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Bone Mass from Childhood to Adulthood

Christian Buttazzoni, MD

DOCTORAL DISSERTATION

By due permission of the Faculty of Medicine, Lund University, Sweden.

To be defended at the Lecture Hall, Level 5, Department of Orthopedics, Skåne University Hospital, Malmö, 20 March 2015, 09.00

Faculty opponent

Associate Professor Diana Swolin-Eide, The Queen Silvia Children's Hospital, Sahlgrenska University Hospital/Östra, Gothenburg, Sweden

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Bone Mass from Childhood to Adulthood

Abstract

Attaining high peak bone mass (PBM), the highest bone mass value in life which is reached in young adulthood, is important as it reduces the risk of having low bone mass in old age. Osteoporosis is the result of bone loss, a physiological process related to aging and/or low PBM and is associated with high fracture risk. It would therefore be of great value to identify children at risk of reaching low PBM for possible interventions. But the level of correlation, in the thesis referred to as "tracking", in bone mass from childhood to adulthood is unclear. Making predictions about adult bone mineral density (BMD) from childhood measurements is difficult as bone properties change rapidly during growth. Most studies that have evaluated the question are either cross-sectional, have a short follow-up time or end close to the final growth spurt, making reliable predictions difficult.

In this thesis, with a long-term prospective study design, we have evaluated the "tracking", i.e. correlation of bone mass from childhood to an adult age when PBM occurs. We have also specifically evaluated two risk factors linked to low BMD in both childhood and adulthood.
Our aim was to evaluate (i) whether a bone mass scan in childhood can be used to predict bone mass in adulthood, (ii) whether children who sustain a fracture are at increased risk of reaching low adult BMD and (iii) whether prematurely born children, either AGA or SGA, are at increased risk of reaching low adult BMD.

We prospectively evaluated bone traits in 214 individuals with single-photon absorptiometry (SPA). They consisted of three cohorts: healthy control subjects, children with fracture during childhood and children born preterm, either small for gestational age (SGA) or appropriate for gestational (AGA). The study subjects were at study start at a mean age of 10 years (range 3–17) and were re-measured a mean 27 (range 25–29) years later.
We also evaluated bone traits prospectively by dual-energy X-ray absorptiometry (DXA) in 121 children from the Pediatric Osteoporosis Prevention (POP) study, an exercise intervention study that is primarily designed to assess musculoskeletal development and fracture risk in response to increased physical education in school children. The study subjects were at study start at a mean age of 8 years (range 7–9) and were re-measured a mean 11 (range 10–12) years later.

This thesis shows that an individual pediatric bone mass scan, regardless of whether it is evaluated with SPA or DXA and independent of the measured skeletal region, has poor ability to predict an adult bone mass value. We also show that a childhood fracture in men was associated with low BMD and smaller bone size in young adulthood and that prematurity and being born SGA is another risk factor for low bone mass in young adulthood.

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Bone Mass from Childhood to Adulthood

Christian Buttazzoni, MD

LUND UNIVERSITY

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To Annelie
-Te amo

"An expert is a person who has made all the mistakes that can be made in a very narrow field."

Niels Bohr

Table of contents

Abstract 9

Abbreviations 11

Original papers 13

Introduction 15

- Objective 15
- Fracture epidemiology 18
  - Childhood 18
  - In elderly (>65 years) 19
- Bone tissue 20
- Bone development 23
- Bone growth and peak bone mass 24
- Bone remodeling 25
- Minerals 27
- Vitamins 27
- Hormones 28
- Bone development in adults and elderly 28
- Preventing fractures and treatment of osteoporosis 31
- Biomechanical aspects of bone 32

Bone mass measurements 35

- Single-photon absorptiometry (SPA) 37
- Dual-photon absorptiometry (DPA) 39
- Dual-energy X-ray absorptiometry (DXA) 39
- Quantitative ultrasound (QUS) 40
- Peripheral Computed Tomography (pQCT) 41
- Magnetic Resonance Imaging (MRI) 43

Biochemical analysis of bone markers 43
Aims of the thesis 45
Hypothesis 47
Material and methods 49
The Landin follow-up study (Papers I, II, IV) 49
Drop-out evaluation 50
Measurements 50
Anthropometrics and questionnaires 51
The Pediatric Osteoporosis Prevention (POP) Study (Paper III) 52
Drop-out evaluation 52
Measurements 53
Anthropometrics, maturation and questionnaires 53
Study subjects 55
Paper I 55
Paper II 55
Paper III 56
Paper IV 56
Statistical analysis 57
Summary of the papers 59
Paper I 59
Paper II 60
Paper III 61
Paper IV 62
General discussion 63
Tracking of bone mass in a growing population 63
Are fractures in childhood a risk factor for low PBM? 66
Development of bone mass in preterm-born individuals 67
Strengths and limitations of the thesis 71
Conclusions 75
Future perspectives 77
Summary in Swedish – Populärvetenskaplig sammanfattning 79
Acknowledgments 81
References 83

Abstract

Attaining high peak bone mass (PBM), the highest bone mass value in life which is reached in young adulthood, is important as it reduces the risk of having low bone mass in old age[69, 80]. Low bone mass is associated with high fracture risk[3, 60]. Osteoporosis is the result of bone loss, a physiological process related to aging and/or low PBM. It would therefore be of great value to identify children at risk of reaching low PBM for possible interventions. But the level of correlation, in the thesis referred to as "tracking", in bone mass from childhood to adulthood is unclear. Making predictions about adult bone mineral density (BMD) from childhood measurements is difficult as bone properties change rapidly during growth[59]. Most studies that have evaluated the question are either cross-sectional, have a short follow-up time or end close to the final growth spurt, making reliable predictions difficult. There are some reports suggesting that a childhood excess[62, 76] or deficit[77, 116] in BMD remains in adulthood, and the few prospective studies that have addressed the question infer that there is a partial "tracking" in BMD during growth. Longitudinal studies with serial measurements that cover both the pre- and post-pubertal phases and that follow the participants until peak bone mass (PBM) would provide data with a higher level of evidence and thereby increase our knowledge.

In this thesis, with a long-term prospective study design, we have evaluated the "tracking" of bone mass from childhood to adulthood, and specifically evaluated two risk factors linked to low BMD. The first is a fracture in childhood which has been an event identified as associated with low BMD both in childhood[31] and in adulthood[54]. The second is premature birth in relation to low birth weight, since both traits have been associated with low PBM[67, 84].

We invited subjects from three previous studies[63, 86-87] published during 1981–1985 to be re-measured almost three decades after the initial measurement. The study subjects with a mean age of 10 years (range 3–17) at the first measurement were re-measured a mean 27 (range 25–29) years later. Bone traits were prospectively evaluated with single-photon absorptiometry (SPA) in 214 individuals consisting of three cohorts: healthy control subjects, children with fracture during childhood and children born preterm, either small for gestational age (SGA) or appropriate for gestational (AGA). In the second cohort we evaluated bone traits prospectively by dual-energy X-ray absorptiometry (DXA) in 121 children from the Pediatric Osteoporosis Prevention (POP) study, an exercise intervention study that is primarily designed to assess

musculoskeletal development and fracture risk in response to increased physical education in school children. The study subjects with a mean age of 8 years (range 7–9) at the first measurement were re-measured a mean 11 (range 10–12) years later.

Our aim was to evaluate (i) whether a bone mass scan in childhood can be used to predict bone mass in adulthood, (ii) whether children who sustain a fracture are at increased risk of reaching low adult BMD and (iii) whether prematurely born children, either AGA or SGA, are at increased risk of reaching low adult BMD.

The correlation coefficients (r) between pre-pubertal and young adulthood measurements for distal radius BMC and BMD varied between 0.35 and 0.64 and for femoral neck BMC, BMD and bone area it varied between 0.37 and 0.65. A childhood fracture in men was associated with a low BMC Z-score (–0.4 (95% CI –0.6, –0.1)) and low BMD Z-score (–0.4 (95% CI –0.7, –0.1)) at baseline and with a low BMC Z-score (–0.5 (95% CI –0.8, –0.2)) and low BMD Z-score (–0.4 (95% CI –0.7, –0.1)) at follow-up. Preterm-born children were still shorter in adulthood (p=0.03), they also had lower femoral neck (FN) BMC, FN BMD, tibial cortical BMD, tibial cross-sectional area and SSI than controls (all p-values 0.001 to <0.05). The deficits were driven by lower bone traits in preterm SGA individuals, while no differences were seen in preterm AGA individuals compared to controls.

This thesis shows that an individual pediatric bone mass scan, regardless of whether it is evaluated with SPA or DXA and independent of the measured skeletal region, has poor ability to predict an adult bone mass value. We also show that a childhood fracture in men was associated with low BMD and smaller bone size in young adulthood and that prematurity and being born SGA is another risk factor for low bone mass in young adulthood.

Abbreviations

AGA	Appropriate for gestational age
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
BMC	Bone mineral content
BMD	Bone mineral density
BMI	Body mass index
BUA	Broadband ultrasound attenuation
CI	Confidence interval
CSA	Cross-sectional area
CV	Coefficient of variation
DPA	Dual photon absorptiometry
DXA	Dual-energy X-ray absorptiometry
FN	Femoral neck
FRAX	Fracture risk assessment tool
LS	Lumbar spine
PBM	Peak bone mass
PHV	Peak height velocity
POP	Pediatric Osteoporosis Prevention (study)
pQCT	Peripheral quantitative computed tomography
QUS	Quantitative ultrasound
RCT	Randomized controlled trial
ROI	Region of interest
TBLH	Total body less head

SD	Standard deviation
SGA	Small for gestational age
SOS	Speed of sound
SPA	Single-photon absorptiometry
SSI	Stress-strain index
vBMD	Volumetric bone mineral density

Original papers

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

I. **Does a Childhood Fracture Predict Low Bone Mass In Young Adulthood? – A 27 Year Prospective Controlled Study.**

Buttazzoni C, Rosengren E. B, Tveit M, Landin L, Nilsson J-Å, Karlsson K.M. J Bone Miner Res. 2013 Feb;28(2):351–9.

II. **A Pediatric Bone Mass Scan Has Poor Ability to Predict Adult Bone Mass: A 28-Year Prospective Study in 214 Children.**

Buttazzoni C, Rosengren E. B, Tveit M, Landin L, Nilsson J-Å, Karlsson K.M. *Calcif Tissue Int*. 2014 Feb;94(2):232–9.

III. **A Pediatric Bone Mass Scan Has Poor Ability to Predict Peak Bone Mass– An 11 Year Prospective Study in 121 Children**

Buttazzoni C, Rosengren E. B, Karlsson C, Dencker M, Nilsson J-Å, Karlsson K.M, Accepted *Calcif Tissue Int*. Feb 2015

IV. **Preterm Children Born Small for Gestational Age Are at Risk for Low Adult Bone Mass – A 27-Year Prospective Controlled Study**

Buttazzoni C, Rosengren E. B, Tveit M, Landin L, Nilsson J-Å, Karlsson K.M, *Submitted to Pediatrics*. Feb 2015

Introduction

Objective

Fractures are a general health problem in society, with fractures having a bimodal peak incidence curve in the population (Figure. 1)[41]. Half of all boys and a third of all girls will sustain a fracture before the age of 18[73, 85], and around half of all women and a quarter of all men after the age of 50[105].

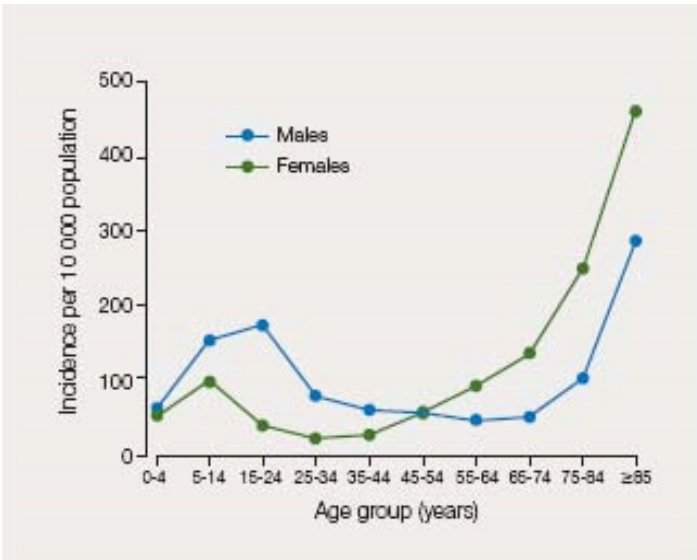

Figure. 1
Example of fracture incidence in a British population[41]

The fracture incidence in childhood has been fairly stable in the last few decades, while the increased life expectancy in developed countries, such as Sweden, increases the prevalence of osteoporosis and also the number of fragility fractures[58]. Osteoporosis-related fractures are associated with large health care costs, in Sweden comparable to the total health care costs for diabetes mellitus[58]. Fragility fractures, especially hip fractures, are also associated with significant individual suffering, increased morbidity

and mortality[85, 88]. Research is therefore focusing on identifying risk factors and minimizing fracture risk throughout life. The cause of a fracture is multifactorial as it depends on the forces applied to the bone and the strength of the skeleton. Bone strength is also multifactorial as it depends not only on bone mass but also on the physical properties of component material, and their geometric arrangement in space. In vivo measurements of the latter two parameters are however difficult to conduct in the clinical situation. This thesis therefore focuses on bone mass, as it has been identified as an important factor of bone resistance to fracture and because the trait is used in the clinical estimation of bone strength. In vivo measurements were made possible 40 years ago with the introduction of single-photon absorptiometry (SPA), and bone mass has since then been the primary focus of interest in the research field of clinical bone biology[23]. These measurements are of the highest clinical relevance since low bone mass is associated with increased fracture risk in both children and adults.

Transient low bone mass in children develops during puberty when the bone increases in size relatively more than the additional mineralization. Low bone mass in old individuals is due to age-related bone loss and or low PBM[4]. There is a correlation between the level of bone mass throughout adult life, as 50% of the variance of BMD at age 65 is estimated to be explained by the level of PBM[68, 79]. The degree and regulation in the accrual of bone mineral during growth until PBM has therefore gradually attracted more interest. To date, however, there are no prospective studies on the accrual of bone mass from childhood until PBM. The aim of this thesis is to determine whether it is possible to predict adult bone mass from childhood measurements, as it would be advantageous to identify individuals at high risk of low PBM early in life.

Fracture epidemiology

Childhood

Since it is actually not low bone mass but fractures which are the clinically relevant end point in this research field, it is also relevant to discuss the magnitude of the problem. During the latter part of the 20th century, accidents took over as the leading cause in mortality and morbidity rates in children. It is therefore imperative to focus on risk reduction and the initiation of preventive strategies. Population-based fracture epidemiology in children was described early by Landin, who presented fracture patterns in children when analyzing 8682 fractures sustained between 1975 and 1979 in Malmö, Sweden. He also reported a twofold increase in fracture incidence from the 1950's to the late 1970's (reaching 212 per 10 000)[88]. More recent studies have reported a similar incidence[25, 32, 61] or a slight decrease[98] compared to the Landin data. Fractures in children are therefore still a huge community problem.

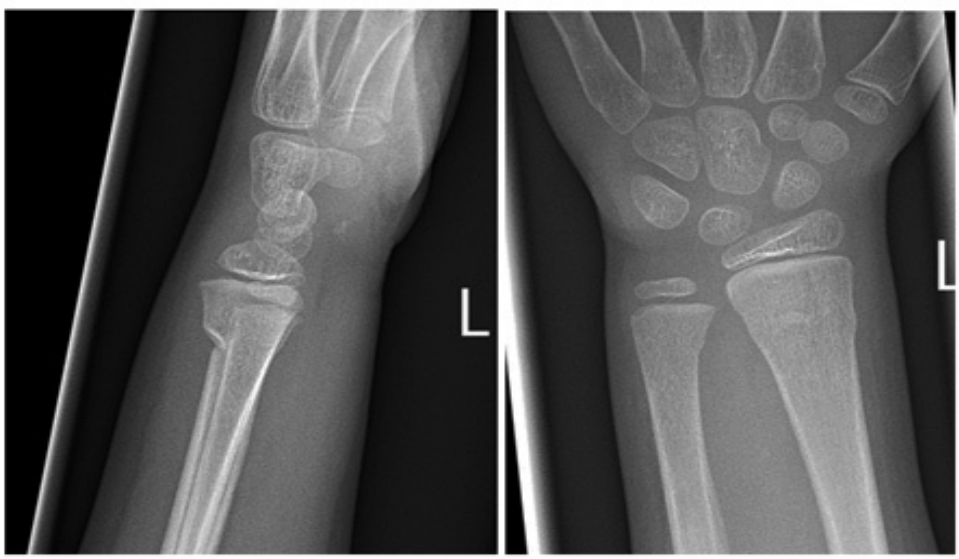

Figure. 2
Greenstick fracture of the distal radius in a child

The incidence of fractures in children varies with seasonal changes, with cultural and environmental factors and with age. Boys have a peak in fracture incidence at age 14 and girls at age 11. These incidence rates are only surpassed later in life at 85 years of age in women but never in men[32]. Most fractures in children occur in the upper extremities, followed by lower extremities, while less than 5% occur in the axial

skeleton. The most common fracture site in both genders is the distal radius (Figure. 2), accounting for 30% of all fractures in children[90].

Approximately two thirds of accidents leading to a fracture are due to low-energy trauma (falling <0.5m, most sports), 20% due to moderate-energy trauma (falling from 0.5–3 meters or falling from bicycle, swings or slides) and up to 10% due to high-energy trauma (fall > 3 meters, traffic accidents)[88].

In elderly (>65 years)

Osteoporosis is common in Sweden as a third of all Swedish women aged 70 to 79 years are expected to have osteoporosis when scanned at the hip with DXA[58]. The consequence of osteoporosis is a weaker bone, leading to an increased fracture risk. In Sweden there are 70 000 osteoporosis-related fractures each year, mainly caused by low-energy trauma[58]. The most common osteoporosis-related fractures are hip fractures (Figure. 3), closely followed by distal forearm and vertebral fractures[58]. Together they constitute almost two thirds of all osteoporosis-related fractures in Sweden[58]. Due to an increasing number of elderly in the population, the total numbers of hip fractures in Sweden are increasing, but the incidence, although the highest in the European Union, has remained relatively stable or even decreased in the last few decades [58, 109, 120].

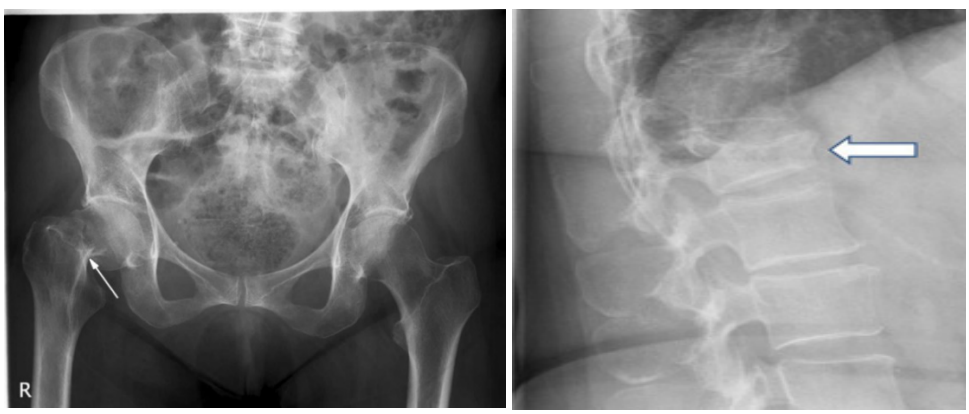

Figure. 3
Osteoporosis-related hip fracture (left picture) and vertebral compression fracture (right picture)

Bone tissue

The skeletal system includes a variety of vital functions. It is not only a framework for muscles and soft tissue but it also offers protection for vital inner organs, it acts as storage for minerals and lipids and blood cell production in the marrow cavity. The bones are complex, dynamic organs that constantly change and adapt to current demands. This also accounts for the actual mechanical load, since the skeletal properties change through modification of the architecture of bones as well as through an increase or decrease of bone mass based on the current load.

Each bone in the skeleton contains two forms of osseous tissue. Cortical (dense or compact) bone is robust and strong and always located on the surface of the bone (Figure. 4). Trabecular bone (cancellous or spongy) is more porous bone located in the interior of bone, primarily found in the metaphysis of long bones and in the vertebrae.

