc P. Bone density, geometry, and fracture in elderly men. Current Osteoporosis Reports. 2006;4(2):57-63.

93. Kokoroghiannis C, Charopoulos I, Lyritis G, Raptou P, Karachalios T, Papaioannou N. Correlation of pQCT bone strength index with mechanical testing in distraction osteogenesis. Bone. 2009;45(3):512-6.

94. Siu WS, Qin L, Leung K. pQCT bone strength index may serve as a better predictor than bone mineral density for long bone breaking strength. Journal of bone and mineral metabolism. 2003;21:316-22.

95. Moisio KC, Podolskaya G, Barnhart B, Berzins A, Sumner DR. pQCT provides better prediction of canine femur breaking load than does DXA. J Musculoskelet Neuronal Interact. 2003;3(3):240-5.

96. Jämsä T, Jalovaara P, Peng Z, Väänänen HK, Tuukkanen J. Comparison of three-point bending test and peripheral quantitative computed tomography analysis in the evaluation of the strength of mouse femur and tibia. Bone. 1998;23(2):155-61.

97. Sheu Y, Zmuda JM, Boudreau RM, Petit MA, Ensrud KE, Bauer DC, et al. Bone strength measured by peripheral quantitative computed tomography and the risk of nonvertebral fractures: the osteoporotic fractures in men (MrOS) study. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. 2011;26(1):63-71.

98. Frontera WR, Ochala J. Skeletal muscle: a brief review of structure and function. Calcif Tissue Int. 2015;96(3):183-95.

99. Greising SM, Gransee HM, Mantilla CB, Sieck GC. Systems biology of skeletal muscle: fiber type as an organizing principle. Wiley Interdiscip Rev Syst Biol Med. 2012;4(5):457-73.

100. MacIntosh BR, Gardiner PF, McComas AJ. Skeletal muscle : form and function. 2nd ed. Champaign, IL: Human Kinetics; 2006. viii, 423 p. p.

101. Galpin AJ, Raue U, Jemiolo B, Trappe TA, Harber MP, Minchev K, et al. Human skeletal muscle fiber type specific protein content. Anal Biochem. 2012;425(2):175-82.

102. Andersen JL, Klitgaard H, Saltin B. Myosin heavy chain isoforms in single fibres from m. vastus lateralis of sprinters: influence of training. Acta Physiol Scand. 1994;151(2):135-42.

103. Gallagher P, Trappe S, Harber M, Creer A, Mazzetti S, Trappe T, et al. Effects of 84-days of bedrest and resistance training on single muscle fibre myosin heavy chain distribution in human vastus lateralis and soleus muscles. Acta Physiol Scand. 2005;185(1):61-9.

104. Klitgaard H, Mantoni M, Schiaffino S, Ausoni S, Gorza L, Laurent-Winter C, et al. Function, morphology and protein expression of ageing skeletal muscle: a cross-sectional study of elderly men with different training backgrounds. Acta Physiol Scand. 1990;140(1):41-54.

105. Klitgaard H, Zhou M, Schiaffino S, Betto R, Salviati G, Saltin B. Ageing alters the myosin heavy chain composition of single fibres from human skeletal muscle. Acta Physiol Scand. 1990;140(1):55-62.

106. Cronholm F. Thesis: Physical activity, musculoskeletal traits and fractures in childhood and in old men. Lund University. 2019.

107. Kell RT, Bell G, Quinney A. Musculoskeletal fitness, health outcomes and quality of life. Sports Med. 2001;31(12):863-73.

108. Purves D. Neuroscience. Sixth edition. ed. New York: Oxford University Press; 2018. 1 volume (various pagings) p.

109. Houk J, Rymer W. Neural Control of Muscle Length and Tension. Vol. II2011.

110. Cruz-Jentoft AJ, Bahat G, Bauer J, Boirie Y, Bruyère O, Cederholm T, et al. Sarcopenia: revised European consensus on definition and diagnosis. Age and Ageing. 2019;48(1):16-31.

111. Abellan van Kan G. Epidemiology and consequences of sarcopenia. J Nutr Health Aging. 2009;13(8):708-12.

112. Campbell MJ, McComas AJ, Petito F. Physiological changes in ageing muscles. J Neurol Neurosurg Psychiatry. 1973;36(2):174-82.