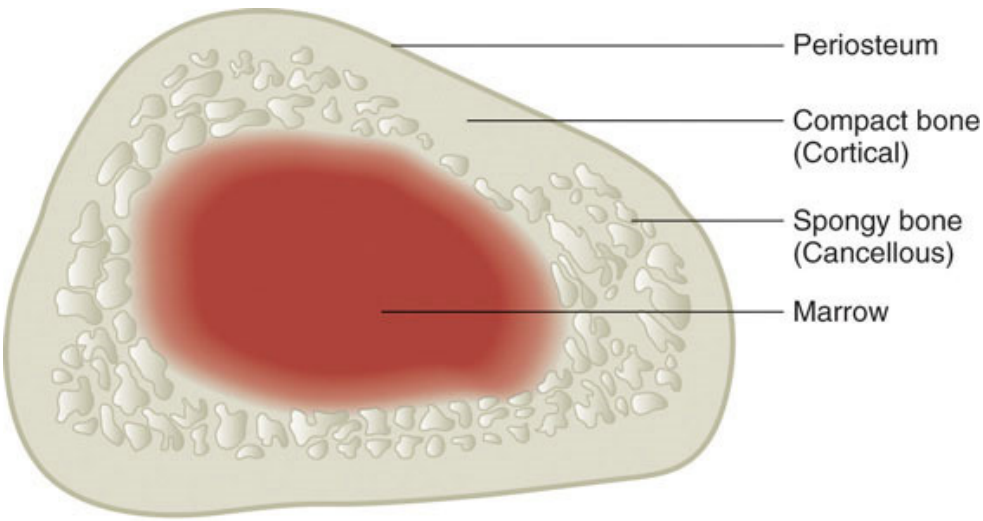

Figure. 4
Bone structure by courtesy of Georgetown Hospital System

Their relative proportions vary with the shape and region of the bone and also affect their resistance to fractures. Cortical bone constitutes 80% of the total bone mass while trabecular bone has a larger area (90%) that is exposed to the surrounding tissue and bone marrow. This makes trabecular bone more susceptible to a larger bone mineral turnover[95]. The superficial layer of the cortical bone is called periosteum, a structure which provides a route for circulatory and nervous supply and a region that actively participates in bone growth and repair. The endosteum, located at the inner surface of the cortical bone is a structure with an incomplete cellular layer, consisting of, among

other things, osteoprogenitor cells that are active during bone repair and remodeling. The composition of the matrix of the bone is the same in compact and trabecular bone but differs in the arrangement of osteocytes, canaliculi and lamellae.

Bone is further composed of cells and a matrix of extracellular protein fibers (predominantly type 1 collagen) which is mineralized by the deposition of calcium salts forming calcium hydroxyapatite, $Ca_{10}(PO4)_6(OH)_2$, a structure that gives the bone considerable rigidity and strength[123]. Calcium hydroxyapatite is hard but brittle, collagen fibers are flexible yet strong, and together they contribute to a strong and flexible unit with properties comparable to steel-reinforced concrete[95]. Their relative contribution together with the differing architecture of compact and trabecular bone gives bone its unique properties of being both light and strong[104].

There are three specific types of cells in the bone: osteoblasts, osteocytes and osteoclasts. Osteprogenitor cells are a type of mesenchymal stem cells that are few in number and can differentiate into osteoblasts. These cells are important in fracture healing and are located in the endosteum, the inner periosteum or the lining of vascular passageways in the matrix. Osteoblasts produce bone matrix that eventually surrounds the cells; a non-mineralized bone matrix is called an osteoid. Osteoblasts later promote the deposition of calcium salts, converting osteoid to mature bone. When fully surrounded by matrix, some of the osteoblasts develop into osteocytes, which account for 90–95% of all bone cells[82]. Each osteocyte occupies a lacuna and is sandwiched between layers of matrix, called lamellae (Figure. 5). Narrow passageways called canaliculi connect different lacunae with each other, and the dendrites of the osteocytes use these canaliculi to connect to each other in a large connecting network. These osteocytic structures regulate the protein and mineral content of the surrounding matrix. The dendrites in the canaliculi are embedded in fluids, and mechanical loading initiates currents in this fluid which transfer the loading signal to a cellular response. Osteoclasts, derived from monocytes, are large multinuclear phagocytic cells capable of eroding bone and are, along with osteoblasts, in charge of the constant turnover and restructuring of bone. Together, these cells form coupled units called basic multicellular units (BMU)[51].

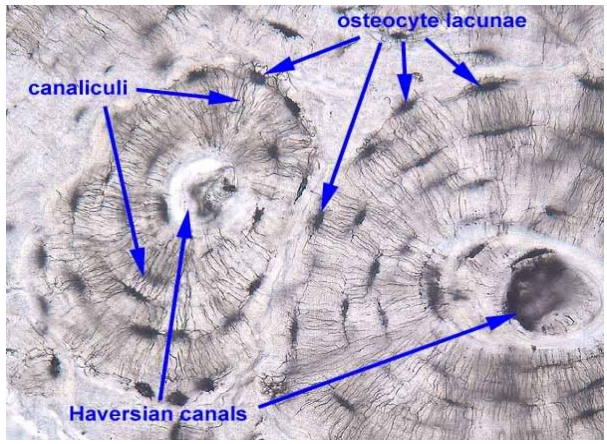

Figure. 5
Structure of an osteon

The basic functional unit of cortical bone includes an osteon (or Haversian system)[34] where octeocytes are arranged in concentric layers around a central canal, called the Haversian canal which contains small blood vessels (Figure. 6). The lamellae of each osteon are cylindrical and parallel so that they collectively resemble a bull's eye around the central canal. Haversian canals run parallel to the length of a long bone while the canals of Volkman extend perpendicular to the surface, supplying blood to osteons deep in the bone as well as in the bone marrow. Interstitial lamellae fill the spaces between the osteon in cortical bone. In trabecular bone, lamellae are not arranged in osteons. Instead the matrix forms rods and struts called trabeculae. These are thin branch structures which create an open network that involves a large area. Nutrients reach osteocytes through canaliculi that open into the surfaces of the trabeculae[123].

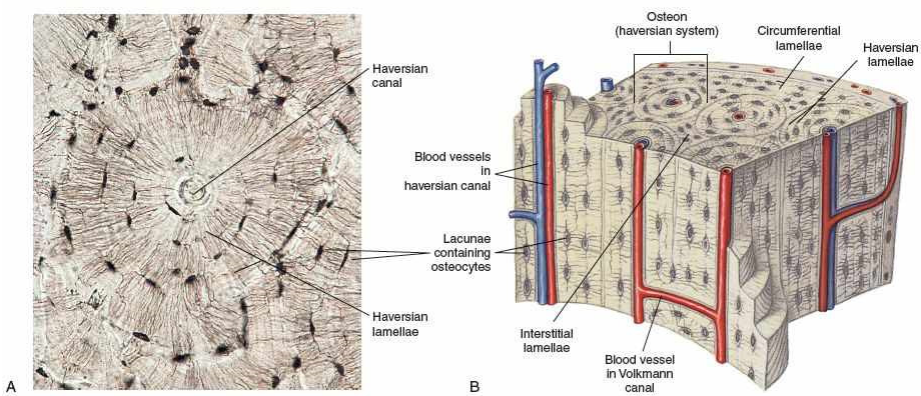

Figure. 6
Cortical bone structure and internal organization by courtesy of Academic, 2000-2014

Bone development

The growth of the skeleton determines to a large extent the size and proportions of the body. Changes in skeletal size and form that occur during growth are called modeling (Figure. 7). There are two major forms of ossification: (1) intramebranous, which occurs in the deeper layers of the dermis creating flat bones of the skull, mandible and clavicle, and (2) endochondral ossification, forming bones from a hyaline cartilage model. Most bones in the body form through endochondral ossification and the bony skeleton starts to take its form from the sixth week of gestation, at which stage the elements are entirely cartilaginous. Growth is achieved through the production of new cartilage at the surface (appositional growth) as well as the expansion of cartilage matrix (interstitial growth). Both types of growth result in a thickening of matrix leading to a deprivation of nutrients to the chondrocytes in the middle, as they rely on diffusion to obtain nutrients. Lucanae are formed in the middle as dead chondrocytes disintegrate. Bone formation then starts as fibroblasts migrate to the middle of the cartilage with penetrating blood vessels creating a primary center of ossification. Osteoblasts then migrate toward the end of the bone, toward the epiphysis consisting of growing cartilage. As the bone enlarges, osteoclasts appear, eroding trabeculae at the center of the diaphysis and creating a marrow cavity.

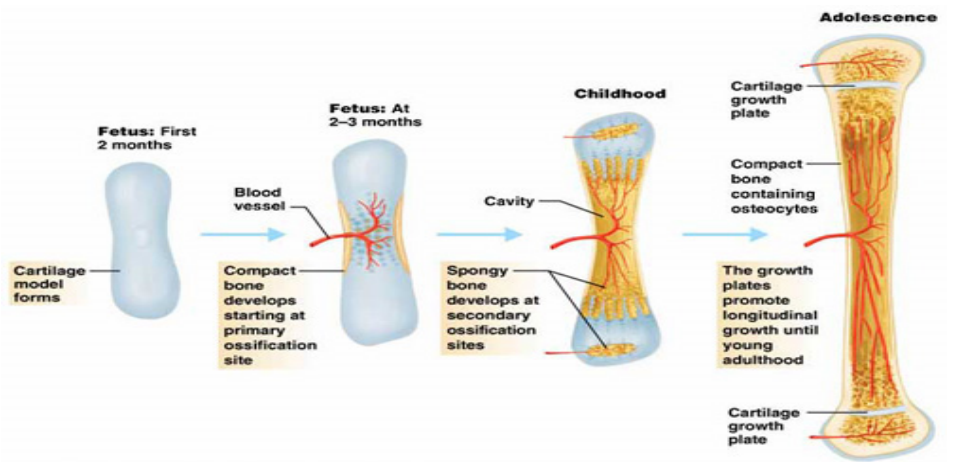

Figure. 7
Bone development by courtesy of Pearson Education Inc.

From here on growth involves both increase in length and enlargement in diameter through appositional growth. In the epiphysis a secondary ossification center develops during growth, creating an epiphyseal cartilaginar plate in the metaphysis. Both the osteoblasts and the cartilage are moving away from the ossification centers, and as long as they move at the same speed the bone grows in length.

Bone growth and peak bone mass

The rate of bone growth in a growing individual is not uniform. Growth is fast after birth, then decreases to a lower but linear rate that continues during the first decade in life[95]. During the pre-pubertal years growth is mainly regulated by growth hormone (GH) and insulin-like growth factor-1 (IGF-1), while in puberty the rising levels of sex hormones, testosterone and estrogen stimulate production of GH and IGF-1 as well as thyroid hormones. This results in a stimulation which increases the growth of bone[95]. Osteoblasts stimulated by the hormones differentiate and proliferate and begin to produce bone at a faster pace than the epiphyseal cartilage expansion. As a result, the epiphyseal growth plate gradually closes, ending longitudinal growth. During puberty there are obvious gender differences in the development, which result in boys ending up with stronger bones[14, 60]. The reason is the later onset of puberty in boys compared with girls, which creates a larger "window of opportunity", i.e. a longer time period to be receptive to growth factors. The bones in boys also increase in size and mass through periosteal apposition. The mass moves away from the neutral axis, creating a stronger bone since the strength of a tubular structure increases with the fourth power of the diameter[14, 21, 60]. In girls, estrogen levels increase during puberty and this hormone limits the periosteal bone formation. It however stimulates the endosteal formation, resulting in an increase of bone mineral content due to a cortical enlargement, but without positioning the mass further away from the neutral axis[21]. Bone mineral deposition continues after the longitudinal growth spurt. The most rapid accrual of bone mineral occurs between 11 and 14 years of age in girls and between 13 and 17 years of age in boys. During this period, the skeleton acquires 36% of peak levels in adult life, referred as PBM[8-9]. PBM is however reached at different ages in different skeletal regions. For example, the mineralization in the hip and lumbar spine ends during the late second or early third decade of life and in the distal radius as late as the third or fourth decade. In some locations such as the skull, slow increase in mineralization may continue throughout adulthood[60].

There are no prospective studies that have followed BMD from growth into old age, but estimations infer that 50% of the variance in BMD at age 65 could be predicted by PBM[69, 80]. Individuals with high bone mass at age 30 are likely to have high bone mass also at age 70[97]. A 10% increase in PBM is therefore predicted to delay the development of osteoporosis by 13 years[15] and as a result is believed to be a useful factor for predicting the development of osteoporosis. Genetic factors explain 50–85% of the variance in PBM[57, 108, 117] and the heredity is polygenic in nature[60]. At the same time it is important to understand that heredity and environment are not totally separable. For example, genetic factors influence processes such as the efficiency with which an individual utilizes and conserves the nutrients needed for bone building and maintenance (Figure. 8). On marginal intakes an individual genetically equipped with efficient utilization may come closer to the "full genetic potential" than one who utilizes nutrients

inefficiently. At high intakes, however, the two individuals may be indistinguishable. In this way, manipulation of an environmental factor such as diet may influence the expression of a genetic influence[60].

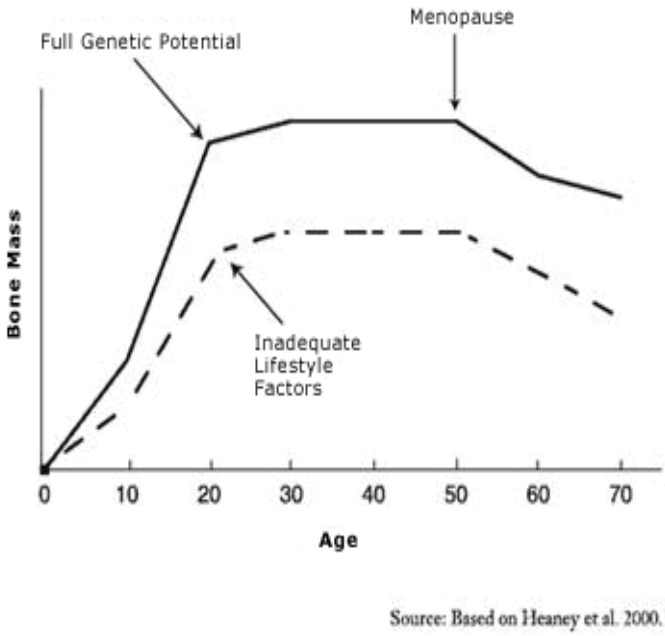

Figure. 8
Bone mass development, figure produced by Heaney et al[60]

Bone remodeling

There is a constant maintenance of bone as it is persistently renewed and recycled through a process called remodeling. Remodeling (Figure. 9) provides fresh bone but does not alter the shape or size of the bone. For normal bone maintenance, there is a need for adequate nutritional and hormonal factors. The turnover rate is also quite high, as up to 10% of the adult skeleton is replaced each year. The turnover rate is different in different bones but also different within a bone. For example, trabecular bone in the femur can be replaced two to three times a year, whereas the cortical bone in the diaphysis remains relatively unchanged[95]. Remodeling is a characteristic that involves osteoblasts, osteocytes and osteoclasts and enables the bone to adapt to

surrounding stress or micro-injuries. This can be beneficial, for example the effect of training on bone as it reacts by becoming thicker and stronger[76], but also degenerative when bone is not stressed, for example in immobilized patients. Actually, one third of bone mass can be lost during such conditions[95].

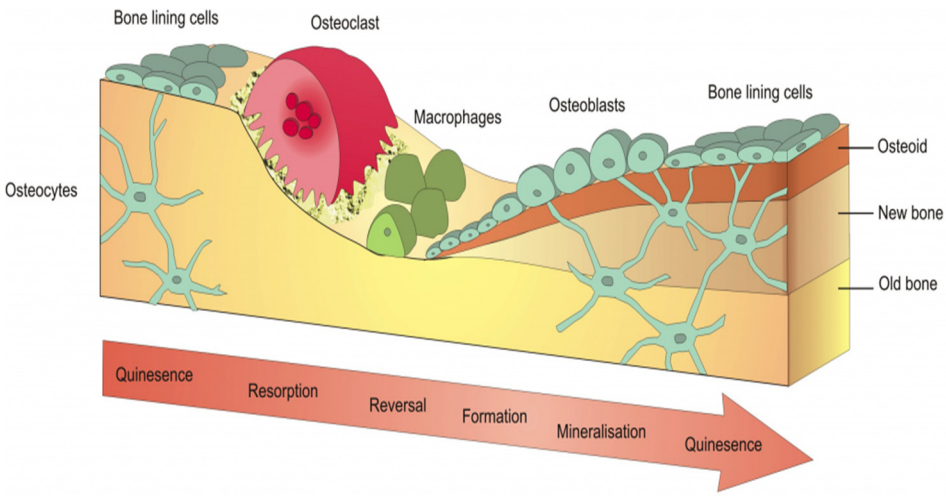

Figure. 9
Bone remodeling © Biomedical Tissue Research, University of York

Minerals

Even if smaller amounts of minerals such as magnesium, fluoride, iron and manganese are required in the bone building process, calcium is the most dominant mineral[95]. A sufficient supply of calcium is therefore essential for the bone-building capacity, and daily dose recommendations vary with age and gender. For example during pregnancy and lactation the need is higher. The amount of calcium in the body at maturity is approximately 1200 g and 1400 g in adult women and men, respectively. In men, this level remains relatively constant until the onset of age-related bone loss later in life, and in women until the onset of menopause[58]. Milk and dairy products are the main sources of calcium in the Nordic countries and adults are recommended 800 mg/day, pregnant women 900 mg/day and women during breastfeeding 1200 mg/day[58]. PTH and Vitamin D are the most important hormones regulating the concentration of calcium in plasma and are kept constant, within a narrow limit (2.1–2.6 mmol/L)[101].

Vitamins

Vitamins play an important role in bone formation and remodeling. Most important is Vitamin D3 (cholecalciferol), which is a steroid-like molecule that can be synthesized from 7-dehydrocholesterol in the skin under the influence of ultraviolet B light[95]. The basic requirement for vitamin D can be satisfied by exposing the skin to the sun. Experience demonstrates, however, that under the living conditions at the latitude of the Nordic countries (55° N–72° N), vitamin D deficiency can occur if the diet (mainly dairy products and fish) is devoid of the vitamin. Infants can develop rickets and elderly people can develop osteomalacia[64]. For this reason vitamin D is considered a micronutrient. Vitamin D is also a pro-hormone because it is converted to a hormone; the liver takes up vitamin D from the skin where it is transformed to 25OH vitamin D, from where it is further transported to the kidney and transformed to 1,25-dihydroxyvitamin D (calcitriol)[64]. Calcitriol is an important hormone that regulates calcium and phosphate ion absorption in the digestive tract and stimulates osteoblasts and the formation of matrix proteins[95]. Vitamin D regulates calcium homeostasis in an intricate interplay with PTH, where low vitamin D levels lead to low serum calcium, in turn stimulating PTH to release calcium from the skeleton[95].

Several other vitamins are involved in bone regulation. Vitamin C acts as a building block in enzymatic reactions in collagen synthesis and for stimulating the activity of osteoblasts[95]. Vitamin A also stimulates osteoblasts and is especially important for bone growth in children[95]. Vitamin K and B12 are also important in bone protein synthesis[95].

Hormones

The thyroid gland is important as it produces thyroxin which stimulates metabolism in cells and the rate of osteoblast activity. In the C-cells of the thyroid gland calcitonin is produced, which inhibits osteoclast activity and reduces calcium ion concentrations in body fluids. The parathyroid hormone (PTH) is produced in the parathyroid gland and works as the antagonist to calcitonin, as it elevates calcium ion concentration and stimulates osteoclast activity[95]. Growth hormone (GH) is anabolic and regulates osteoblast activity and synthesis of the bone matrix. Sex hormones also play an important role as estrogen and testosterone are osteoblast activators and osteoclast inhibitors. At puberty they affect the timing of epiphyseal closure, as men reach puberty later than women they have a longer time at growth, resulting in larger bones and higher PBM[60]. After menopause the loss of estrogen in women results in a decrease in activation of osteoblasts and inhibition of osteoclasts, resulting in higher resorption of bone. During the first postmenopausal years women lose 10–20% of bone mineral, at age 70 the rate of decline lessens to around 3% per decade and mimics the loss seen in men[2, 58, 121].

Bone development in adults and elderly

After PBM, resorption dominates over bone formation, resulting in an annual bone loss of around 0.5–1% until the age of menopause. Achieving a high PBM in young adulthood is therefore important since it predicts a relatively higher bone mass later in life, which results in fracture risk reduction[60]. The physiological process related to aging includes an endosteal resorption and a periosteal apposition of bone. In spite of the increasing bone width, the skeleton grows weaker due a decrease in BMD[4, 60]. Trabecular bone has a higher turnover rate due to a larger surface to mass ratio compared to cortical bone. The bone loss is therefore registered first in regions rich of trabecular bone, for example in the vertebrae or at the end of the long bones. This leads to a weaker skeleton and the increased risk of having a fracture is therefore first seen in trabecular regions (Figure. 10)[45].

Figure. 10
Normal and osteoporotic bone

Osteoporosis was first described in the early 1800's and was initially a generic term that summarized several different conditions which resulted in lower bone mass and changed bone architecture, leading to an increased risk of sustaining fractures[112]. In 1994 the World Health Organization (WHO) further defined osteoporosis as a BMD with a T-score of –2.5 standard deviations or more below the mean value of young healthy adults. It should be noted that the WHO definition was only stated for women and with measurements done with dual-energy X-ray absorptiometry (DXA)[1]. The diagnosis of osteoporosis in children and adolescents does not have this strict definition but is instead based on DXA results as well as a significant fracture history, defined as one of the following: (i) a vertebral compression fracture, (ii) one lower extremity long bone fracture, or (iii) two or more upper extremity long bone fractures[12]. When measuring bone mass in growing individuals the BMD scores should be compared to reference data for the same sex and age (Z-score). Low BMC or BMD in children is defined as a Z-score equal to or less than –2.0[12]. Osteoporosis, however, is a rare diagnosis in children and adolescents, and is often secondary to an underlying medical disorder or medications used to treat the disorder.

Osteoporosis is sometimes divided into primary osteoporosis, a condition caused by natural aging, menopause and lifestyle factors, while secondary osteoporosis is caused by diseases and medications that lead to low BMD. In adults several risk factors for

osteoporosis have been identified. These risk factors could be further divided into modifiable and non-modifiable (Table 1). All risk factors could be used to predict high-risk individuals for developing osteoporosis, but the modifiable is also possible to influence by intervention strategies.

Table 1.
Examples of risk factors for osteoporosis

Non-avoidable		Avoidable	
High age	Heredity	Physical inactivity	Smoking
Earlier fracture	Ethnicity	Low weight/BMI	Alcohol consumption
Female gender	Stature	Cortisone treatment	Low sun exposure
Age of menopause		Low bone mass	Reduced visual capacity
		Agility	Low vitamin D and Ca intake

Although low BMD is highly correlated to fracture risk, osteoporosis itself makes an individual more prone to sustain a fracture but does not cause them. High age is the most important risk factor for osteoporosis. But age is also, independently of BMD, a risk factor for fractures. Other risk factors for fractures worth mentioning are falls, impaired vision, several medicines (e.g. long-lasting benzodiazepines and psychoactive drugs), low muscle strength, neuromuscular function and balance. As osteoporosis is highly associated with both falls and fractures, most risk factor are the same for these conditions[58]. Based on this knowledge, WHO has developed an epidemiologically derived risk-assessment tool called the Fracture Risk Assessment Tool (FRAX). The model has been constructed and validated using primary data from population-based cohorts around the world and is frequently updated as new validated risk indicators for fractures become available[99]. It is based on individual patient models that integrate the risks associated with different clinical risk factors with or without BMD. The output is a 10-year probability of sustaining a major osteoporotic or hip fracture. FRAX therefore provides physicians with a way to effectively choose candidates for therapy[99].

Preventing fractures and treatment of osteoporosis

Preventive strategies for fracture risk reduction include lifestyle recommendations regarding nutrition, alcohol intake, physical activity and smoking. As falls are the most common accident in society and as there is an increase in absolute numbers of fallers in society[58], mainly due to the fact that the population is getting older, fall prevention strategies to create a safer environment are of great importance. Examples of such strategies includes walking aids, reduction of medications that cause dizziness, reduction of multi-pharmacy, implementation of exercise programs to improve neuromuscular function and balance, reduction of home hazards, eyesight controls and anti-slip shoe devices in slippery areas[78]. A structured approach, aimed especially at high risk groups, has been shown in randomized controlled trials (RCT) to reduce both the number of falls and the number of fallers[52], and this accounts both for individuals living in society and institutionalized individuals[22, 52]. Another fracture-prevention device that has been shown to be effective in RCT is hip protectors[52], but the problem with this method is the adherence of the patient.

There are also pharmacological treatments that not only prevent or reduce the progress of osteoporosis but actually increase BMD. Today there is a common use of supplementary Calcium and Vitamin D. The next line of treatment is drugs that reduce the resorption of bone such as bisphosphonates, estrogens, selective estrogen receptor modulators (SERM) and selective antibody blockers. The only current drug that stimulates bone formation is parathyroid (PTH), which is given intermittently.

Biomechanical aspects of bone

Bone successfully combines seemingly contradictory mechanical properties such as being stiff yet flexible and light yet strong. It is determined by bone's material composition and how the material is fashioned into a three-dimensional structure with geometric properties that confer structural strength. Bone has a unique advantage compared to man-made load-bearing material as it can adapt in response to changes in demands[24]. The strength of bone is determined by its resistance of breaking when exposed to external mechanical forces creating either compressive, tensile or shear stresses on bone. Engineers have studied this in detail using load-deformation tests where bone is loaded and its deformation is recorded as a function of the applied load (Figure. 11). The typical load and deformation are linearly proportional until the yielding point. To this point the deformation is plastic, i.e. can return to its original form when unloaded. After the yielding point the structure begins to permanently deform, causing micro fractures, finally reaching the failure point that results in a complete fracture[93].

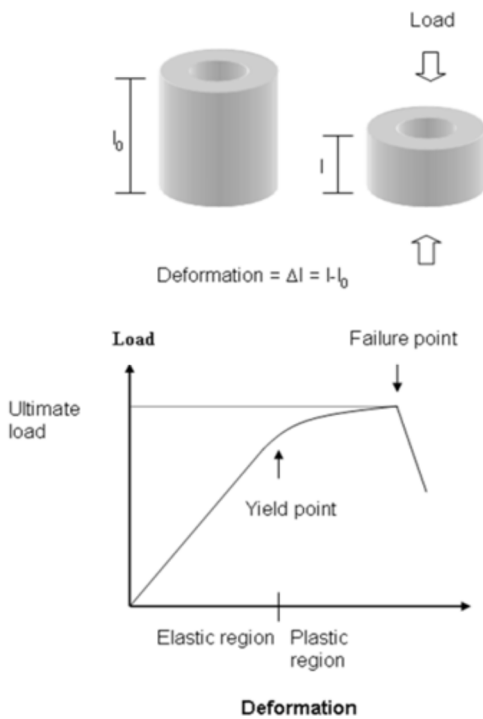

Figure. 11
Load-deformation curve. By courtesy of Henrik Ahlborg

The geometry of a bone is important in respect of the ability to withstand stress. A wider bone withstands bending and shear forces better than thin bone. Long bones shift the cortical shell outward using a marrow cavity. The displacement of the cortical shell from the neutral axis increases bending strength, and as the area increases without increasing the amount of building material, the weight stays low[24]. In trabecular bone, spring-like shock absorbers, in which stiffness and peak load-bearing are sacrificed for flexibility, show an open-celled porous cancellous structure able to deform and return to its original size and shape without cracking[114]. Size is important in trabecular bone too, as it has been shown that larger vertebral cross-sectional area withstands compressive forces better than smaller vertebral bodies[24].

Bone mass measurements

Bone mass is a general term without specific definition. The expression includes: (i) bone mineral content (BMC; g), which is one-dimensional and refers to the amount mineral detected when scanned, irrespective of width and depth, or (ii) bone mineral density (BMD), which can either be presented as areal BMD (aBMD; g/cm^2), which is two-dimensional and refers to bone mineral detected over a projected area, or (iii) volumetric BMD (vBMD; g/cm^3), which is three-dimensional and takes both width and depth of a bone into account (Figure. 12). However, it is important to realize that this too is only an estimate of the true density since these scanning techniques do not take the porosity of the bone into account. In adults and in clinical practice aBMD is the preferred and most frequently used variable. In children and adolescents who constantly increase in bone size, BMC and bone size are often reported separately[59]. The current standard for reporting DXA results is the aBMD Z-score, which provides an estimate of the SD(s) away from the mean for chronologic age and sex[35]. Measurements can be performed in the total body or in specific regions of the skeleton such as the spine, hips, legs and arms. Furthermore, since small children have a proportionally larger head than older children, instead of total body bone mass (TB), total body bone mass less head (TBLH) is nowadays recommended, meaning all skeleton excluding the head.

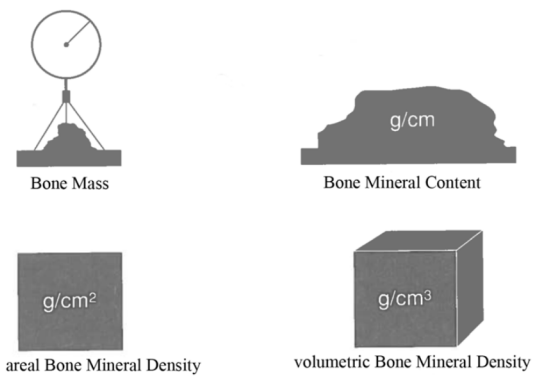

Figure. 12
Definition of bone mass properties. By Courtesy of the Swedish Council of Technology Assessment in Health Care

To assess whether a patient has high or low bone mass requires a method of high accuracy. Accuracy is defined as the difference between a measured value and its true physical value. When longitudinal changes are to be evaluated, a method of high precision is required. Precision is the ability to make reproducible measurements without regard to their accuracy (Figure. 13); and can be determined by duplicate measurements on a phantom or a patient. High precision requires an exact repositioning of subjects and a stable method without long-term drift.

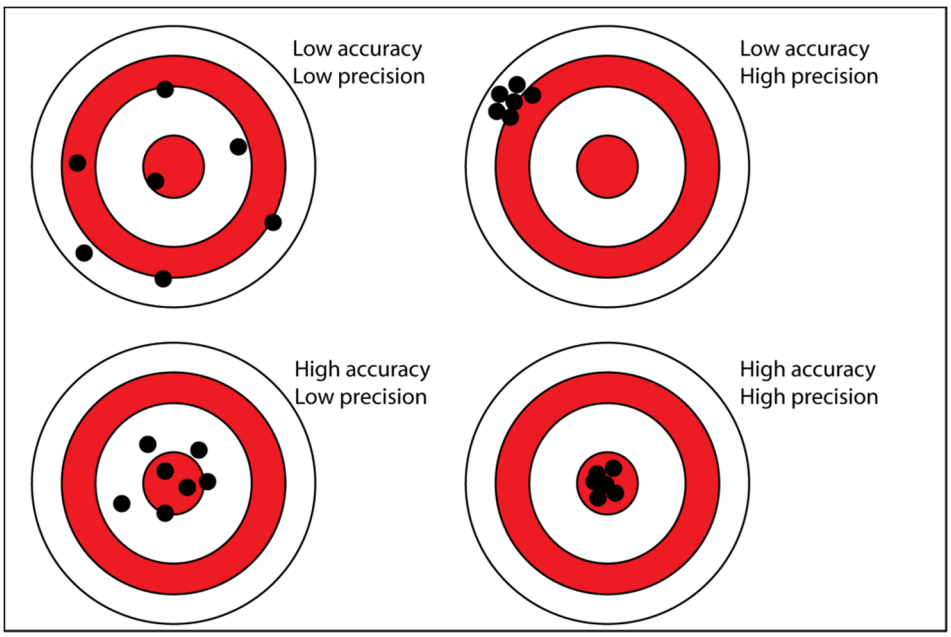

Figure. 13.
Illustration of precision and accuracy in bone densitometry. By courtesy of the Swedish Council of Technology Assessment in Health Care

Initially, ordinary radiographs were used to assess the mineralization of bone. But this method only captured large deficits in bone mass. Due to this there was a development of various measuring techniques, some utilizing ionizing methods while others were non-ionizing, all with the aim of reflecting the amount of bone mineral in the bone. Examples of non-ionizing methods are either magnetic resonance imaging (MRI) or quantitative ultrasound sonography (QUS). The ionizing radiation can either be from X-rays or gamma radiation using different isotopes.

Single-photon absorptiometry (SPA)

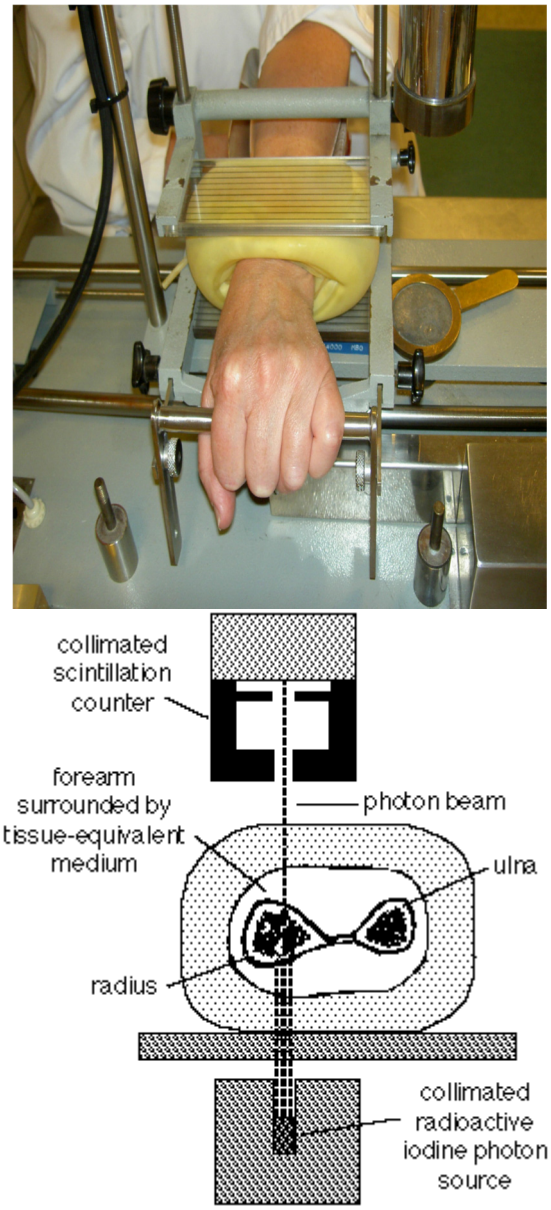

Figure. 14
Measurement of bone mineral density in the distal forearm using single-photon absorptiometry (SPA).

Single-photon absorptiometry (SPA) was invented in the early 1960's by Cameron and Sorensen[23] and was soon thereafter followed by Nilsson et al in Malmoe, Sweden[106]. The technique was able to estimate the amount of mineral in the bone non-invasively and in vivo, and the method revolutionized bone research at the time. The radiation source was usually Iodine-125 or Americium-241, where a detector measures the radiation that is going through the bone in relation to the radiation going through the soft tissue or water surrounding the wrist. The calculation of the thickness of mineral in the pathway of the beam depends on the assumption that the thickness of the soft tissue measures is constant. Since there is a low degree of soft tissue around the wrist, this is ensured by a cuff of the same density as the soft tissue placed around the measured wrist. The thickness of the mineral can then be estimated by calculating the relation between the absorption in the soft tissue and the bone tissue. This method is limited to measuring the appendicular skeleton, most frequently the forearm. Areal BMD (g/cm^2) is calculated by dividing the BMC by area.

The SPA technique has a 9% accuracy and a 1–2% precision[106] the high precision, especially at the cortical site of the forearm, makes the method reliable for the long-term assessment of changes of bone mass[7]. The method has also been shown to give reliable estimates of fracture risk at population level[37].

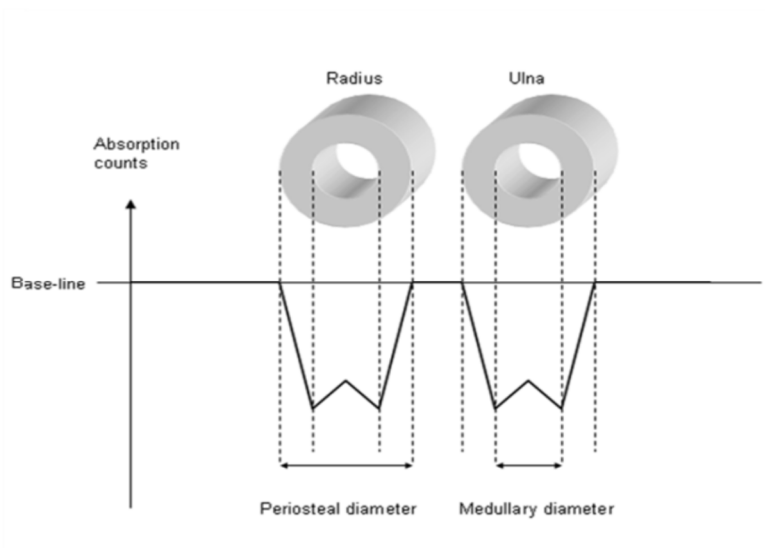

Figure. 15
The graphical representation of the output of a SPA scan. The absorption counts indicate how much radiation passes through the tissues, i.e. the radiation that is not absorbed. The baseline represents attenuation in the cuff and soft tissues, which is subtracted when calculating the density of the bone mineral. The trace also marks the contours of the bones and thus allows estimation of the total width, medullary width and cortical thickness. By courtesy of Henrik Ahlborg.

Dual-photon absorptiometry (DPA)

Dual-photon absorptiometry (DPA) is an advance on the SPA which uses two photon sources, also making it also possible to measure the central parts of the body such as the spine or hip, without the need to submerge the measured skeletal part in water. DPA has to a large extent been replaced by DXA.

Dual-energy X-ray absorptiometry (DXA)

Dual-energy X-ray absorptiometry (DXA) was introduced in 1987[36] and has become the "gold standard" for clinical bone densitometry techniques. Photons are produced from a low-dose X-ray tube instead of from radionuclide source. Two peak energies are produced and are selected to optimize separation of the mineralized and soft tissue components of the sites scanned, enabling accurate measurement of the axial skeleton such as the spine or hip and also total body. The higher photon flux produced by X-rays increases scanning speed, produces lower radiation and enhances the spatial resolution. This creates a higher accuracy (3–9% depending on the measured site)[58] than for radionuclide sources, while the precision (0.7–1.3)[7] is comparable to that of SPA. The method is considered safe, as the effective radiation dose received by the patient during a scan is 1–8 μSv, corresponding to 1/1000 of yearly background radiation[58]. DXA is the preferred method for assessing BMC and aBMD in children and the recommended scanning sites are the total body less head (TBLH) and the posterioanterior lumbar spine. However, data from the Bone Mineral Density in Childhood Study (BMDCS) suggest that age-related precision of the total hip and femoral neck is comparable to both that of the spine and TBLH[35].

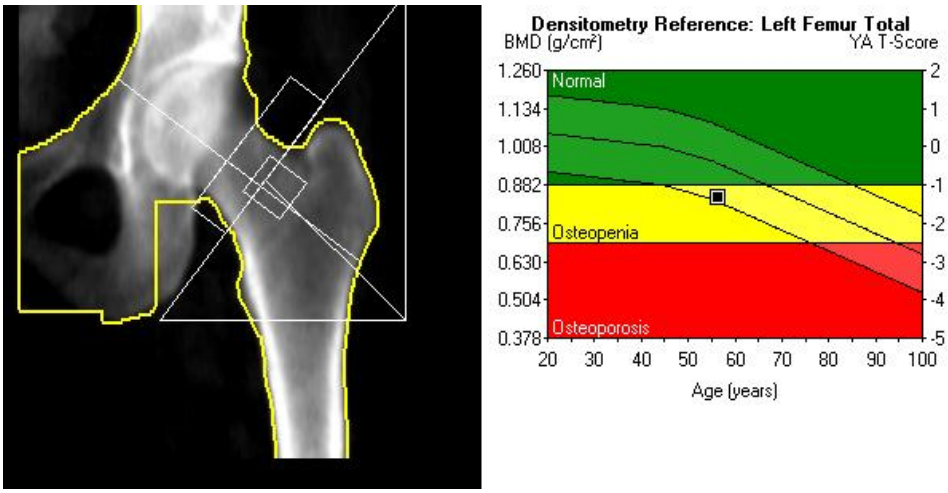

Figure. 16
Example of DXA output of a hip scan

Quantitative ultrasound (QUS)

Quantitative ultrasound (QUS) uses the speed of sound (SOS; m/s) to reflect, at least partly, the architecture and elasticity of bone and broad band attenuation (BUA; dB/MHz) to reflect the density of the bone. Hence no ionizing radiation is used. QUS has been proposed to give reliable estimates of skeletal traits but cannot discriminate between cortical and trabecular bone[58]. It is also limited to peripheral measurements, most commonly the calcaneus, as bones covered by a thicker layer of soft tissue cannot be examined[58]. The precision is about 1.5–6%[58] and the predictive ability for fractures using QUS is similar to that of DXA[53].