113. Tomlinson BE, Irving D. The numbers of limb motor neurons in the human lumbosacral cord throughout life. J Neurol Sci. 1977;34(2):213-9.

114. Rosengren BE, Ribom EL, Nilsson JA, Mallmin H, Ljunggren O, Ohlsson C, et al. Inferior physical performance test results of 10,998 men in the MrOS Study is associated with high fracture risk. Age Ageing. 2012;41(3):339-44.

115. Karlsson MK, Ribom E, Nilsson J, Ljunggren Ö, Ohlsson C, Mellström D, et al. Inferior physical performance tests in 10,998 men in the MrOS study is associated with recurrent falls. Age Ageing. 2012;41(6):740-6.

116. Metter EJ, Talbot LA, Schrager M, Conwit RA. Arm-cranking muscle power and arm isometric muscle strength are independent predictors of all-cause mortality in men. J Appl Physiol (1985). 2004;96(2):814-21.

117. Hunter SK, Pereira HM, Keenan KG. The aging neuromuscular system and motor performance. Journal of applied physiology (Bethesda, Md : 1985). 2016;121(4):982-95.

118. Fritz J, Rosengren BE, Dencker M, Karlsson C, Karlsson MK. A seven-year physical activity intervention for children increased gains in bone mass and muscle strength. Acta Paediatr. 2016;105(10):1216-24.

119. Fiatarone MA, O'Neill EF, Ryan ND, Clements KM, Solares GR, Nelson ME, et al. Exercise training and nutritional supplementation for physical frailty in very elderly people. N Engl J Med. 1994;330(25):1769-75.

120. Gabriel DA, Kamen G, Frost G. Neural adaptations to resistive exercise: mechanisms and recommendations for training practices. Sports Med. 2006;36(2):133-49.

121. Phillips SM. A brief review of critical processes in exercise-induced muscular hypertrophy. Sports Med. 2014;44 Suppl 1:S71-7.

122. Yan Z, Lira VA, Greene NP. Exercise training-induced regulation of mitochondrial quality. Exerc Sport Sci Rev. 2012;40(3):159-64.

123. Osternig LR. Isokinetic dynamometry: implications for muscle testing and rehabilitation. Exerc Sport Sci Rev. 1986;14:45-80.

124. Scafoglieri A, Clarys JP. Dual energy X-ray absorptiometry: gold standard for muscle mass? J Cachexia Sarcopenia Muscle. 2018;9(4):786-7.

125. Caspersen CJ, Powell KE, Christenson GM. Physical activity, exercise, and physical fitness: definitions and distinctions for health-related research. Public Health Rep. 1985;100(2):126-31.

126. Weaver CM, Gordon CM, Janz KF, Kalkwarf HJ, Lappe JM, Lewis R, et al. The National Osteoporosis Foundation's position statement on peak bone mass development and lifestyle factors: a systematic review and implementation recommendations. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 2016;27(4):1281-386.

127. Koerhuis C, Heide F, Hof A. Energy consumption in static muscle contraction. European journal of applied physiology. 2003;88:588-92.

128. Sallis JF, Bull F, Guthold R, Heath GW, Inoue S, Kelly P, et al. Progress in physical activity over the Olympic quadrennium. Lancet. 2016;388(10051):1325-36.

129. World Health Organization (WHO). Assessing national capacity for the prevention and control of noncommunicable diseases: report of the 2017 global survey. 2018.

130. Ding D, Lawson KD, Kolbe-Alexander TL, Finkelstein EA, Katzmarzyk PT, van Mechelen W, et al. The economic burden of physical inactivity: a global analysis of major non-communicable diseases. Lancet. 2016;388(10051):1311-24.

131. Thivel D, Tremblay A, Genin PM, Panahi S, Riviere D, Duclos M. Physical Activity, Inactivity, and Sedentary Behaviors: Definitions and Implications in Occupational Health. Front Public Health. 2018;6:288.

132. Rezende LFM, Sa TH, Mielke GI, Viscondi JYK, Rey-Lopez JP, Garcia LMT. All-Cause Mortality Attributable to Sitting Time: Analysis of 54 Countries Worldwide. Am J Prev Med. 2016;51(2):253-63.

133. Raustorp A, Ekroth Y. Tracking of pedometer-determined physical activity: a 10-year follow-up study from adolescence to adulthood in Sweden. J Phys Act Health. 2013;10(8):1186-92.