Figure. 17
A quantitative ultrasound apparatus

Peripheral Computed Tomography (pQCT)

Peripheral Computed Tomography (pQCT) uses a technique where X-rays and detector rotate around the measured subject, giving a three-dimensional (3D) image of the bone and enabling visualization of the microarchitecture, distinguishing cortical and trabecular bone. pQCT is used in the appendicular skeleton, such as the radius or tibia where the radiation dose is relatively low (<10 µSv)[39]. Central measurements in children are not acceptable as the radiation dose can be up to 250 µSv[39, 58]. With the recent introduction of high-resolution pQCT systems (HR-QCT), even more refined assessments are possible, with estimations of the micro-architectural properties such as trabecular size and number. The precision of pQCT is 0.3 to 2.2% and for high-resolution pQCT, 0.3 to 3.9%[7] .

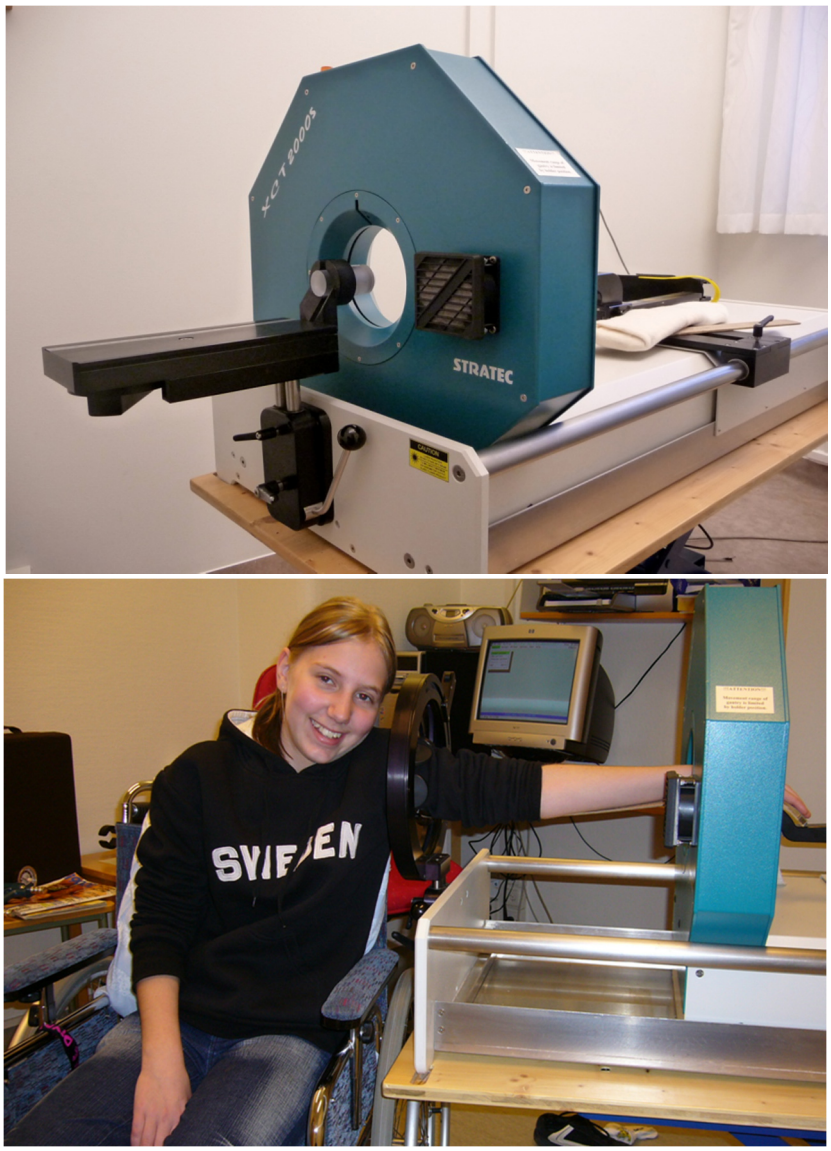

Figure. 18
pQCT machine used in Malmö. By courtesy of Bjarne Löfgren

Magnetic Resonance Imaging (MRI)

Magnetic Resonance Imaging (MRI) is another three-dimensional technique that has been used for research purposes when evaluating bone traits. The advantage compared to pQCT is that the method does not use ionizing radiation. The disadvantage so far is high cost and low availability.

Biochemical analysis of bone markers

There are several biochemical markers of bone metabolism that are released from the cells and matrix during the remodeling of bone. Both formation and resorption of bone create traceable markers circulating in the blood that are later released through urine. Formation markers (Table 2) are found in serum and are mainly derived from osteoblast activity during the formation of matrix. Resorption markers (Table 2) can be analyzed both in serum and in urine and are products of type 1 collagen breakdown. It is hoped to be possible to find markers that could help us to predict which individuals are at risk of osteoporosis and also to evaluate whether treatment of osteoporosis and fractures has any effect. The results of research so far show a correlation in postmenopausal women between bone markers and low bone mass, especially in markers of resorption where high levels are correlated with low bone mass. The variance, however, is so large that it is difficult to draw any conclusions for the specific individual[58]. No bone markers were evaluated in this thesis.

Table 2.
Overview of biochemical bone markers

Bone markers	
Bone formation	Procollagen Type 1 N-terminal propeptide (s-PINP), Alkaline phosphatase (s-ALP) Procollagen Type 1 C-terminal propeptide (s-PICP), Osteocalcin
Bone resorption	Cathepsin K, Bone sialoprotein (s-BSP), Cathepsin L, total Pyrodinoline (u-PYD), free deoxypyridinoline (u-DPD), Tartrate resistant alkaline phosphatase (TRAP), Ctelopeptide cross-link of type 1 collagen (CTX)

Aims of the thesis

The aim of this thesis was to evaluate bone mass from childhood to adulthood. Specifically we wanted to answer the following research questions:

1. Does axial and appendicular bone mass track from childhood to peak bone mass?
2. Is there a gender discrepancy in the level of "tracking" in bone mass?
3. What are the sensitivity and specificity of a pediatric bone scan to predict low peak bone mass?
4. Is movement from one BMD quartile in childhood to another in adulthood due to different accrual of bone mineral or different gain in bone size?
5. Is a fracture in childhood associated with low adult BMD?
6. Is preterm birth AGA and/or SGA a risk factor for low adult BMD?

Hypothesis

We hypothesized that a childhood bone mass measurement would correlate with adult bone mass measurement and that the correlation between adult bone mass values ought to be higher in old than young children. We also hypothesized that a fracture in childhood and premature birth would be associated with low BMD in young adulthood.

Material and methods

The Landin follow-up study (Papers I, II, IV)

Between the years 1979 and 1985, Dr. Lennart Landin invited 296 children in Malmö, a city in southern Sweden, to a skeletal evaluation performed by single-photon absorptiometry (SPA). The children were recruited as three separate cohorts. One consisted of reference children used as a control cohort, the second of children with a recent fracture and the third of children who were born preterm. The children were measured at a mean age of 10 years (range 3 to 17). The baseline data has been presented by Landin in three separate articles between 1981 and 1985[63, 86-87]. No follow-up measurements were planned at study start, but from 2006 to 2009 our research group initiated follow-up examinations of all subjects who agreed to be re-measured. Out of the 296 original participants, 214 were re-measured with the same SPA apparatus a mean 28 years (range 25 to 29) later when they were at a mean age of 37 years (range 28 to 44). At the follow-up, measurements were also performed by DXA, pQCT and QUS. At the initiation and baseline of the study there were no requirements for Ethics Committee approval. This was later obtained before follow-up (Lund University (LU 646-02; October 16, 2002) and the study was conducted according to the Helsinki Declaration of 2000. The original cohorts are described below:

1. The control cohort consists of 65 boys and 66 girls with a mean age of 10.8 years (range 4–16) where none of the children at the baseline examination had had recent fractures, signs or symptoms of metabolic disease, malnutrition or other stigmata that could be expected to influence their bone mineral content or growth. Most of the children were volunteers from kindergarten or school, but a few were also patients without any conditions known to be associated with changes in bone mass or growth.

2. The fracture cohort consists of 57 boys and 33 girls with a mean age of 10 years (range 3–16). Scans were performed 40 ± 25 days (mean ± SD) after they had sustained a fracture. All types of fractures were accepted, except hand, finger, skull, tooth and rib fractures. Fifty-five children were reported with a fracture due to low-energy trauma, 31 due to moderate-energy trauma and 4 due to high-energy trauma. All children with a fracture were without diseases or medications known to affect bone metabolism and none were malnourished or had impairment in growth.

3. The preterm cohort consists of 44 boys and 31 girls with a mean age of 10 years (range 4-17) who had been born premature. They were further classified according to the growth charts compiled by Karlberg et al[75]. as born preterm, appropriate for gestational age (AGA) or small for gestational age (SGA). All infants were born at Malmö General Hospital (now called Skåne University Hospital (SUS) in Malmö) between 1964 and 1979, all were without disease or medication known to affect bone metabolism. None were malnourished, had chromosomal abnormalities or were when included considered to have any intrauterine growth retardation[63].

Drop-out evaluation

The drop-out analyses in papers **I, II and IV** revealed that age, height, weight, body mass index (BMI), gender distribution and lifestyle factors were similar in the three cohorts. Children in the control cohort who did not participate in the follow-up examination were older than those who did (11.7 ± 4.0 versus 10.2 ± 3.6 years, p=0.04). In all three cohorts no other differences were seen in those individuals who attended the follow-up examination and those who did not (data not shown).

Measurements

Single-photon absorptiometry (SPA)

Bone mineral content (BMC; g) and bone mineral density (BMD; g/cm^2) were measured in the distal forearm both at baseline and at follow-up. The scan was at baseline done on a level corresponding to 25% of the ulnar length measured from the tip of the ulnar styloid and at follow-up 6 cm proximal to the ulnar styloid process. The apparatus constructed by Professor Bo Nilsson in 1964 was used for all bone measurements with replacement only of the radiation source in 1980. Both arms were scanned, after which the mean value was used. In individuals with a history of a forearm fracture, the non-fractured arm was used. The coefficient of variation (CV) was 2% with a standardized phantom and 4% after repeated measurements in 14 subjects after repositioning. The long-term drift during the study period, evaluated by a standardized phantom, was 0.1% per year (95% CI –0.2 to 0.4)[4]. Since the radiation source was replaced in 1980, all bone mass measurements thereafter were recalculated with a correction factor provided from the phantom measurement data. One technician performed all baseline measurements, another all follow-up measurements and one of the authors analyzed all plots.

Dual-energy X-ray absorptiometry (DXA), peripheral computed tomography (pQCT) and quantitative ultrasound (QUS)

BMC and BMD were also measured at follow-up by DXA (Lunar® DPX-L scanner, software version 1.3z; Lunar, Madison, WI, USA) in total body by a total body scan, in the first to fourth lumbar vertebra (L1–4) by a lumbar spine scan and in the femoral neck and total hip by a hip scan. Daily calibration of the apparatus was done with the Lunar® phantom. The CV evaluated in 14 individuals after repositioning was 0.4% to 3.0% for BMD depending on the measured region. QUS evaluated broadband ultrasonic attenuation (BUA; db/MHz) and speed of sound (SOS; m/s) in both calcanei, after which the mean value was used. The CV evaluated in 14 individuals after repositioning was 2.2% for BUA and 0.3% for SOS. pQCT (pQCT; XCT 2000; Stratec, Pforzheim, Germany) measured BMD (g/cm^2), Cross-Sectional Area (CSA; mm^2) and Stress Strain Index (SSI, mm^3) in the left radius and left tibia. We measured at the 4% and 38% level from the ankle joint and at 6% and 66% level from the wrist. Daily calibration of the apparatus was done with a standard phantom. The CV evaluated in 14 individuals after repositioning was 1.1 to 4.6% for CSA and 0.3 to 1.2% for BMD depending on the measured region. Three research technicians performed all the DXA, QUS and PQCT measurements and analyzed all the scans.

Anthropometrics and questionnaires

Body weight was measured to the nearest 0.1 kg with an electric scale and body height to the nearest 0.5cm by a wall-tapered height meter. Questionnaires registered lifestyle factors, diseases and medications both at baseline and at follow-up. Body mass index was calculated as weight divided by height squared (kg/m^2).

The Pediatric Osteoporosis Prevention (POP) Study (Paper III)

The *Bunkeflo study* or in English *the Pediatric Osteoporosis Intervention (POP) study* was initiated by our research group in 1999. The POP study is a population-based, prospective, controlled exercise intervention study, which originally was designed to prospectively assess musculoskeletal development in response to a school-based physical activity program in children aged 6 to 9 years at baseline and through the compulsory school years. Four government-funded schools in the same socioeconomic area in the city of Malmö in southern Sweden were invited to participate in the study. At baseline, the school curriculum in children assigned to the physical activity intervention group was changed from the Swedish standard of 60 min of physical education per week to 40 minutes per day (200min per week). The control cohort continued with an average of 60 min provided as 1 to 2 lessons of physical education per week. The lessons were led by the regular teachers and included general activities within the standard school curriculum such as ball games, running, jumping and climbing activities. As school physical education is compulsory, all children had to participate. All students participated at a level so that they acquired a "pass" grade in the subject physical education. During vacation periods, no additional exercise training was provided. The study was conducted according to the Helsinki Declaration of 2000 and was approved by the Ethics Committee of Lund University (LU 453-98; September 15, 1998). Informed written consent was obtained from parents or guardians of all participating children before study start.

Drop-out evaluation

Age, height, weight and body mass index (BMI), bone traits, gender distribution and lifestyle factors were similar at baseline in the intervention and the control schools. Furthermore, at baseline these traits were also similar in children who attended the follow-up and those who did not (data not shown). Finally, since we found no statistically significant difference in the correlations of bone mass at study start and adulthood between the intervention and the control groups (data not shown), all data were pooled.

Measurements

There was one intervention school and three control schools. All students who started the first grade in the intervention school from 1998 to 2000 and in the control schools from 1999 to 2000 were invited to have their femoral neck, total body and lumbar spine bone mineral content (BMC; g), bone mineral density (BMD; g/cm^2), and bone area (cm^2) measured by DXA. All participants were without any diseases or medications known to affect bone metabolism, and all were of Caucasian ancestry. The study protocol included yearly bone scanning until age 15 years, then an additional follow-up at age 19 years (range 18–19). This rendered a mean follow-up period of 11 years (range 10–12). To be included in this report, participants had to participate in the first and the last measurements. Baseline measurements were done at a mean age of 8 years (range 6–9) when all children were pre-pubertal in Tanner stage I. The second follow-up in this report was done a mean 5 years (range 4.8–5.2) later when the children were a mean 13 years old (range 12–13). At this measurement, 5% were in Tanner stage I, 49.5% in Tanner stage II–III and 42.9% in Tanner stage IV–V. The third (and last) measurement in this report was done a mean 11 years old (range 10–12) after the baseline examination when the participants were mean 19 years (range 18–19) and all in Tanner stage V. The last follow-up corresponds to the age when femoral neck peak bone mass is reached in our target population[5], while PBM in TB and L1–4 has been shown to occur at higher ages[5].

Dual-energy X-ray absorptiometry (DXA)

Bone mineral content (BMC, g) and bone mineral density (BMD, g/cm^2) were measured by DXA (DXA; DPX-L version 1.3z; Lunar, Madison, WI) in the femoral neck (FN) by a standard hip scan, total body (TB) by a total body scan and first to fourth lumber vertebra (L1–4) by a standard lumbar spine scan. The area (cm^2) of FN and L1–4 was estimated at each scan. For the last measurement we changed scanner from DPX-L to a Lunar Prodigy, Compaq DP, cross-calibration was performed and the last measurements were corrected accordingly. Our research technicians calibrated the machine daily with a Lunar phantom and performed all measurements and all software analyses. The coefficients of variation (CV %), evaluated by duplicate measurements in 13 healthy children, in the measured regions were BMD 1.4–3.8%, BMC 1.3–3.2%, area 1.1–2.2%.

Anthropometrics, maturation and questionnaires

Height (Holtain stadiometer) and weight (Avery Berkel HL120 electric scale) were measured by standard equipment repeatedly in all children in the subgroup when wearing light clothes without shoes. Body mass index (BMI) was calculated as weight divided by height squared (kg/m^2). Pubertal maturation was assessed by our research

nurse in younger ages and self-assessed with the assistance of our research nurse if problems arose in older ages. This method has been validated by Duke et al[42]. Questionnaires registered lifestyle factors, diseases and medications both at baseline and at follow-up.

Study subjects

Paper I

Out of the original 221 participants in the fracture and control cohort, 157 were re-measured a mean 27 years (range 25–29) after the baseline evaluation. This corresponded to a participation rate of 71%. The participation rate in the fracture cohort was 47/57 (82%) boys and 26/33 (79%) girls and in the control cohort 41/65 (63%) boys and 43/66 (65%) girls. Two men and 1 woman had then died, 13 men and 7 women had relocated, 12 men and 15 women could not be found, 7 men and 6 women declined further measurements due to unwillingness to participate and 1 man was unable to attend due to illness. One additional fracture case was excluded since we were not able to verify the type of trauma severity. In the re-measured fracture cohort, 28 boys and 19 girls had experienced the index fracture due to a low-energy trauma, 16 boys and 7 girls due to a moderate-energy trauma and 3 boys and no girl due to a high-energy trauma.

Paper II

Of the original 296 participants in the fracture, control and preterm cohorts, 214 were re-measured 28 years (range 25–29) after the baseline evaluation. This corresponds to a participation rate of 72%. The participation rate was 41/65 (63%) boys and 43/66 (65%) girls in the control cohort, 47/57 (82%) boys and 28/33 (85%) girls in the fracture cohort and 31/44 (70%) boys and 25/31 (81%) girls in the premature-born cohort. Among the non-participants, 5 men and 2 women had died, 13 men and 9 women had relocated, 19 men and 15 women could not be located, 9 men and 8 women declined further participation, and 2 men were unable to attend due to illness. The two girls in the fracture cohort with missing trauma severity and the 9 children who was erroneously classified as being born preterm was included in this study.

Paper III

Out of the 338 children in the POP study at baseline, 121 underwent the last measurement a mean 11 (range 10–12) years later. This corresponds to a participation rate of 36%. The participation rate was 65/189 (34%) in boys and 56/149 (38%) in girls. Among the drop-outs, 1 boy and 1 girl had died, 20 boys and 15 girls had relocated, 44 boys and 24 girls could not be located, 58 boys and 51 girls had declined further participation during the study period, and 1 boy and 2 girls were excluded due to low age or growth hormone medication. Seventy-eight of the original 207 individuals (38%) from the intervention school with daily school physical education attended the last follow-up and 43 out of the original 131 individuals (33%) in the control school.

Paper IV

Out of the original 206 participants in the preterm and control cohorts, 130 were re-measured after a mean 27 years (range 22–29). This corresponds to a participation rate of 63%. The participation rate was 41/65 (63%) boys and 43/66 (65%) girls in the control cohort and 26/44 (59%) boys and 20/31 (65%) girls in the preterm-born cohort. At follow-up, five men and one woman from the original study had died, 11 men and 12 women had relocated out of our region, 20 men and 15 women could not be located or declined further participation, 2 women were unable to attend the follow-up examination due to illness, 1 man had missing baseline data and 4 men and 5 women originally classified as preterm were found to be born full-term (born > 37th week) when we re-examined the birth cards.

Statistical analysis

We used SPSS® version 18.0, 19.0 and 20.0 for the statistical calculations. Data are presented as numbers (n), means with 95% confidence interval (95% CI) and as proportions (%).

In **Paper I**, group differences were depending on analysis evaluated by chi-square test or analyses of covariance (ANCOVA) with adjustment for age. Individuals Z scores, the number of standard deviations above or below the age-predicted mean, were gender-specifically derived by linear regression using the control cohort as a reference population.

In **Paper II**, group differences depending on analysis were evaluated by chi-square test, Student's t-test or ANCOVA with adjustment for age, height and weight. As there were no existing reference data at baseline individual and age-specific Z-scores (the number of standard deviation above or below the age-predicted mean) were gender-specifically derived by linear regression at baseline and at follow-up, using the baseline control cohort as reference population. "Tracking" (i.e. the correlation) of the Z-scores between baseline (age 3–17) and follow-up (age 28–44) was evaluated by Pearson's correlation coefficient, partial correlation was used to adjust for height and weight. We also stratified the Z-scores of each bone trait in quartiles and (i) examined the proportion of individuals that left their original quartile during the study period, (ii) estimated the sensitivity of a pediatric bone scan with a result in the lowest quartile to predict an adult result in the same quartile, and (iii) the specificity for a scan outside the lowest quartile to predict an adult result outside the lowest quartile.

In **paper III**, group differences were depending on analysis evaluated by chi-square test, Student's t-test or ANCOVA with adjustment for age. Individual Z-scores (the number of standard deviation above or below the age predicted mean) were gender-specifically derived by linear regression using all 121 individuals as reference population. "Tracking" (i.e. the correlation) of the Z-scores between the baseline measurements at mean age 8 years and follow-up measurement at mean age 19 years and between 13 and 19 years was evaluated by Pearson's correlation coefficient; partial correlation was used to adjust for height and weight at age 8 and 13 years. We also stratified the Z-scores of each bone trait in quartiles and (i) examined the proportion of individuals that left their original quartile during the study period, (ii) estimated the sensitivity of a pediatric bone scan with a result in the lowest quartile to predict a follow-up

measurement in the same quartile, and (iii) the specificity for a scan outside the lowest quartile to predict an adult result outside the lowest quartile.

In **Paper IV**, group differences were depending on analysis evaluated by ANCOVA with adjustment for gender and age. Sidak was chosen for pairwise comparisons between groups to adjust for multiple comparisons.

Summary of the papers

Paper I

Introduction

The aim of this study was to gender-specifically evaluate whether children with a fracture are at increased risk of reaching low BMD in young adulthood.

Subjects and methods

Distal forearm BMD was measured by SPA in 47 boys and 26 girls (mean age 10 years, range 3–16) with an index fracture and 41 boys and 43 girls (mean age 10 years, range 4–16) with no fracture. BMD was re-measured a mean 27 years later with the same SPA apparatus and with DXA, QUS and pQCT. Individual Z-scores were gender-specifically calculated using the control cohort as reference population.

Results

A childhood fracture in men was associated with a low BMC Z-score (–0.4 (95% CI –0.6, –0.1)) and low BMD Z-score (–0.4 (95% CI –0.7, –0.1)) at baseline and with a low BMC Z-score (–0.5 (95% CI –0.8, –0.2)) and low BMD Z-score (–0.4 (95% CI –0.7, –0.1)) at follow-up. There were no statistically significant changes in the BMC or BMD Z-scores from growth into adulthood. A statistically significant BMD deficit in adult men with a former index fracture was statistically captured by all scanning techniques with the largest Z-score deficit registered by DXA (total hip Z-score –1.0 (95% CI –1.3, –0.7)). Men with an index fracture also had smaller cross-sectional area (CSA), with the largest deficit in tibia (Z-score –0.5 (95% CI –0.7, –0.3)). The deficit in women did not reach statistical significance at baseline or at follow-up.

Discussion

Our results imply that a childhood fracture in men could be used as a risk factor for low BMD in young adulthood.

Conclusion

A childhood fracture in men was associated with low BMD and smaller bone size in young adulthood while the deficit in women did not reach statistical significance.

Paper II

Introduction

As the level of correlation of bone mass from childhood to adulthood is unclear, we conducted a long-term prospective observational study to determine whether a pediatric bone mass scan could predict adult bone mass.

Subjects and methods

We measured cortical BMC, BMD and bone width in the distal forearm by SPA in 120 boys and 94 girls with a mean age of 10 years (range 3–17) and a mean 28 years (range 25–29) later. We calculated individual and age-specific bone mass Z-scores, using the control cohort included at baseline as reference, and evaluated correlations between the two measurements. Individual Z-scores were also stratified in quartiles to register movements between quartiles from growth to adulthood. We also calculated the sensitivity of a pediatric bone scan with a result in the lowest quartile to predict an adult result in the same quartile, and the specificity for a scan outside the lowest quartile to predict an adult result outside the lowest quartile.

Results

There were correlations between Z-scores in childhood and adulthood for BMC ($r=0.56$, $p<0.001$), BMD ($r=0.42$, $p<0.0001$), and bone width ($r=0.58$, $p<0.001$), evident also in the gender-specific analyses. Of the children in the lowest quartile of BMD, 58% had left the lowest quartile in adulthood. A pediatric bone scan with a BMD value in the lowest quartile had a sensitivity of 48% (95% CI 27 to 69) to identify individuals who would remain in the lowest quartile also in adulthood. The specificity for a scan with a BMD value outside the lowest quartile to predict an adult BMD value outside the lowest quartile was 76% (95% CI 66 to 84).

Discussion

The higher correlation for BMC than for BMD is supported by previous reports[26, 48, 90] and could reflect the way that BMC estimates the amount of mineral while BMD reflects two separate estimates, the amount of bone mineral and areal bone size. Childhood BMD was able to explain only 12% of the variance in adult BMD in men and 25% in women.

Conclusion

The data suggest that a childhood BMD scan is of limited use for prediction of adult BMD, at least in children within fairly normal BMD values. The reason seems to be a discrepancy in the accrual of bone mineral accrual and the gain in bone size at growth.

Paper III

Introduction

There is low correlation between childhood and adult appendicular BMD measured by SPA. The aim of this study was to follow BMD from childhood to adulthood by DXA in the axial skeleton.

Subjects and methods

Femoral neck, total body and lumbar spine BMC, BMD and bone area were measured by DXA in a population-based cohort including 65 boys and 56 girls with a mean age of 8 years (range 6–9) and a mean 11 years (range 10–12) later when the participants had a mean age of 19 years (range 18–19), an age range that corresponds to peak bone mass in the femoral neck in our target population[5]. We gender-specifically estimated individual bone mass and bone size Z-scores, using all participants at each measurement as reference and evaluated correlations between the two measurements. Individual Z-scores were also stratified in quartiles to register movements between quartiles from pre-pubertal age to peak bone mass.

Results

The correlation coefficients (r) between pre-pubertal and young adulthood measurements for femoral neck BMC, BMD and bone area varied between 0.37 and 0.65. The BMC value at age 8 explained 42% of the variance in the BMC peak bone mass value, the corresponding value for BMD was 31% and for bone area 14%. Among the participants with femoral neck BMD in the lowest childhood quartile, 52% had left this quartile at peak bone mass. A pediatric bone scan with a femoral neck BMD value in the lowest quartile had a sensitivity of 47% (95% CI 28 to 66) to identify individuals who would remain in the lowest quartile at peak bone mass. The specificity for a scan with a BMD value outside the lowest quartile to predict an adult BMD value outside the lowest quartile was 82% (95% CI 72 to 89).

Discussion

The sensitivity of a childhood bone mass scan to predict peak bone mass in a normal population is low and a large proportion of individuals move between the quartiles of BMC and BMD during growth.

Conclusion

The pre-pubertal femoral neck BMD explained only 31% of the variance in femoral neck peak bone mass. A pre-pubertal BMD scan in a population-based sample has poor ability to predict individuals who are at risk of low peak bone mass.

Paper IV

Introduction

Cross-sectional studies suggest that premature birth may be associated with low peak bone mass (PBM). Since no prospective studies are available, we followed bone traits in preterm children born appropriate for gestational age (AGA), defined as weight ± 2SD for the gestational age, small for gestational age (SGA), defined as weight below -2SD for the gestational age and controls until adulthood.

Subjects and methods

We measured distal forearm BMC and BMD with SPA in 46 preterm children (born before completion of the 37th gestational week) (31 AGA and 15 SGA) at a mean age of 10.1 years (range 4–17) and in 84 healthy age-matched children. The measurements were repeated a mean 27 years later with the same SPA apparatus but then also with DXA and peripheral computed tomography pQCT.

Results

Preterm-born children were still shorter in adulthood (p=0.03), they also had lower femoral neck (FN) BMC, FN BMD, tibial cortical BMD, tibial cross-sectional area and SSI than controls (all p-values 0.001 to <0.05). The deficits were driven by lower bone traits in preterm SGA individuals, while no differences were seen in preterm AGA individuals compared to controls. The gain in forearm BMC from childhood to adulthood was also lower in preterm SGA individuals than in controls (p=0.005) but not in comparison with preterm AGA individuals (p=0.18).

Discussion

Preterm SGA individuals had similar BMD to controls in childhood but lower in adulthood. This indicates that preterm SGA individuals have a deficit in the accrual of bone mineral during growth. The data supports the fetal programming hypothesis[11] which infers that intrauterine events can specifically influence the pubertal development, findings that have previously been shown for traits other than bone mass.

Conclusion

Preterm SGA individuals are at increased risk for reaching low adult BMD. In our cohort we were unable to find increased risk for obtaining low BMD in preterm AGA individuals.

General discussion

Tracking of bone mass in a growing population

PBM is reached in early adulthood and is thought to explain 50% of the variance of bone mineral density (BMD) at age 65 years[60, 69, 80]. PBM is also a is a major determinant of fracture risk later in life[60]. BMD during growth and PBM are to a large extent determined by genetic factors as there are data demonstrating a strong resemblance between mother-daughter bone traits and showing that this resemblance is present even before the daughters have reached puberty[47, 115]. The level of BMD at growth may also be important in the long term perspective since cross-sectional studies infer that a childhood excess[62, 76] or deficit[77, 116] in BMD remains in adulthood. Recent prospective studies support this view, inferring that there is a partial correlation between bone mass in growth and PBM, also described as a partial "tracking" of bone mass during growth[26, 48, 50, 72, 122]. This implies that individuals may possibly maintain their ranked position in the distribution curve in bone mass over time, or in other words, if they have a low bone mass value in childhood these individuals are also at high risk of reaching low PBM.

Making predictions of adult BMD from childhood measurements is however difficult, as bone properties change rapidly during growth[60]. It is therefore important to perform serial measurements that cover both the pre-pubertal and the post-pubertal phases, including the time of PBM, to make accurate predictions. During the last decade several prospective studies have followed bone mass short term during growth[26, 48, 50, 55, 72-74, 90]. The longest follow-up described so far is 8.5 years, and although most of the studies begin at pre-pubertal stages, the follow-up measurements are usually done around the termination of growth. It is therefore unlikely that PBM is captured in these studies. The age of PBM has also been thoroughly discussed. Most estimates indicate that this event is reached at different ages in different anatomical regions[3, 15, 60]. Several studies also suggest that BMD in most regions does not significantly increase after the third decade of life[15, 60]. In some regions, however, such as the distal radius, reports have shown that PBM may be reached as late as age 40 years[3]. In **paper II** we followed our study subjects with distal forearm SPA measurements to a mean age of 37 years (range 28–44), making it probable that PBM was captured. In contrast, PBM in femoral neck has in girls been found in ages 16 to 18 years and in boys in ages 18 to 20 years[15, 60, 96]. In a large normative pediatric bone mass study from our region[5] these findings have

been confirmed, and also that peak total body BMD and peak lumbar spine BMD are expected to occur 5 to 10 years later than in the femoral neck[5]. For this reason, in **paper III** where study subjects were followed to a mean age of 18 years (range 18–19), we used the femoral neck as the region of interest when evaluating tracking until PBM. But we also presented data for total body and lumbar spine for completeness, with the knowledge that these regions probably have remaining growth after our last follow-up evaluation. In **paper II** we found lower correlation in BMC (r=0.48 for boys and r=0.63 for girls, both $p < 0.001$) than the only other longitudinal distal forearm SPA study to date (r=0.69 for boys and r=0.78 for girls, both $p < 0.001$, which followed children from age 11 to 17[90]. In **paper III** we found a statistically significantly lower correlation of FN compared to TB and LS. **Papers II and III** therefore support the view that correlations between childhood and young adulthood BMD are lower in regions that have reached PBM than in regions with remaining growth. A plausible explanation would be that a region in which the peak value has been reached has undergone larger changes in bone mass from baseline than a region with remaining growth[100, 103]. It would have been of great interest to follow the study participants until they had reached PBM in the spine and total body, to be able to evaluate whether the correlation in these regions are similar to the femoral neck.

Reports in the literature also suggest that the correlation of bone mass from childhood to young adulthood may be higher in old compared to young children [74] and in girls compared to boys[19, 50, 122]. This could reflect a longer remaining growth period in young children than in old children and suggest that boys with the same chronological age had longer remaining growth potential due to later puberty than girls[74, 90]. The inclusion of pre-, peri-, and post-pubertal children could also influence our inferences since bone properties change rapidly in puberty[60]. In **paper II** girls experienced menarche at a mean age of 12.7 years (range 10–18) while boys are known to reach puberty approximately 1.5 years later[92]. We therefore stratified children below and above age 10 years to capture children before they reached the fast pre-pubertal growth spurt[92] in the stratum of children <10 years. In **paper III** baseline measurements in this sub-cohort were performed at a mean age of 8 years (range 6–9), when all children were pre-pubertal in Tanner stage I. In contrast to several other reports, we found in both **papers II and III** that there were no statistically significant differences in correlation when comparing the BMD in young or older children with the final adult BMD or in boys compared to girls. Since the sample sizes in these subgroup analyses were small, we must raise the concern of a statistical type II error.

In **papers II and III** we stratified the Z-scores of each bone trait in quartiles and (i) examined the proportion of individuals that left their original quartile during the study period and (ii) estimated the sensitivity of a pediatric bone scan with a result in the lowest quartile to predict a follow-up measurement in the same quartile. We found that there was a large proportion of individuals moving from the lowest quartile from baseline to follow-up measurements. This resulted in a low sensitivity for a childhood

scan in the lower quartile to predict an adult bone mass value in the lowest quartile. But we must highlight that our study included children within fairly normal ranges of bone mass and bone size. We therefore cannot state whether there is a higher correlation or better prediction with higher sensitivity and specificity to predict peak bone mass in children with more extreme deficits in their pediatric bone traits. Wren et al., for example, reported that individuals with markedly low bone mass (<–1.5 SD) are at higher risk of reaching low (<–1.0 SD) at follow-up[74]. As we had only 5 children in **paper III** with a Z-score below –1.5, we were not able to evaluate individuals with more marked deficits in our study. Future studies that include children with lower BMD values are therefore necessary. Further long-term longitudinal studies, preferably with modern measuring techniques such as DXA and pQCT, and with different sites such as spine and hip, as well as inclusion of children with BMD below –2.5 SD, are needed before any definite clinical inferences can be drawn regarding the use of childhood BMD measurements to predict the PBM value.

The correlation coefficients in **papers II and III** were higher in absolute values for BMC than for BMD, both in the distal forearm and in the femoral neck. This might be explained by the heterogeneity of bone mineral accrual and gain in bone size in a growing individual, a theory supported by several other studies[26, 48, 90]. Individuals who improved from the lowest quartiles of BMD to higher quartiles in both **papers II and III** had a statistically significant higher accrual of bone mineral (BMC) but no statistically significant gain in bone size. In contrast, those deteriorating from the highest quartile of BMD to lower quartiles had a statistically significant smaller accrual of bone mineral but no statistically significant difference in bone size. The heterogeneity of bone mineral accrual and bone size is thus a probable explanation why a pediatric BMC value could explain a larger part of the variance in adult BMC (which only evaluates the amount of mineral) than a pediatric BMD value for the variance in adult BMD (which in addition to the amount of mineral also reflects bone size).

We conclude that the correlation of bone mass from childhood to adulthood in the distal forearm measured by SPA is low and in the femoral neck measured by DXA is moderate. A pediatric bone mass scan with values within fairly normal ranges in both the distal forearm and the femoral neck has poor ability to predict adult BMD in the individual person. This seems attributable to the heterogeneity of bone mineral accrual and gain in bone size during growth.

Are fractures in childhood a risk factor for low PBM?

The increase in fracture incidence during puberty could partly be explained by an increase in participation in physical activities[30] as well as more risk-prone behavior. However, the peak of fracture incidence occurs at the same time as peak height velocity (PHV)[70], a period when there is an increase in skeletal size but without an accompanying increase in mineralization. One hypothesis suggests that a childhood fracture is associated with a maturational pattern that creates transiently reduced BMD[9, 29, 46, 72] and thus a relatively weaker bone, prone to fractures. Low BMC and BMD is found to be associated with increased fracture risk in adults[71, 91], as a one standard deviation (SD) lower BMD is usually reported to be associated with a doubled fracture risk[91]. Furthermore, a previous forearm fracture in childhood is suggested as an independent risk factor for sustaining a new fractures[56]. A systematic review and meta-analysis that included all relevant articles on the subject published 1965–2005 found that children with a fracture have a mean BMD deficit of –0.3 SD compared to children with no fracture[31]. In **paper I** we found a –0.4 SD deficit in the boys and a –0.2 SD deficit in the girls at fracture event, indicating that our cohort includes a representative study population so that our inferences can be generalized.

However, the studies included in the meta-analysis were predominantly retrospective case-control studies[31], and the authors of the review summarized their publication by concluding that there is a need for well-conducted prospective studies that evaluate whether the deficit in BMD at fracture event is transient or retained into adulthood. Only a few prospective studies have investigated this, and these publications infer that the BMD deficit found at fracture event are retained years after the injury[27, 48, 55, 72-73]. The end point in these studies was also set at ages (range 14-18 years) when it could be questioned whether PBM had been reached. Furthermore, the European Prospective Osteoporosis Study (EPOS) presented results from a large (over 12 000 subjects) and well-conducted study in 2009 where they could not find a statistical difference in BMD in adults (>50 years) with or without self-reported fractures in childhood (8–18 years)[107].

As data are conflicting, the aim of **paper I** was to evaluate whether the deficit in BMD found at baseline was transient or not. Measurements were performed by the same scanner at the same skeletal region (distal forearm) both at fracture event and at follow-up close to three decades later, corresponding to the timing of PBM in the measured region. In **paper I**, we found that a childhood fracture in boys was associated with low BMD and smaller bone size in adult men, while the deficit in adult women did not reach statistical significance. Furthermore, we found that there were no significant changes in the BMD Z-scores in the children with an index fracture during the follow-up period. This implies that a childhood fracture in males ought to be regarded as a risk factor associated with low BMD in young adulthood. According to published prospective observational studies, a deficit of –0.4 SD in BMD would be associated

with a 40% higher fracture risk than expected by age[81]. However, as bone size also influences who will sustain further fractures, we speculate that the fracture risk in men with an index fracture possibly could be even larger than 40% given that they also had a smaller than expected adult bone size. We did examine all X-rays taken from study start until follow-up in our study subjects. We found a non statistically significant increase in relative risk (RR) of incident fractures in those with a childhood fracture RR 1.32 (0.69 to 2.25), compared to controls.

The association between low BMD and fractures has generally been related to low-energy trauma, both in elderly (i.e. fragility fractures)[54] and in children[86]. A large prospective study from the UK has however challenged this view, reporting that there is also an association between low BMD and childhood fractures due to high-energy trauma[28]. We therefore tried to address this also in **paper I**, where we found that the BMD deficit in boys with a low-energy-related index fracture, at fracture event as well as at follow-up, was statistically significant for boys but not for girls. Additionally, the deficit in boys with a high energy related index fracture did not reach statistical significance. Comparison between girls with a high energy fracture and controls was not tested due to small sample size and all subgroup analyses are difficult to interpret due to the low power. More longitudinal studies including larger cohorts are therefore needed.

Development of bone mass in preterm-born individuals

Premature birth is defined as being born before the end of the 37th gestational week[118]. The definition is based on the fact that many of the organs of the fetus reach adequate maturity for birth between gestation weeks 34 and 37[95]. Between 5% and 18% of all children worldwide and 6% of all children in Sweden are born preterm[13]. There is a variety of reasons for preterm birth, the most common being multiple pregnancies, infections and chronic conditions in the mother such as diabetes or high blood pressure[13]. Genetic influence on premature birth has also been established but often no cause is found[40].