134. Telama R, Yang X, Leskinen E, Kankaanpaa A, Hirvensalo M, Tammelin T, et al. Tracking of physical activity from early childhood through youth into adulthood. Med Sci Sports Exerc. 2014;46(5):955-62.

135. Caldwell HA, Proudfoot NA, King-Dowling S, Di Cristofaro NA, Cairney J, Timmons BW. Tracking of physical activity and fitness during the early years. Appl Physiol Nutr Metab. 2016;41(5):504-10.

136. Lahti A. Thesis: Physical Activity in Childhood and Adolescence. Lund University. 2019.

137. Guthold R, Stevens GA, Riley LM, Bull FC. Global trends in insufficient physical activity among adolescents: a pooled analysis of 298 population-based surveys with 1·6 million participants. Lancet Child Adolesc Health. 2020;4(1):23-35.

138. Raustorp A, Froberg A. Comparisons of pedometer-determined weekday physical activity among Swedish school children and adolescents in 2000 and 2017 showed the highest reductions in adolescents. Acta Paediatr. 2019;108(7):1303-10.

139. Ewart CK, Young DR, Hagberg JM. Effects of school-based aerobic exercise on blood pressure in adolescent girls at risk for hypertension. Am J Public Health. 1998;88(6):949-51.

140. Dencker M, Thorsson O, Karlsson MK, Linden C, Svensson J, Wollmer P, et al. Daily physical activity and its relation to aerobic fitness in children aged 8-11 years. Eur J Appl Physiol. 2006;96(5):587-92.

141. Korczak DJ, Madigan S, Colasanto M. Children's Physical Activity and Depression: A Meta-analysis. Pediatrics. 2017;139(4).

142. Coe DP, Pivarnik JM, Womack CJ, Reeves MJ, Malina RM. Effect of physical education and activity levels on academic achievement in children. Med Sci Sports Exerc. 2006;38(8):1515-9.

143. Kall LB, Nilsson M, Linden T. The impact of a physical activity intervention program on academic achievement in a Swedish elementary school setting. J Sch Health. 2014;84(8):473-80.

144. Cöster ME, Fritz J, Karlsson C, Rosengren BE, Karlsson MK. Extended physical education in children aged 6-15 years was associated with improved academic achievement in boys. Acta Paediatr. 2018;107(6):1083-7.

145. World Health Organization (WHO). Global recommendations on physical activity for health. 2010.

146. Frost HM. The mechanostat: a proposed pathogenic mechanism of osteoporoses and the bone mass effects of mechanical and nonmechanical agents. Bone Miner. 1987;2(2):73-85.

147. Robling AG, Castillo AB, Turner CH. Biomechanical and molecular regulation of bone remodeling. Annu Rev Biomed Eng. 2006;8:455-98.

148. Daly RM, Bass SL. Lifetime sport and leisure activity participation is associated with greater bone size, quality and strength in older men. Osteoporos Int. 2006;17(8):1258-67.

149. Karlsson MK, Nordqvist A, Karlsson C. Sustainability of exercise-induced increases in bone density and skeletal structure. Food Nutr Res. 2008;52.

150. Nilsson M, Ohlsson C, Oden A, Mellstrom D, Lorentzon M. Increased physical activity is associated with enhanced development of peak bone mass in men: a five-year longitudinal study. J Bone Miner Res. 2012;27(5):1206-14.

151. Sievanen H, Kannus P. Physical activity reduces the risk of fragility fracture. PLoS Med. 2007;4(6):e222.

152. Skelton DA, Beyer N. Exercise and injury prevention in older people. Scand J Med Sci Sports. 2003;13(1):77-85.

153. Beck TJ, Oreskovic TL, Stone KL, Ruff CB, Ensrud K, Nevitt MC, et al. Structural adaptation to changing skeletal load in the progression toward hip fragility: the study of osteoporotic fractures. J Bone Miner Res. 2001;16(6):1108-19.

154. Coupland C, Wood D, Cooper C. Physical inactivity is an independent risk factor for hip fracture in the elderly. J Epidemiol Community Health. 1993;47(6):441-3.

155. Macdonald HM, Kontulainen SA, Khan KM, McKay HA. Is a school-based physical activity intervention effective for increasing tibial bone strength in boys and girls? J Bone Miner Res. 2007;22(3):434-46.