Preterm as well as full term born children can be divided into those born appropriate for gestational age (AGA) defined as weight ± 2SD for the gestational age and those born small for gestational age (SGA), defined as birth weight and/or length at least 2 SD below the mean for gestational age (<or=-2 SD)[89]. Being born SGA is either predetermined, i.e. genetically determined, or a result of pathological processes; the latter can be subdivided into maternal, fetal or placental causes. Preterm infants usually show physical signs of prematurity in inverse proportion to the gestational age. As a result they are at risk of numerous medical problems affecting different organ systems such as the neurological, pulmonary and cardiac systems. By improving neonatal care,

however, many children adjust well during childhood and adolescence and the prognosis to be visibly healthy during young adulthood is good.[110]

The full effect of premature birth on the aging process is not yet known but has gradually attracted interest. The English epidemiologist David Barker has established a connection between low birth weight and hypertension, cardiovascular disease and diabetes in adulthood[44]. He developed the *"Barker hypothesis"* or the *"fetal programming theory",* stating that abnormal fetal growth is strongly associated with a number of chronic conditions later in life[11]. The word "programming" illustrates the idea that during critical periods in early fetal development, there are persisting changes in the body structure and function that are caused by environmental stimuli[20]. This relates to the concept of developmental plasticity where our genes can express different ranges of physiological or morphological states in response to the environmental conditions during fetal development.

Osteoporosis is an important and increasing cause of morbidity and mortality in developed countries, and the possibility that osteoporosis risk could be programmed by factors in fetal life or infancy is an interesting and important public health issue. The clinically important consequence of reduced bone mass is fracture, and data are now available which directly link growth rates in childhood to a hip fracture later in life[33]. Bone mass and strength in later life depend upon the peak attained during skeletal growth, and the subsequent rate of bone loss. For this reason, increasing attention has been focused on influences operating in early life[111]

During the last trimester bone is mineralized at a rapid pace, as 80% of the bone mass formation in a newly born infant is acquired during this period. Preterm birth together with difficulty in ensuring adequate mineral intake during the neonatal period therefore leads to a period with under mineralized bones[102]. The under mineralization seems to be transient, as preterm children have been shown to a have a rapid accrual of bone mineral, leading to similar bone mineral at 1 or 2 years of age compared to full-term born children[65]. Several studies imply that there is a difference in BMC and BMD in preterm compared to full-term children during childhood and adolescence but that the difference disappears when corrected for body size[17, 43, 63]. Other researchers report no bone mass differences at all[83, 119]. It is therefore unclear whether or not prematurity is a risk factor for low bone mass in childhood and adolescence, and if so, whether low bone mass is the result of being preterm or being SGA. When investigating this question, further subdivision of preterm children into SGA or AGA has shown that the birth deficit seen in preterm SGA children may be retained longer throughout infancy but with a normalization during childhood[49]. This is interesting, as a unique longitudinal Finnish study observed that objects with hip fracture were found to be shorter at birth, but of average height by age 7 years[33]. These findings do not contradict the fetal programming hypothesis.

There are no studies that have longitudinally followed preterm children from childhood and adolescence until they reach PBM, but there are several studies which have used cross-sectional evaluation to identify a relation between birth weight and adult bone mass, regardless of preterm birth or not. Three systemic reviews and meta-analyses evaluating cross-sectional studies were published between 2009 and 2011 and found that higher birth weight was associated with greater BMC of the lumbar spine and hip in adulthood[10, 94, 113]. Assessments of the effect of premature birth on PBM also rely on cross-sectional studies[38, 49, 67, 84]; these studies cannot distinguish between deficits developed pre- or post-natal or later during the pubertal growth spurt. To summarize, it is currently unclear what effect preterm birth and being born SGA or AGA has on PBM.

Our main objective in **paper IV** was to answer whether preterm children are at greater risk of reaching low adult BMD and if there is a difference in risk between preterm SGA and AGA children. With longitudinal data we also wanted to find out whether any deficits in PBM in SGA individuals existed because of persisting low bone mass or because of deterioration from childhood. In **paper IV** we could show with longitudinal bone mass data that there is a deficit in bone mineral accrual during the growth period and that this is transferred to a lower BMD in adulthood. We also found that this deficit is only driven by preterm SGA individuals. This knowledge could provide a possible explanation for discrepancies in published studies[18, 38, 67, 84], since the proportion of included preterm SGA and AGA children could then affect the outcome. In our study preterm SGA born children had similar BMD to controls in childhood but lower BMD accrual during later growth, including puberty, resulting in lower adult BMD. The responsible factors are unknown but this finding could at least in part be explained by the fetal programming hypothesis[11]. A young child would then develop normal BMD until puberty, where a deficit in the pubertal growth spurt could result in low PBM.

To summarize, preterm SGA individuals are at increased risk for reaching low adult BMD. In our cohort we were unable to find increased risk for obtaining low BMD in preterm AGA individuals. Our data support that this is the result of a lower accrual of bone mineral from childhood to adulthood. Future studies that evaluate bone traits should not only report data from children born preterm but stratify these into those born SGA and AGA.

Strengths and limitations of the thesis

The main strength of our studies is the unique length of the follow-up period, providing SPA data collected over 28 years and DXA data over 11 years. In **papers I, II and IV** measurements were performed with the same SPA scanner at the same skeletal region, continuous validation of the apparatus by a phantom was performed every second week during the entire study period, making it possible to exclude bias introduced by long-term drift. Exact positioning when measuring a subject is vital and therefore all measurements on each occasion were performed by just one technician at baseline and exclusively by another technician at follow-up. All graphical analyses were conducted by one of the authors. In **paper III** we conducted serial measurements with DXA in skeletal regions where bone mass measurements are used to predict future fracture risk. The study has the longest follow-up period to date and spans the timing of femoral neck PBM in the target population. The measurements were conducted by the same technicians at the same skeletal regions and with continuous validation of the apparatus by a phantom during the entire study period. In **paper I** measurements were performed in close conjunction with the fracture event, which avoids a major influence of posttraumatic osteopenia, as it is well known that posttraumatic osteopenia may have influences on bone mass both locally and generally[16, 28, 48]. Both arms were normally scanned but in individuals with a history of forearm fracture, the non-fractured arm was used. The long duration of the follow-up period in **papers I, II and IV** makes it probable that PBM was reached, even if we cannot state this for sure since we had no serial measurements. At follow-up, however, our cohort was in the same age range as the reference population usually used when calculating T-scores according to the definition of osteoporosis. The T-score is usually regarded as an estimation of PBM. In **papers I and IV** we were able at follow-up to verify deficits measured by SPA with modern scanners and at different regions using different measuring techniques. This strengthens the view that there actually were remaining deficits in young adulthood. The attendance rate of 71% in **paper I**, 72% in **paper II** and 63% in **paper IV** after almost three decades is also superior to other published studies[9, 16, 48, 50, 72, 74, 90]. Finally, drop-out analysis in **papers I, II, III and IV** revealed similarity between cohorts as well as between participants and drop-outs as regards anthropometry and lifestyle, thus reducing the risk of selection bias and further strengthening the quality of the data.

Study limitations include the sample size that renders the risk of committing type II errors in subgroup analyses. In **papers I, II, III and IV** sample size prevents us from making gender-specific evaluations either in all or in some of the subgroup evaluations. The attendance rate of 35% in **paper III** is comparable to other studies with a shorter follow-up period[122], but must still also be considered a limitation. The different participation rate among subgroups is also a weakness. A low participation rate with low number of participants will increase the risk of a type II error when evaluating the outcome and also increase the risk of achieving a non-representative cohort due to bias in those who declined further participation. In **papers I, II** and **IV** it would have been beneficial to have prospective DXA and pQCT data at other anatomical regions, especially the hip and spine, commonly used for clinical evaluation of osteoporosis[6], but these techniques were not available at study start. Serial measurements enabling us to pinpoint the exact timing of peak bone mass would have been preferable in all papers. In **papers I, II and IV** a registration of pubertal maturity to stratify the children by true pubertal status would have been preferable, as well as individual registration of menopause, which would have given reasonable estimates of individuals at risk of post-menopausal bone loss. As the oldest woman in our cohort was 44 years of age, the mean age of menopause in Scandinavia is 51 (95% CI 45 to 55) years[66], and bone loss in the cortical region of the distal forearm is initiated after age 40 years[2], there is a low risk of any significant age-related bone loss in our data. In **paper III** Tanner stage classification by self-assessments in the follow-up evaluation rather than expert classification must also be regarded as a study weakness. Worth discussing is the use of the proximal femur region in **paper III**, which is commonly assessed by DXA in adults but is considered to be more challenging to evaluate in children. Skeletal landmarks, which guide proper positioning, may not be well developed in young children, which can lead to errors in positioning and placement of the region of interest (ROI) using standard software. However, data from the Bone Mineral Density in Childhood Study (BMDCS) suggest that age-related precision of the total hip and femoral neck is comparable to both that of the spine and TBLH[35]. Further there are limitations to DXA measurements in children, as size and developmental status must be considered before interpretation on BMD. Growth in size will influence the BMD value as the BMD estimate is a function of BMC and bone area, which changes non-linearly during growth. The situation is therefore not the same as in adults. This is one of the reasons why we used the current standard recommended by ISCD 2013[35] for reporting DXA results, BMD Z-score, which provides an estimate of the SD(s) away from the mean for chronologic age and sex. In **paper III** it would have been preferable to have a cohort without any intra-curricular physical activity, but as the exercise cohort and the controls were similar in anthropometry and since we found no statistically significant difference in the correlations of bone mass at study start and adulthood between the intervention and the control groups (data not shown), all data were pooled. As growth occurred and ended during the study period in **papers I, II and IV**, this could hypothetically influence the location of the position of measurement and influence the acquired

absolute bone mass value. In **papers I and II** we therefore calculated and estimated tracking between Z-scores instead of absolute values. No calculations of Z-scores were performed in **paper IV** as the aim of this paper was to compare group differences and not conduct individual correlations. In **paper IV** we tried to classify the control cohort into SGA and AGA, but this could not be done since available information on either gestational week or birth weight was missing in 69/84 individuals. We consider this a limitation because it refrain us to differentiate if the bone mass deficits found in premature SGA children are the result of being born prematurely or because of pre natal growth retardation. Further we could not establish the underlying reason of neither premature birth nor why the child was born prematurely SGA or AGA. We could therefore not distinguish if the children born SGA are constitutionally small or include a proportion that we by today's definition would classify as intra uterine growth restriction (IUGR) infants. Further, data on socio-economic position (SEP) and lifestyle factors (diet, physical activity, smoking) during the pre- and postnatal period would have been valuable as they act as confounders both to premature birth as well as to growth.

Conclusions

This thesis shows that:

- In children with fairly normal values of bone mass there is low to moderate correlation of distal forearm BMC and BMD from childhood to adulthood when measured with SPA.
- In children with fairly normal values of bone mass there is a moderate to high correlation of total body, lumbar spine and femoral neck BMC and BMD between childhood and adulthood when measured by DXA.
- A pediatric bone mass scan in children with fairly normal values of bone mass has poor ability to predict the adult bone mass value.
- A childhood fracture is associated with low BMD and smaller bone size in adult men.
- Preterm SGA born children are at increased risk of reaching low adult BMD.

Future perspectives

To prove casual relationships and long-term effects of risk factors on low bone mass and fragility fractures, the perfect study should be prospective and follow a large cohort from birth to death. Registration of numerous confounders, fractures, comorbidity and mortality data should optimally be carried out along the way and the study should have enough statistical power to be able to compare the registered data. Serial measurements with three-dimensional densitometry should be used to register changes in material, geometrical and micro-architectural properties from birth through growth, with the possibility to pinpoint the exact timing on PBM. This cohort should also be followed into old age when fragility fractures occur to be able to evaluate the clinically relevant end point of osteoporosis, fractures. Such a study must be regarded as utopian, but nonetheless worth aiming for.

With the results from this thesis in mind, it would also be interesting to evaluate whether there is a difference in full-term SGA and AGA born individuals in PBM. It would also be interesting to evaluate correlations in bone mass between childhood and adulthood in a cohort with more extreme bone mass values, as we in this study only evaluated healthy children with fairly normal bone mass values.

Summary in Swedish – Populärvetenskaplig sammanfattning

Osteoporos (benskörhet) drabbar främst äldre och innebär en försvagning av skelettet som leder till en ökad risk för frakturer. Hälften av alla kvinnor och en fjärdedel av alla män i Sverige råkar under sin livstid ut för en fragilitetsfraktur, vilket innebär stort personligt lidande såväl som att det ger stora samhällsekonomiska kostnader. Det är känt att 50–85 % av mängden mineral i skelettet (BMD) är genetiskt betingat men att resterande del kan påverkas av miljöfaktorer främst under tillväxtfasen. Mängden benmassa (bentäthet) är störst vid 20–40-årsåldern hos både kvinnor och män och därefter sker en naturlig åldersrelaterad minskning som i vissa fall leder till osteoporos. Att nå en hög högsta nivå av benmassa (peak bone mass, PBM) har visats ha en skyddande effekt mot osteoporos och det är därför viktigt att identifiera barn med risk för att nå lågt PBM så att förebyggande åtgärder kan sättas in tidigt. Vårt mål med detta arbete var att utvärdera om det går att förutspå vuxen benmassa från mätningar i barnaår, och specifikt om en fraktur i barndomen samt låg vikt i förhållande till födelsevecka hos prematura, är en riskfaktor att nå låg PBM.

För att studera hur benmassan utvecklar sig från barndomen till vuxen ålder har vi i **delarbete I, II och IV** följt benmassa i underarmen hos en grupp med 214 friska individer från i 27 år från en medelålder på 10 år till 37 års ålder. I **delarbete III** har vi följt benmassa i rygg och höft hos en grupp med 121 individer i 11 år, från 8 år till 19 års ålder.

I **delarbete I** följde vi benmassa hos 47 pojkar och 26 flickor med fraktur i barndomen och 41 pojkar och 43 flickor utan fraktur i barndomen. En fraktur i barndomen var hos män kopplat till en lägre benmassa i vuxenlivet men vi kunde inte säkerställa några skillnader mellan grupperna hos kvinnor. Våra resultat antyder att en fraktur i barndomen kan ses som en riskfaktor för lågt PBM hos män.

I **delarbete II** följde vi med mätmetoden single-photon absorptiometry (SPA) BMD hos 120 pojkar och 94 flickor för att utvärdera om det går att förutspå låg vuxen benmassa från mätningar i barnaår. Resultaten visade att benvärde från bentäthetsmätning i barndomen endast kunde förklara en liten del av vuxenvärdet.

I **delarbete III** följde vi 65 pojkar och 56 flickor med dual-energy X-ray absorptiometry (DXA) för att utvärdera om det går att förutspå vuxen benmassa från mätningar i barnaår med en modernare mätmetod än SPA. Vi fann även med denna mätmetod att resultaten från bentäthetsmätning i barndomen endast kan förklara en liten del av vuxenvärdet och att sensitiviteten av en bentäthetsmätning, med lågt benvärde i barndomen att förutspå låg benmassa i vuxenlivet, var låg.

I **delarbete IV** följde vi benmassa hos 46 prematurt födda barn och 84 friska kontroller för att utvärdera om låg vikt i förhållande till födelsevecka hos prematura är en riskfaktor att nå låg PBM. Vi fann att tidigt födda barn, som är små i storlek i förhållande till födelsevecka, har ökad risk att nå lågt BMD som vuxen.

Sammantaget ser det ut som att en bentäthetsmätning i en frisk population är ett dåligt kliniskt redskap för att förutspå benmassa hos vuxna. Vi har däremot kunnat identifiera två möjliga riskfaktorer för lågt PBM, en fraktur i barndomen hos män och prematuritet med låg födelsevikt i förhållande till födelsevecka.

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Paper I

ORIGINAL ARTICLE

JBMR

Does a Childhood Fracture Predict Low Bone Mass In Young Adulthood?—A 27-Year Prospective Controlled Study

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ABSTRACT

A fracture in childhood is associated with low bone mineral density (BMD), but it is debated whether a fracture at growth also predicts low BMD in young adulthood. The purpose of this work was to gender-specifically evaluate whether children with a fracture are at increased risk of low BMD in young adulthood. Distal forearm BMD (g/cm^2) was measured with single-photon absorptiometry (SPA) in 47 boys and 26 girls (mean age 10 years, range 3–16 years) with an index fracture and in 41 boys and 43 girls (mean age 10 years, range 4–16 years) with no fracture. BMD was re-measured mean 27 years later with the same SPA apparatus and with dual-energy absorptiometry (DXA), quantitative ultrasound (QUS), and peripheral computed tomography (pQCT). Individual *Z*-scores were calculated using the control cohort as reference population. Data are presented as means with 95% confidence intervals (95% CI) within brackets and correlation with Pearson's correlation coefficient. Boys with an index fracture had at fracture event a distal forearm BMD *Z*-score of −0.4 (95% CI, −0.7 to −0.1) and at follow-up −0.4 (95% CI, −0.7 to −0.1). Corresponding values in girls were −0.2 (95% CI, −0.5 to 0.1) and −0.3 (95% CI, −0.7 to 0.1). The deficit in absolute bone mass was driven by men with index fractures in childhood due to low energy rather than moderate or high energy. There were no changes in BMD *Z*-score during the follow-up period. The BMD deficit at follow-up was in boys with an index fracture verified with all advocated techniques. A childhood fracture in men was associated with low BMD and smaller bone size in young adulthood whereas the deficit in women did not reach statistical significance. © 2013 American Society for Bone and Mineral Research.

KEY WORDS: BONE MASS; BMD; GROWTH; CHILDREN; FRACTURES

Introduction

Fractures are a general health problem because around one-half of all women and 25% of all men will sustain a fracture after the age of 50 years.[1] But because close to one-half of all children will sustain a fracture before the age of 18 years,[2,3] fractures are also a huge pediatric problem, associated with large health care costs and significant individual suffering.[3,4] Therefore, it is imperative to identify risk factors for fractures in all ages, enabling identification of high-risk individuals. One such risk factor is low bone mineral density (BMD), found to be associated with increased fracture risk in both adults[5,6] and children,[7–16] and 1 SD lower BMD is usually reported to be associated with doubled fracture risk.[5] Research has therefore focused on factors that influence BMD, both the loss during aging[17] and the accrual during growth.[18] Osteoporosis has long since been attributed to predominantly high bone loss in adult life, but because 50% of BMD at age 65 years has been estimated to be predicted by peak bone mass,[19,20] the accrual of BMD during growth has gradually attracted interest. This especially accounts for the peripubertal period, because 36% of the total amount of adult BMD is acquired during the 4 peripubertal years, similar to the total amount of loss in adult life.[21] Recent data have also inferred that both benefits[22,23] and deficits[24,25] in BMD acquired during growth may be retained into adulthood. So, if children with low BMD could be identified, this would open possibilities for targeted interventions.[22,23]

A childhood fracture is one such risk for low BMD,[7–8,13,26] predominantly fractures following a low-energy trauma,[13] but possibly also moderate- to high-energy trauma.[26] Because there

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is a peak in fracture incidence in childhood after a period with increased skeletal size but without an accompanying similar increase in mineralization, one hypothesis suggests that a childhood fracture is associated with a delayed maturational pattern that creates transient reduced BMD.[8,21,27,28] This period has, however, been shown to be followed by an extended period of mineralization that will lead to normal peak bone mass.[21]

Another hypothesis infers that maturational delayed children are overrepresented among children with fractures, but that an extended growth period after the fracture event would lead to normal peak bone mass.[29,30] A third hypothesis infers that BMD tracks from childhood to adulthood so that any BMD deficit in childhood would also be reflected by low peak bone mass.[16] But up to now no study has prospectively been able to shown that children with a fracture actually reach low peak bone mass.[13,26] The current knowledge is based on cross-sectional studies[19,20] and short-term prospective observational studies.[16,28,31] But because peak bone mass is reported to be reached at the end of the second or even third decade in life,[30] it is debated whether peak bone mass was actually reached in the published studies.[16,28,31] Therefore, there is a need for long-term prospective controlled data. That is the reason why this study was designed as a prospective controlled study with the aim of following BMD in children with an index fracture for close to three decades. We hypothesized that a childhood fracture would be associated with low BMD in young adulthood.