156. Petit MA, McKay HA, MacKelvie KJ, Heinonen A, Khan KM, Beck TJ. A randomized school-based jumping intervention confers site and maturity-specific benefits on bone structural properties in girls: a hip structural analysis study. J Bone Miner Res. 2002;17(3):363-72.

157. Baptista F, Janz KF. Habitual Physical Activity and Bone Growth and Development in Children and Adolescents: A Public Health Perspective. In: Preedy VR, editor. Handbook of Growth and Growth Monitoring in Health and Disease. New York, NY: Springer New York; 2012. p. 2395-411.

158. Alwis G, Karlsson C, Stenevi-Lundgren S, Rosengren BE, Karlsson MK. Femoral neck bone strength estimated by hip structural analysis (HSA) in Swedish Caucasians aged 6-90 years. Calcif Tissue Int. 2012;90(3):174-85.

159. Turner CH, Robling AG. Designing exercise regimens to increase bone strength. Exerc Sport Sci Rev. 2003;31(1):45-50.

160. Khan K, McKay HA, Haapasalo H, Bennell KL, Forwood MR, Kannus P, et al. Does childhood and adolescence provide a unique opportunity for exercise to strengthen the skeleton? J Sci Med Sport. 2000;3(2):150-64.

161. Robling AG, Hinant FM, Burr DB, Turner CH. Improved bone structure and strength after long-term mechanical loading is greatest if loading is separated into short bouts. J Bone Miner Res. 2002;17(8):1545-54.

162. Clark EM, Ness AR, Tobias JH. Vigorous physical activity increases fracture risk in children irrespective of bone mass: a prospective study of the independent risk factors for fractures in healthy children. J Bone Miner Res. 2008;23(7):1012-22.

163. Bloemers F, Collard D, Paw MC, Van Mechelen W, Twisk J, Verhagen E. Physical inactivity is a risk factor for physical activity-related injuries in children. Br J Sports Med. 2012;46(9):669-74.

164. Cöster ME, Fritz J, Nilsson J, Karlsson C, Rosengren BE, Dencker M, et al. How does a physical activity programme in elementary school affect fracture risk? A prospective controlled intervention study in Malmo, Sweden. BMJ Open. 2017;7(2):e012513.

165. Sallis JF, Saelens BE. Assessment of physical activity by self-report: status, limitations, and future directions. Res Q Exerc Sport. 2000;71(2 Suppl):S1-14.

166. The Swedish National Agency for Education (Skolverket). Timplan för grundskolan 2019 [Available from: https://www.skolverket.se/undervisning/grundskolan/laroplan-och-kursplaner-for-grundskolan/timplan-for-grundskolan.

167. Kannus P, Haapasalo H, Sankelo M, Sievanen H, Pasanen M, Heinonen A, et al. Effect of starting age of physical activity on bone mass in the dominant arm of tennis and squash players. Ann Intern Med. 1995;123(1):27-31.

168. Sundberg M, Gärdsell P, Johnell O, Karlsson MK, Ornstein E, Sandstedt B, et al. Peripubertal moderate exercise increases bone mass in boys but not in girls: a population-based intervention study. Osteoporos Int. 2001;12(3):230-8.

169. Blimkie CJ, Rice S, Webber CE, Martin J, Levy D, Gordon CL. Effects of resistance training on bone mineral content and density in adolescent females. Can J Physiol Pharmacol. 1996;74(9):1025-33.

170. Witzke KA, Snow CM. Effects of plyometric jump training on bone mass in adolescent girls. Med Sci Sports Exerc. 2000;32(6):1051-7.

171. Heinonen A, Sievanen H, Kannus P, Oja P, Pasanen M, Vuori I. High-impact exercise and bones of growing girls: a 9-month controlled trial. Osteoporos Int. 2000;11(12):1010-7.

172. Rowland TW. The biological basis of physical activity. Med Sci Sports Exerc. 1998;30(3):392-9.

173. Gomersall SR, Rowlands AV, English C, Maher C, Olds TS. The ActivityStat hypothesis: the concept, the evidence and the methodologies. Sports Med. 2013;43(2):135-49.

174. Gomersall SR, Maher C, English C, Rowlands AV, Dollman J, Norton K, et al. Testing the activitystat hypothesis: a randomised controlled trial. BMC Public Health. 2016;16:900.