Subjects and Methods

Children at baseline

A skeletal evaluation was performed by single-photon absorptiometry (SPA) in 90 children with an index fracture between 1979 and 1981,[3] 57 boys and 33 girls with a mean age of 10 (range, 4–16) years. The scans were performed 40 ± 25 days (mean $\pm$ SD) after they had sustained the fracture. All types of fractures except hand, finger, skull, tooth, and rib fractures were included. Fifty-five children were reported with a fracture due to low-energy trauma, 31 due to moderate energy trauma, and 4 due to high-energy trauma.[3] A control cohort that included 131 volunteers within the same ages with no index fracture, 65 boys and 66 girls with a mean age of 10 (range, 4–16) years, was measured during the same period. All participants were white without diseases or medications known to affect the bone metabolism, none were malnourished or had impairment in growth. In the original report, children with an index fracture were reported with significantly lower BMD than the children with no index fracture.[13]

Follow-up evaluation

The follow-up evaluation was performed a mean 27 (range, 25–29) years after the fracture event. At follow-up, 2 men and 1 woman had died, 13 men and 7 women had relocated, 12 men and 14 women could not be found, 7 men and 6 women declined further measurements due to unwillingness to participate, 1 man was unable to attend due to illness, and 1 additional fracture case was excluded because we were not able to verify the type of trauma severity. Thus 157 of the original 221 were finally included in this report when they were at a mean age of 37 (range, 30–44) years. This corresponded to a 71% participation rate in total, 47 of 57 (82%) boys and 28 of 33 (85%) girls in the fracture cohort, and 41 of 65 (63%) boys and 43 of 66 (65%) girls in the control cohort. In the re-measured fracture cohort, 28 boys and 19 girls had experienced the index fracture due to a low-energy trauma, 16 boys and 7 girls due to a moderate-energy trauma, and 3 boys and 0 girls due to a high-energy trauma. The dropout analysis revealed that there were no statistically significant group differences regarding, age height, weight, or body mass index (BMI) registered between participants and nonparticipants.

Bone mass measurements

Bone mineral content (BMC; g/cm) and BMD (g/cm^2) were measured both at baseline and at follow-up on the forearm 6 cm proximal to the ulnar styloid process by the same SPA apparatus; the scanning technique is described in detail in previous reports.[13,17] Both arms were scanned, after which the mean value was used. In individuals with a history of forearm fracture, the nonfractured arm was used. Twenty-eight children had a fractured upper extremity, 11 on the right side and 17 on the left side. The coefficient of variation (CV) was 2% with a standardized phantom and 4% determined by double measurements after the subject was repositioned. The long-term drift was 0.1%/year (95% confidence interval [CI], -0.2 to 0.4), evaluated by a standardized phantom every second week during the entire study.[17] One technician performed all baseline measurements and one performed all follow-up measurements, and one of the authors analyzed all the plots.

At follow-up, BMC and BMD were also measured by dual X-ray absorptiometry (DXA) (Lunar DPX-L scanner, software version 1.3z; Lunar, Madison, WI, USA) in total body by a total body scan, in the first to fourth lumbar vertebra (L_1–L_4) by a lumbar spine scan and in the femoral neck and total hip by a hip scan. Daily calibration of the apparatus was done with the Lunar phantom. The CV evaluated in 14 individuals after repositioning was 0.4% to 3.0% for BMD depending on the measured region. Qualitative ultrasound (QUS) evaluated broadband ultrasonic attenuation (BUA; db/MHz) and speed of sound (SOS; m/s) in both calcanei, after which the mean value was used. The CV evaluated in 14 individuals after repositioning was 2.2% for BUA and 0.3% for SOS. Peripheral quantitative computed tomography (pQCT) (XCT 2000; Stratec, Pforzheim, Germany) measured BMD, cross-sectional area (CSA; mm^2) and stress-strain index (SSI, mm^3) in the left radius and left tibia. We measured at the 4% and 38% level from the ankle joint and at 6% and 66% level from the wrist. Daily calibration of the apparatus was done with a standard phantom. The CV evaluated in 14 individuals after repositioning was 1.1% to 4.6% for CSA depending on the measured region. Three research technicians performed all the DXA, QUS, and pQCT measurements and analyzed all the scans.

Anthropometric measurements and registration of lifestyle factors and incident fractures

We measured body weight to the nearest 0.1 kg with an electric scale and body height to the nearest 0.5 cm by a wall-tapered

height meter. Questionnaires registered lifestyle factors, diseases, and medications both at baseline[13] and at follow-up.[32]

Statistical evaluation

Statistical calculations were performed with PASW Statistic software SPSS (version 18.0, SPSS, Inc., Cicago, IL, USA). Data are presented as numbers (*n*), means with 95% CI, and as proportions (%). Group differences were evaluated by chi-square test and ANCOVA with adjustment for age. Individual *Z*-scores, the number of SDs above or below the age-predicted mean, were derived by linear regression using the control cohort as a reference population.

Results

BMC and BMD in individuals with an index fracture, independent of trauma type

There were no differences in age or anthropometrics between boys and girls with or without an index fracture, neither at baseline nor at follow-up (Table 1). Boys with an index fracture had at fracture event a distal forearm BMC *Z*-score of −0.4 (95% CI, −0.6 to −0.1) and BMD *Z*-score of −0.4 (95% CI, −0.7 to −0.1) and at follow-up of −0.5 (95% CI, −0.8 to −0.2) and −0.4 (95% CI, −0.7 to −0.1), respectively. Thus there were no changes in the BMC or BMD deficit from growth into adulthood (Table 2). The BMD deficit in adult men with a former index fracture was statistically captured by all scanning techniques with the largest *Z*-score deficit registered by DXA (total hip *Z*-score −1.0; 95% CI, −1.3 to −0.7) (Tables 3 and 4). Men with an index fracture also had smaller CSA with the largest deficit in tibia (*Z*-score −0.5; 95% CI, −0.7 to −0.3), resulting in a lower SSI (*Z*-score −0.5; 95% CI, −0.7 to −0.3) (Tables 3 and 4). Girls with an index fracture had at fracture event a distal forearm BMC *Z*-score of −0.3 (95% CI, −0.7 to 0.1) and BMD *Z*-score of −0.2 (95% CI, −0.5 to 0.1) and at follow-up of −0.3 (95% CI, −0.6 to 0.1) and −0.3 (95% CI, −0.7 to 0.1), respectively. Thus there were no changes in the BMC and BMD deficit from childhood into adulthood. The deficits in adulthood when measured by the other scanning techniques did not reach statistical significance (Table 3), even though pQCT measured a cortical radius BMD *Z*-score of −0.4 (95% CI, −0.9 to −0.0) (Table 4).

All group differences in Tables 1 and 3 remained after adjusting for height, weight, and age (data not shown).

BMC and BMD in children with an index fractures due to low-energy trauma

Boys with an index fracture due to a low-energy–related trauma had a distal forearm BMC *Z*-score of −0.4 (95% CI, −0.7 to −0.0) and BMD *Z*-score of −0.5 (95% CI, −0.9 to −0.1) and at follow-up of −0.5 (95% CI, −0.9 to −0.2) and −0.5 (95% CI, −0.9 to −0.1), respectively. Thus, there were no changes in the BMC or BMD deficit from growth into adulthood (Table 2). The BMD deficit in adult men with a former low-energy–related index fracture was statistically captured by all scanning techniques, with the largest *Z*-score deficit registered by DXA (total hip *Z*-score −1.2; 95% CI, −1.6 to −0.8) (Tables 3 and 4). Men with an index fracture also had smaller CSA with the largest deficit in tibia (*Z*-score of −0.6; 95% CI, −0.8 to −0.3), resulting in a lower SSI (*Z*-score −0.6; 95% CI, −0.8 to −0.3) (Tables 3 and 4). Girls with an index fracture due to a low-energy–related trauma had a distal forearm BMC *Z*-score of −0.3 (95% CI, −0.8 to 0.1) and BMD *Z*-score of −0.3 (95% CI, −0.6 to 0.1) and at follow-up of −0.4 (95% CI, −0.9 to 0.1) and −0.4 (95% CI, −0.8 to −0.0), respectively. Thus, there were no changes in the BMC or BMD deficit from growth into adulthood (Table 2). The deficits in adulthood when measured by the other scanning techniques did not reach statistical significance (Table 3), even though DXA measured a total body BMD *Z*-score of −0.5 (95% CI, −1.0 to 0.0) (Table 4). All group differences in Tables 1 and 3 remained after adjusting for height, weight, and age (data not shown).

BMC and BMD in children with an index fracture due to moderate- or high-energy trauma

Boys with a moderate- or high-energy–related index fracture had a distal forearm BMC *Z*-score of −0.3 (95% CI, −0.7 to 0.0) and BMD *Z*-score of −0.2 (95% CI, −0.7 to 0.3) and at follow-up of −0.3 (95% CI, −0.8 to 0.3) and −0.2 (95% CI, −0.8 to 0.4). Thus, there were no changes in the BMC or BMD deficit from growth into adulthood (Table 2). The BMD deficit in adult men with a former moderate- or high-energy–related index fracture was statistically captured by DXA with the largest *Z*-score deficit found with a total body *Z*-score of −0.7 (95% CI, −1.3 to −0.2) (Tables 3 and 4). The deficits in adulthood when measured by the other scanning techniques did not reach statistical significance (Table 3), even though pQCT measured a tibial CSA *Z*-score of −0.4 (95% CI, −0.8 to −0.1)), resulting in a SSI *Z*-score of −0.4 (95%CI, −0.8 to −0.0) (Table 4).

There were only 7 girls with an index fracture due to a moderate- or high-energy–related trauma; therefore, no further statistical evaluation was done in this group.

Discussion

A childhood fracture in men was associated with low BMD and smaller bone size whereas the deficit in women did not reach statistical significance. It is widely accepted that low BMD in adults is associated with an increased fracture risk.[10] Recently, a systematic review and meta-analysis that included all relevant articles published in 1965–2005 found that this also accounts for children,[7] concluding that children with a fracture have a mean BMD deficit of −0.3 SD compared to children with no fracture.[7] This is in close accordance with the −0.4 SD deficit in the boys and the −0.2 SD deficit in the girls at fracture event in the current study, a finding which indicates that our cohort includes a representative study population so that our inferences can be generalized. However, the studies included in the meta-analysis were predominantly retrospective case-control studies[7] and the authors of the review summarized their publication by concluding that there is a need for well-conducted prospective studies that evaluate whether the deficit in BMD at fracture event is transient or retained into adulthood. Our study has this design, with measurement performed with the same scanner and in the same skeletal region both at fracture event and at follow-up close to three decades later, indicating that the participants

Table 1. Age, Height, Weight, BMI, BMC, and BMD Were Measured in 47 Boys and 26 Girls When They Sustained a Fracture in Childhood and at a Mean 27 Years Later

	Baseline			Follow-up		
	Cases	Controls	*p*	Cases	Controls	*p*
Fractures due to all types of trauma						
Men	$n = 47$	$n = 41$		$n = 47$	$n = 41$	
Age (years)	9.7 ± 4.0	9.8 ± 3.5	0.93	36.4 ± 4.1	37.4 ± 3.6	0.25
Height (cm)	140,5 ± 25.6	140.8 ± 22.8	0.95	180.1 ± 7.0	181.4 ± 8.2	0.42
Weight (kg)	35.2 ± 18.0	36.7 ± 19.0	0.71	85.7 ± 12.9	88.7 ± 15.0	0.31
BMI (kg/m^2)	17.1 ± 3.2	17,3 ± 3.2	0.82	26.4 ± 3.2	26.9 ± 3.8	0.50
BMC (g/cm)	0.44 ± 0.19	0.48 ± 0.18	**0.05**	1.00 ± 0.14	1.07 ± 0.13	**0.04**
BMD (g/cm^2)	0.41 ± 0.11	0.43 ± 0.09	0.09	0.65 ± 0.07	0.68 ± 0.07	0.08
Women	$n = 26$	$n = 43$		$n = 26$	$n = 43$	
Age (years)	9.8 ± 3.4	10.6 ± 3.7	0.37	36.3 ± 3.6	38.2 ± 3.7	0.04
Height (cm)	139.5 ± 17.9	142.9 ± 20.4	0.49	167.9 ± 5.5	166.7 ± 6.6	0.46
Weight (kg)	33.6 ± 11.0	38.5 ± 14.6	0.14	74.4 ± 15.9	72.6 ± 16.2	0.65
BMI (kg/m^2)	16.7 ± 2.1	18.0 ± 2.4	**0.03**	26.4 ± 5.3	26.1 ± 5.5	0.82
BMC (g/cm)	0.40 ± 0.13	0.45 ± 0.16	0.17	0.70 ± 0.09	0.73 ± 0.09	0.30
BMD (g/cm^2)	0.39 ± 0.09	0.42 ± 0.09	0.38	0.53 ± 0.06	0.55 ± 0.06	0.34
Fractures due to low-energy trauma						
Men	$n = 28$	$n = 41$		$n = 28$	$n = 41$	
Age (years)	9.8 ± 4.3	9.8 ± 3.5	0.99	36.6 ± 4.5	37.4 ± 3.6	0.41
Height (cm)	141.2 ± 29.1	140.8 ± 22.8	0.95	179.5 ± 6.6	181.4 ± 8.2	0.30
Weight (kg)	35.2 ± 19.6	36.7 ± 19.0	0.71	83.8 ± 13.2	88.7 ± 15.0	0.17
BMI (kg/m^2)	17.1 ± 2.9	17.3 ± 3.2	0.83	26.0 ± 3.3	26.9 ± 3.8	0.31
BMC (g/cm)	0.45 ± 0.21	0.48 ± 0.18	0.12	1.00 ± 0.13	1.07 ± 0.13	**0.02**
BMD (g/cm^2)	0.40 ± 0.11	0.43 ± 0.09	**0.05**	0.65 ± 0.07	0.68 ± 0.07	**0.04**
Women	$n = 19$	$n = 43$		$n = 19$	$n = 43$	
Age (years)	10.1 ± 3.7	10.6 ± 3.7	0.37	36.6 ± 3.8	38.2 ± 3.7	0.12
Height (cm)	141 ± 18.2	142.9 ± 20.4	0.49	168.0 ± 4.9	166.7 ± 6.6	0.48
Weight (kg)	34.1 ± 11.5	38.5 ± 14.6	0.14	74.4 ± 16.1	72.6 ± 16.2	0.68
BMI (kg/m^2)	16.6 ± 2.3	18.0 ± 2.4	**0.03**	26.3 ± 5.3	26.1 ± 5.5	0.86
BMC (g/cm)	0.41 ± 0.14	0.45 ± 0.16	0.19	0.69 ± 0.09	0.73 ± 0.09	0.13
BMD (g/cm^2)	0.40 ± 0.09	0.42 ± 0.09	0.36	0.53 ± 0.05	0.55 ± 0.06	0.47
Fractures due to moderate/high-energy trauma						
Men	$n = 19$	$n = 41$		$n = 19$	$n = 41$	
Age (years)	9.4 ± 3.6	9.8 ± 3.5	0.69	36.0 ± 3.5	37.4 ± 3.6	0.16
Height (cm)	137.9 ± 20.1	140.8 ± 22.8	0.65	181.0 ± 7.9	181.4 ± 8.2	0.85
Weight (kg)	34.6 ± 16.1	36.7 ± 19.0	0.67	88.6 ± 12.5	88.7 ± 15.0	0.99
BMI (kg/m^2)	17.2 ± 3.7	17.3 ± 3.2	0.88	27.0 ± 3.2	26.9 ± 3.8	0.89
BMC (g/cm)	0.43 ± 0.15	0.48 ± 0.18	0.18	1.03 ± 0.15	1.07 ± 0.13	0.35
BMD (g/cm^2)	0.41 ± 0.1	0.43 ± 0.09	0.06	0.66 ± 0.08	0.68 ± 0.07	0.56
Women	$n = 7$	$n = 43$		$n = 7$	$n = 43$	
Age (years)	9.0 ± 2.7	10.6 ± 3.7	–	35,6 ± 2.9	38.2 ± 3.7	–
Height (cm)	135.5 ± 17.8	142.9 ± 20.4	–	167.7 ± 7.1	166.7 ± 6.6	–
Weight (kg)	32.2 ± 10.0	38.5 ± 14.6	–	74.4 ± 16.8	72.6 ± 16.2	–
BMI (kg/m^2)	17.1 ± 1.8	18.0 ± 2.4	–	26.4 ± 5.8	26.1 ± 5.5	–
BMC (g/cm)	0.38 ± 0.12	0.45 ± 0.16	–	0.72 ± 0.1	0.73 ± 0.09	–
BMD (g/cm^2)	0.38 ± 0.08	0.42 ± 0.09	–	0.55 ± 0.08	0.55 ± 0.06	–

Comparisons were made with 41 boys and 43 girls with no index fracture at baseline. Bone mass measurements were done by SPA in distal forearm. Data are shown as unadjusted means ± SD. Comparisons of the two groups are adjusted for age, and statistically significant differences ($p < 0.05$) are in bold. No group comparison was made in girls with moderate/high-energy trauma versus controls due to the small sample size.

BMI = body mass index; BMC = bone mineral content; BMD = bone mineral density; SPA = single-photon absorptiometry.

Table 2. BMC and BMD Measured by SPA in 47 Boys and 26 Girls When They Sustained a Fracture in Childhood and a Mean 27 Years Later

	Individuals with fractures due to all types of trauma		Individuals with fractures due to low-energy trauma		Individuals with fractures due to moderate/high-energy trauma	
	Men	Women	Men	Women	Men	Women
	Cases ($n = 47$)	Cases ($n = 26$)	Cases ($n = 28$)	Cases ($n = 19$)	Cases ($n = 19$)	Cases ($n = 7$)
Baseline						
BMC Z-score	−0.38 (−0.62 to −0.14)	−0.31 (−0.66 to 0.05)	−0.36 (−0.67 to −0.04)	−0.34 (−0.78 to 0.10)	−0.34 (−0.73 to 0.04)	−0.22 (−1.0 to 0.56)
BMD Z-score	−0.37 (−0.67 to −0.07)	−0.21 (−0.53 to 0.10)	−0.50 (−0.90 to −0.10)	−0.25 (−0.60 to 0.11)	−0.17 (−0.68 to 0.34)	−0.13 (−1.03 to 0.78)
Follow-up						
BMC Z-score	−0.45 (−0.76,−0.15)	−0.31 (−0.73 to 0.12)	−0.54 (−0.92 to −0.16)	−0.39 (−0.88 to 0.10)	−0.26 (−0.81 to 0.30)	−0.08 (−1.17 to 1.00)
BMD Z-score	−0.40 (−0.73,−0.07)	−0.31 (−0.72 to 0.10)	−0.49 (−0.89 to −0.09)	−0.42 (−0.83 to −0.01)	−0.19 (−0.80 to 0.42)	−0.03 (−1.13 to 1.26)
Delta Z-score						
BMC Z-score	0.07 (−0.22 to 0.37)	−0.00 (−0.34 to 0.34)	0.18 (−0.18 to 0.54)	0.05 (−0.33 to 0.43)	−0.08 (−0.64 to 0.47)	−0.14 (1.08 to 0.80)
BMD Z-score	0.03 (−0.36 to 0.41)	0.10 (−0.30 to 0.49)	−0.01 (−0.44 to 0.43)	0.17 (−0.28 to 0.63)	0.02 (−0.07 to 0.74)	−0.10 (−1.13 to 0.92)

Data are shown as mean Z-scores with 95% confidence interval within brackets. The sample mean in the controls (zero) was calculated based on data in 41 boys and 43 girls who were followed during the same period but had no index fracture at baseline. Individuals' Z-scores, the number of SDs above or below the age predicted mean, were derived by linear regression using our control cohort (with a mean Z-score of zero) as a reference population.

BMC = bone mineral content; BMD = bone mineral density; SPA = single-photon absorptiometry.

actually had reached peak bone mass, something that has been discussed in previous prospective reports with shorter follow-up periods.[16,28,31]

To our knowledge there are to date only five case-control studies that have prospectively followed children with fractures into older ages, four published articles[2,16,28,31,33] and one abstract.[33] These reports infer that the BMD deficit that was found at fracture event was retained years after the injury. However, the end-point was in these studies set at ages when it could be questioned whether peak bone mass really had been reached, as other studies have suggested that peak bone mass may be reached as late as the third or even fourth decade of life.[17,30] In contrast, our study chose an end-point at older ages, to increase the probability that the participants had reached peak bone mass. With this study design and with the possibility to present longitudinal data with the same scanner, we could actually state that there were no significant changes in the BMD deficits in the children with an index fracture during the follow-up period. This implies that a childhood fracture ought to be regarded as a factor that is associated with low BMD in young adulthood and that low BMD at childhood ought to be regarded as a risk factor for also reaching low peak bone mass.