175. Cronholm F, Rosengren BE, Karlsson C, Karlsson MK. A Physical Activity Intervention Program in School is Also Accompanied by Higher Leisure-Time Physical Activity: A Prospective Controlled 3-Year Study in 194 Prepubertal Children. J Phys Act Health. 2017;14(4):301-7.

176. Cronholm F, Rosengren BE, Karlsson C, Karlsson MK. A comparative study found that a seven-year school-based exercise programme increased physical activity levels in both sexes. Acta Paediatrica. 2018;107(4):701-7.

177. Lindgren E, Karlsson MK, Lorentzon M, Rosengren BE. Bone Traits Seem to Develop Also During the Third Decade in Life—Normative Cross-Sectional Data on 1083 Men Aged 18–28 Years. Journal of Clinical Densitometry. 2017;20(1):32-43.

178. Hoaglin DC, Iglewicz B. Fine-Tuning Some Resistant Rules for Outlier Labeling. Journal of the American Statistical Association. 1987;82(400):1147-9.

179. Linden C, Ahlborg HG, Besjakov J, Gardsell P, Karlsson MK. A school curriculum-based exercise program increases bone mineral accrual and bone size in prepubertal girls: two-year data from the pediatric osteoporosis prevention (POP) study. J Bone Miner Res. 2006;21(6):829-35.

180. Linden C, Alwis G, Ahlborg H, Gardsell P, Valdimarsson O, Stenevi-Lundgren S, et al. Exercise, bone mass and bone size in prepubertal boys: one-year data from the pediatric osteoporosis prevention study. Scand J Med Sci Sports. 2007;17(4):340-7.

181. Hebert JJ, Moller NC, Andersen LB, Wedderkopp N. Organized Sport Participation Is Associated with Higher Levels of Overall Health-Related Physical Activity in Children (CHAMPS Study-DK). PLoS One. 2015;10(8):e0134621.

182. Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. Arch Dis Child. 1969;44(235):291-303.

183. Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in boys. Arch Dis Child. 1970;45(239):13-23.

184. Duke PM, Litt IF, Gross RT. Adolescents' self-assessment of sexual maturation. Pediatrics. 1980;66(6):918-20.

185. Ashby RL, Ward KA, Roberts SA, Edwards L, Mughal MZ, Adams JE. A reference database for the Stratec XCT-2000 peripheral quantitative computed tomography (pQCT) scanner in healthy children and young adults aged 6-19 years. Osteoporos Int. 2009;20(8):1337-46.

186. Neu CM, Manz F, Rauch F, Merkel A, Schoenau E. Bone densities and bone size at the distal radius in healthy children and adolescents: a study using peripheral quantitative computed tomography. Bone. 2001;28(2):227-32.

187. Binkley TL, Specker BL, Wittig TA. Centile curves for bone densitometry measurements in healthy males and females ages 5-22 yr. J Clin Densitom. 2002;5(4):343-53.

188. Moyer-Mileur LJ, Quick JL, Murray MA. Peripheral quantitative computed tomography of the tibia: pediatric reference values. J Clin Densitom. 2008;11(2):283-94.

189. Butz S, Wüster C, Scheidt-Nave C, Götz M, Ziegler R. Forearm BMD as measured by peripheral quantitative computed tomography (pQCT) in a German reference population. Osteoporos Int. 1994;4(4):179-84.

190. Compston J. Bone quality: what is it and how is it measured? Arq Bras Endocrinol Metabol. 2006;50(4):579-85.

191. Fujita T, Fujii Y, Goto B. Measurement of forearm bone in children by peripheral computed tomography. Calcif Tissue Int. 1999;64(1):34-9.

192. Bergh C, Wennergren D, Möller M, Brisby H. Fracture incidence in adults in relation to age and gender: A study of 27,169 fractures in the Swedish Fracture Register in a well-defined catchment area. PLoS One. 2020;15(12):e0244291.

193. Dumith SC, Gigante DP, Domingues MR, Kohl HW, 3rd. Physical activity change during adolescence: a systematic review and a pooled analysis. Int J Epidemiol. 2011;40(3):685-98.

194. Corder K, Sharp SJ, Atkin AJ, Griffin SJ, Jones AP, Ekelund U, et al. Change in objectively measured physical activity during the transition to adolescence. Br J Sports Med. 2015;49(11):730-6.

195. Dalene KE, Anderssen SA, Andersen LB, Steene-Johannessen J, Ekelund U, Hansen BH, et al. Secular and longitudinal physical activity changes in population-based samples of children and adolescents. Scand J Med Sci Sports. 2018;28(1):161-71.

196. Tan VP, Macdonald HM, Kim S, Nettlefold L, Gabel L, Ashe MC, et al. Influence of physical activity on bone strength in children and adolescents: a systematic review and narrative synthesis. J Bone Miner Res. 2014;29(10):2161-81.

197. Larsen MN, Nielsen CM, Helge EW, Madsen M, Manniche V, Hansen L, et al. Positive effects on bone mineralisation and muscular fitness after 10 months of intense school-based physical training for children aged 8-10 years: the FIT FIRST randomised controlled trial. Br J Sports Med. 2018;52(4):254-60.

198. Nogueira RC, Weeks BK, Beck BR. An in-school exercise intervention to enhance bone and reduce fat in girls: the CAPO Kids trial. Bone. 2014;68:92-9.

199. Macdonald HM, Kontulainen SA, Khan KM, McKay HA. Is a School-Based Physical Activity Intervention Effective for Increasing Tibial Bone Strength in Boys and Girls? Journal of Bone and Mineral Research. 2007;22(3):434-46.

200. Meyer U, Romann M, Zahner L, Schindler C, Puder JJ, Kraenzlin M, et al. Effect of a general school-based physical activity intervention on bone mineral content and density: a cluster-randomized controlled trial. Bone. 2011;48(4):792-7.

201. Cöster ME, Rosengren BE, Karlsson C, Dencker M, Karlsson MK. Effects of an 8-year childhood physical activity intervention on musculoskeletal gains and fracture risk. Bone. 2016;93:139-45.

202. Tveit M, Rosengren BE, Nilsson JA, Karlsson MK. Exercise in youth: High bone mass, large bone size, and low fracture risk in old age. Scand J Med Sci Sports. 2015;25(4):453-61.

203. Lord SR, Ward JA, Williams P, Strudwick M. The effect of a 12-month exercise trial on balance, strength, and falls in older women: a randomized controlled trial. J Am Geriatr Soc. 1995;43(11):1198-206.

204. Nordstrom A, Karlsson C, Nyquist F, Olsson T, Nordstrom P, Karlsson M. Bone loss and fracture risk after reduced physical activity. J Bone Miner Res. 2005;20(2):202-7.

205. Gustavsson A, Olsson T, Nordstrom P. Rapid loss of bone mineral density of the femoral neck after cessation of ice hockey training: a 6-year longitudinal study in males. J Bone Miner Res. 2003;18(11):1964-9.

206. Valdimarsson O, Alborg HG, Düppe H, Nyquist F, Karlsson M. Reduced training is associated with increased loss of BMD. J Bone Miner Res. 2005;20(6):906-12.

207. Lahti A, Rosengren BE, Nilsson JA, Karlsson C, Karlsson MK. Long-term effects of daily physical education throughout compulsory school on duration of physical activity in young adulthood: an 11-year prospective controlled study. BMJ Open Sport Exerc Med. 2018;4(1):e000360.

208. Cronholm F, Rosengren BE, Nilsson JA, Ohlsson C, Mellstrom D, Ribom E, et al. The fracture predictive ability of a musculoskeletal composite score in old men - data from the MrOs Sweden study. BMC Geriatr. 2019;19(1):90.

Skeletal Development During the First Three Decades in Life

Around the end of the second decade in life, bone mass in the hip starts to attenuate. Does this lead to weaker bones already at such a young age, or are there parallel changes counteracting the loss of bone mass? This thesis explores the skeletal development in the upper and lower extremities in men aged 18-28. It also investigates how a daily school-based physical activity program during nine compulsory school-years affect musculoskeletal traits from before to after puberty and if possible effects on these traits are retained after the termination of the intervention program.

In 1987, Erik Lindgren was born in Uddevalla. He graduated from Lund University medical school and got his medical license in 2020. Dr Lindgren currently plan to work as a medical doctor at Sahlgrenska University Hospital in Gothenburg. His doctoral studies began near the end of medical school in 2015.

FACULTY OF MEDICINE

LUND UNIVERSITY

9 789180 210539