According to published prospective observational studies, a deficit of −0.4 SD would be associated with a 40% higher fracture risk than expected by age.[34] However, because bone size also influence who will sustain further fractures, we speculate that the fracture risk in adult boys with an index fracture possibly could be even larger than 40% given the smaller than expected bone size in young adulthood.

Opposed to our findings, The European Prospective Osteoporosis Study (EPOS) group presented results from a large (over 12,000 subjects) and well-conducted study in 2009, in which they could not find a statistical difference in BMD in adults (>50 years old) with or without self-reported fractures in childhood (8–18 years old).[35] A possible explanation could be that information on fractures was self-reported, thus introducing recall bias. Another difference was that the cited study included children with an index fracture up to an age of 18 years, thus actually fully grown individuals. There is a need for more longitudinal studies with larger cohorts included and with fractures prospectively registered.

The association between low BMD and fractures in the elderly has generally been related to low-energy trauma, i.e., fragility fractures,[10] as has the association generally described in children.[13] A recent large prospective study from the UK has, however, challenged this view, reporting that there is also an association between low BMD and childhood fractures due to high-energy trauma.[26] In our study we found that the BMD deficit in boys with a low-energy–related index fracture, at fracture event as well as at follow-up, was statistically significant for boys but not for girls.

In addition, the deficit in boys with a high-energy–related index fracture did not reach statistical significance. Comparison between girls with a high-energy fracture and controls was not tested due to small sample size. All subgroup analysis is, however, difficult to interpret due to the power problem; there is therefore a need for more longitudinal studies including larger cohorts.

Table 3. BMD Measured by DXA, SOS and BUA Measured by QUS, and BMD Measured by pQCT in 47 Men and 26 Women a Mean 27 Years After They Sustained an Index Fracture in Childhood

	Fractures due to all types of trauma				Fractures due to low-energy trauma				Fractures due to moderate/high-energy trauma			
	Men		Women		Men		Women		Men		Women	
Modality	Cases ($n = 47$)	Controls ($n = 41$)	Cases ($n = 26$)	Controls ($n = 43$)	Cases ($n = 28$)	Controls ($n = 41$)	Cases ($n = 19$)	Controls ($n = 43$)	Cases ($n = 19$)	Controls ($n = 41$)	Cases ($n = 7$)	Controls ($n = 43$)
DXA												
Total body BMD	**1.24**	1.30	1.18	1.20	**1.23**	1.30	**1.17**	1.20	**1.24**	1.30	1.22	1.20
(g/cm^2)	(1.21–1.26)	(1.27–1.33)	(1.15–1.21)	(1.18–1.22)	(1.20–1.26)	(1.27–1.33)	(1.13–1.20)	(1.18–1.22)	(1.20–1.28)	(1.27–1.33)	(1.14–1.30)	(1.18–1.22)
Total hip BMD	**1.04**	1.16	1.01	1.04	**1.03**	1.16	1.00	1.04	**1.07**	1.16	1.05	1.04
(g/cm^2)	(1.01–1.08)	(1.12–1.19)	(0.96–1.06)	(1.01–1.08)	(0.98–1.07)	(1.12–1.19)	(0.95–1.05)	(1.01–1.08)	(1.01–1.13)	(1.12–1.19)	(0.91–1.19)	(1.01–1.08)
FN BMD	**1.03**	1.13	1.00	1.03	**1.01**	1.13	0.99	1.03	**1.06**	1.13	1.04	1.03
(g/cm^2)	(0.99–1.06)	(1.10–1.17)	(0.95–1.06)	(0.99–1.06)	(0.97–1.05)	(1.10–1.17)	(0.93–1.05)	(0.99–1.06)	(0.99–1.12)	(1.10–1.17)	(0.89–1.19)	(0.99–1.06)
L_1–L_4 BMD	**1.20**	1.30	1.22	1.24	**1.19**	1.30	1.19	1.24	1.22	1.30	1.31	1.24
(g/cm^2)	(1.16–1.24)	(1.26–1.34)	(1.17–1.27)	(1.20–1.28)	(1.14–1.23)	(1.26–1.34)	(1.13–1.25)	(1.20–1.28)	(1.16–1.29)	(1.26–1.34)	(1.21–1.41)	(1.20–1.28)
QUS												
SOS (m/s)	**1568**	1592	1586	1591	**1561**	1592	**1576**	1591	1578	1592	1609	1591
	(1553–1583)	(1579–1604)	(1575,1599)	(1580–1601)	(1535–1586)	(1579,1604)	(1565–1587)	(1580,1601)	(1562–1593)	(1579,1604)	(1583–1633)	(1580,1601)
BUA (dB/MHz)	**117**	124	123	120	**115**	124	115	120	120	124	146	120
	(113–121)	(119–129)	(113–133)	(115–125)	(109–120)	(119–129)	(109–121)	(115–125)	(113–128)	(119–129)	(114–178)	(115–125)
pQCT												
Trabecular tibia (4%)												
BMD (g/cm^2)	**1.45**	1.56	1.23	1.24	1.45	1.56	1.20	1.24	1.47	1.56	1.32	1.24
	(1.40–1.50)	(1.49–1.62)	(1.15–1.31)	(1.20–1.27)	(1.37–1.53)	(1.49–1.62)	(1.11 1.28)	(1.20–1.27)	(1.40–1.53)	(1.49–1.62)	(1.13–1.52)	(1.20–1.27)
Cortical tibia (38%)												
BMD (g/cm^2)	**1.97**	2.04	1.81	1.83	**1.97**	2.04	1.80	1.83	1.99	2.04	1.84	1.83
	(1.94–2.00)	(2.00–2.09)	(1.77–1.85)	(1.80–1.87)	(1.92–2.01)	(2.00–2.09)	(1.75–1.84)	(1.80–1.87)	(1.94–2.03)	(2.00–2.09)	(1.75–1.93)	(1.80–1.87)
Cross sectional area	**464**	499	385	387	**460**	499	382	387	470	499	385	387
(mm^2)	(446–482)	(476–522)	(367–403)	(372–402)	(441–478)	(476–522)	(363–400)	(372–402)	(445–495)	(476–522)	(338–432)	(372–402)
SSI (mm^3)	**1942**	2166	1464	1504	**1909**	2166	1440	1504	1993	2166	1523	1504
	(1853–2031)	(2017–2315)	(1368–1560)	(1423–1585)	(1790–2028)	(2017–2315)	(1331–1549)	(1423–1585)	(1837–2149)	(2017–2315)	(1273–1774)	(1423–1585)
Trabecular radius (6%)												
BMD (g/cm^2)	**0.95**	1.00	0.75	0.77	**0.94**	1.00	0.74	0.77	0.97	1.00	0.78	0.77
	(0.92–0.98)	(0.97–1.05)	(0.70–0.79)	(0.75–0.80)	(0.89–0.99)	(0.97–1.05)	(0.71–0.78)	(0.75–0.80)	(0.91–1.03)	(0.97–1.05)	(0.69–0.88)	(0.75 0.80)
Cortical radius (66%)												
BMD (g/cm^2)	1.12	1.16	0.99	1.02	**1.11**	1.16	0.98	1.02	1.15	1.16	1.00	1.02
	(1.10–1.15)	(1.13–1.19)	(0.96–1.02)	(0.99–1.04)	(1.08–1.14)	(1.13–1.19)	(0.95–1.02)	(0.99–1.04)	(1.11–1.19)	(1.13–1.19)	(0.96–1.06)	(0.99–1.04)
Cross sectional area	180	188	130	138	174	188	131	138	190	188	127	138
(mm^2)	(170–189)	(177–199)	(122–139)	(131–145)	(161–186)	(177–199)	(120–143)	(131–145)	(174–205)	(177–199)	(111–144)	(131–145)
SSI (mm^3)	413	440	273	293	406	440	275	293	429	440	268	293
	(382–443)	(407–473)	(250–296)	(274–311)	(363–449)	(407–473)	(246–304)	(274–311)	(380–477)	(407–473)	(229–307)	(274–311)

Comparisons are made with 41 men and 43 women with no index fracture at baseline. Adjustments for differences in age are advocated in the group comparison and statistically significant differences ($p < 0.05$) are in bold. Due to small sample size no group comparison was made between girls with moderate/high-energy trauma and controls. Data are shown as unadjusted means with 95% CI in parentheses.

BMD = bone mineral density; DXA = dual-energy X-ray absorptiometry; SOS = speed of sound; BUA = broadband attenuation; QUS = quantitative ultrasound; pQCT = peripheral computed tomography; FN = femoral neck; L_1–L_4 = lumbar spine vertebrae 1 to 4; SSI = stress strain index; CI = confidence interval.

Table 4. *Z*-Scores for Bone Traits Measured by DXA, QUS, and pQCT in 47 Men and 26 Women a Mean 27 Years After They Sustained an Index Fracture in Childhood

	Individuals with fractures due to all types of trauma		Individuals with fractures due to low-energy trauma		Individuals with fractures due to moderate- or high-energy trauma	
	Men ($n = 47$)	Women ($n = 26$)	Men ($n = 28$)	Women ($n = 19$)	Men ($n = 19$)	Women ($n = 7$)
DXA						
Total body BMD *Z*-score	−0.70 (−0.96 to −0.45)	−0.31 (−0.78 to 0.16)	−0.72 (−1.05 to −0.38)	−0.53 (−1.02 to −0.03)	−0.72 (−1.25 to −0.20)	0.28 (−0.97 to 1.54)
Total hip BMD *Z*-score	−1.01 (−1.33 to −0.70)	−0.30 (−0.76 to 0.17)	−1.16 (−1.57 to −0.75)	−0.42 (−0.93 to 0.09)	−0.62 (−1.03 to −0.20)	0.04 (−1.24 to 1.32)
Femoral neck BMD *Z*-score	−0.92 (−1.23 to −0.62)	−0.19 (−0.70 to 0.32)	−1.04 (−1.41 to −0.68)	−0.32 (−0.89 to 0.25)	−0.67 (−1.23,−0.11)	0.15 (−1.25,1.56)
L_1–L_4 BMD *Z*-score	−0.77 (−1.05 to −0.48)	−0.16 (−0.58 to 0.26)	−0.85 (−1.20 to −0.50)	−0.43 (−0.90 to 0.05)	−0.56 (−1.08 to −0.04)	0.56 (−0.23 to 1.35)
QUS						
SOS *Z*-score	−0.64 (−1.04 to −0.24)	−0.11 (−0.47 to 0.25)	−0.83 (−1.49 to −0.17)	−0.42 (−0.73 to −0.11)	−0.37 (−0.78 to 0.04)	0.73 (−0.14 to 1.60)
BUA *Z*-score	−0.48 (−0.76 to −0.19)	0.21 (−0.44 to 0.87)	−0.64 (−1.02 to −0.26)	−0.33 (−0.74 to 0.09)	−0.26 (−0.74 to 0.22)	1.69 (−0.43 to 3.80)
pQCT						
Trabecular tibia (4%)						
BMD *Z*-score	−0.49 (−0.74 to −0.24)	−0.03 (−0.74 to 0.69)	−0.49 (−0.88 to −0.11)	−0.35 (−1.13 to 0.42)	−0.42 (−0.72 to 0.12)	0.82 (−1.01 to 2.66)
Cortical tibia (38%)						
BMD *Z*-score	−0.50 (−0.73 to −0.27)	−0.20 (−0.54 to 0.14)	−0.54 (−0.87 to −0.21)	−0.31 (−0.71 to 0.09)	−0.42 (−0.77 to 0.07)	0.09 (−0.72 to 0.89)
Cross-sectional area *Z*-score	−0.50 (−0.70 to −0.30)	−0.09 (−0.43 to 0.26)	−0.56 (−0.82 to −0.30)	−0.11 (−0.49 to 0.28)	−0.42 (−0.77 to −0.06)	−0.04 (−1.02 to 0.95)
SSI *Z*-score	−0.49 (−0.68 to −0.29)	−0.15 (−0.53 to 0.21)	−0.56 (−0.82 to −0.30)	−0.25 (−0.68 to 0.18)	−0.38 (−0.71 to −0.04)	0.07 (−0.90 to 1.06)
Trabecular radius (6%)						
BMD *Z*-score	−0.50 (−0.78 to −0.23)	−0.27 (−0.80 to 0.27)	−0.58 (−0.95 to −0.21)	−0.42 (−1.02 to 0.18)	−0.34 (−0.80 to 0.13)	0.15 (−1.26 to 1.57)
Cortical radius (66%)						
BMD *Z*-score	−0.42 (−0.68 to −0.16)	−0.44 (−0.86,−0.01)	−0.58 (−0.92 to −0.23)	−0.52 (−1.03 to −0.01)	−0.14 (−0.55 to 0.26)	−0.21 (−1.14 to 0.73)
Cross-sectional area *Z*-score	−0.25 (−0.56 to 0.05)	−0.35 (−0.76 to 0.05)	−0.42 (−0.82 to −0.03)	−0.31 (−0.84 to 0.22)	0.07 (−0.44 to 0.57)	−0.47 (−1.20 to 0.26)
SSI *Z*-score	−0.28 (−0.60 to 0.04)	−0.34 (−0.73 to 0.05)	−0.36 (−0.79 to 0.08)	−0.31 (−0.83 to 0.21)	−0.10 (−0.60 to 0.41)	−0.41 (−1.06 to 0.24)

Data are shown as means with 95% confidence interval in parentheses. The sample mean in the controls (zero) are calculated based on data in 41 boys and 43 girls with no index fracture at baseline. Individuals' *Z*-scores, the number of SDs above or below the age predicted mean, were derived by linear regression using our control cohort (with a mean *Z*-score of zero) as a reference population.

DXA = dual-energy X ray absorptiometry; QUS = quantitative ultrasound; pQCT = peripheral computed tomography; BMD = bone mineral density; L_1–L_4 = lumbar spine vertebrae 1 to 4; SOS = speed of sound; BUA = broadband attenuation; SSI = stress strain index.

Study strengths include the prospective controlled study design and gender-specific measurements performed in close conjunction with the fracture event, which avoids a major influence of posttraumatic osteopenia, as it is well known that posttraumatic osteopenia may have influences on bone mass both locally and generally.[26,31,36] The long duration of the follow-up period makes it probable that peak bone mass was reached, even though we have not performed serial measurements in adulthood and therefore could not pinpoint the exact peak value for each person. Our cohort is, however, at follow-up, in the same age range as the reference population usually used when calculating *T*-scores in the definition of osteoporosis. The *T*-score is usually regarded as an estimation of peak bone mass. The use of the same apparatus with measurements done at the same region, and with available phantom data during the entire follow-up period, made it possible to exclude bias introduced by long-term drift. The use of modern scanners using different techniques, which all verified the remaining adult bone mass deficit also strengthens the view that there actually is a remaining deficit at young adulthood. An attendance rate of 71%, 27 years after fracture event must also be regarded as advantageous compared with earlier cited studies.[21,28,31,36] A low participation rate will increase the risk of making a type II error when evaluating the outcome and also increase the risk of achieving a nonrepresentative cohort as a result of bias in those who denied further participation Finally, the similarity between the fracture and the control cohort and participants and dropouts with respect to anthropometry and lifestyle reduce the risk of selection bias and increase the possibility of generalizing our inferences.

Study limitations include the sample size, which creates the risk of committing type II errors especially in girls and in individuals with high-energy–related fractures. The different participation rate among the index group and the control cohort is also a weakness. A low participation rate will increase the risk of making a type II error when evaluating the outcome and also increase the risk of achieving a nonrepresentative cohort due to bias in those who denied further participation. It would also have been advantageous to have prospective data with the modern scanning techniques. However, these techniques were not available at study start. It would also have been advantageous to have a registration of Tanner stage at baseline, to be able to correlate prepubertal BMD and bone mass in young adulthood based on maturational stage and not chronological age. It would have been advantageous to register whether any women at follow-up had reached menopause, and then had possibly also experienced a period of postmenopausal bone loss. If so, peak bone mass would not have been captured. But, as none of the women were above age 45 years, this ought to be a minor confounding factor. Finally, it would have been advantageous to have performed serial measurements of bone mass in adulthood to be able to predict actual peak bone mass.

In summary, a childhood fracture in men was associated with low BMD and smaller bone size in young adulthood whereas the deficit in women did not reach statistical significance.

Disclosures

All authors state that they have no conflicts of interest.

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Paper II

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ORIGINAL RESEARCH

A Pediatric Bone Mass Scan Has Poor Ability to Predict Adult Bone Mass: A 28-Year Prospective Study in 214 Children

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Abstract As the correlation of bone mass from childhood to adulthood is unclear, we conducted a long-term prospective observational study to determine if a pediatric bone mass scan could predict adult bone mass. We measured cortical bone mineral content (BMC [g]), bone mineral density (BMD [g/cm^2]), and bone width (cm) in the distal forearm by single photon absorptiometry in 120 boys and 94 girls with a mean age of 10 years (range 3–17) and mean 28 years (range 25–29) later. We calculated individual and age-specific bone mass *Z* scores, using the control cohort included at baseline as reference, and evaluated correlations between the two measurements with Pearson's correlation coefficient. Individual *Z* scores were also stratified in quartiles to register movements between quartiles from growth to adulthood. BMD *Z* scores in childhood and adulthood correlated in both boys ($r = 0.35$, $p < 0.0001$) and girls ($r = 0.50$, $p < 0.0001$) and in both children ≥10 years at baseline (boys $r = 0.43$ and girls $r = 0.58$, both $p < 0.0001$) and children <10 years at baseline (boys $r = 0.26$ and girls $r = 0.40$, both $p < 0.05$). Of the children in the lowest quartile of BMD, 58 % had left the lowest quartile in adulthood. A pediatric bone scan with a value in the lowest quartile had a sensitivity of 48 % (95 % confidence interval [CI] 27–69 %) and a specificity of 76 % (95 % CI 66–84 %) to identify individuals who would remain in the lowest quartile also in adulthood. Childhood forearm BMD explained 12 % of the variance in adult BMD in men and 25 % in women. A pediatric distal forearm BMD scan has poor ability to predict adult bone mass.

Keywords Bone mass · Tracking · Child · Bone mineral density · Bone mineral content

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Introduction

Bone loss is a physiological process related to aging [1, 2] that results in low bone mineral density (BMD) and possibly osteoporosis [2]. There are no prospective studies that have followed bone mass from young adulthood into the ages when osteoporosis becomes a problem of magnitude. However, calculations have inferred that 50 % of the variance in BMD at age 65 could be predicted by peak bone mass [3, 4]; it has also been shown that individuals with high bone mass at age 30 are likely to have high bone mass also at age 70 [5].

This has led to speculations inferring that a reduction of age-related bone loss [2] or optimizing of peak bone mass [1, 4] could possibly reduce the prevalence of osteoporosis. For intervention strategies in adulthood it thus seem feasible to target not only the population at large [6] but also high-risk individuals.

In contrast, the level of bone mass tracking from childhood to adulthood is unclear. There are some reports that indicate a childhood excess [7, 8] or deficit [9, 10] in BMD remains in adulthood, and the few prospective studies that have addressed this question indicate a partial tracking of BMD during growth [11–14]. But as these

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studies have all been shorter than a decade and terminated before the age of 17 and since peak bone mass is reached later [15], it seems unlikely that peak bone mass was actually captured in any of them.

We therefore set up a prospective long-term study to answer the following questions: (1) Does bone mass track from childhood to adulthood? (2) Is tracking more evident in older than younger children, and is there a gender discrepancy? (3) What proportion of individuals remain in the lowest quartile of bone mass in both childhood and adulthood, and what are the sensitivity and specificity of a pediatric bone scan to predict low bone mass also in adulthood? (4) Is movement from one BMD quartile in childhood to another in adulthood due to different accrual of bone mineral or gain in bone size?

Materials

Distal forearm bone mineral content (BMC [g]), BMD (g/cm^2), and bone width (cm) were measured by single-photon absorptiometry (SPA) in 120 boys with a mean age of 9.9 years (range 3–17) and 94 girls with a mean age of 10.7 years (range 4–17). The children were included from three published cohort studies between the years 1979 and 1981: 48 boys and 28 girls with a previous fracture [16], 31 boys and 25 girls with premature birth [17], and 41 boys and 43 girls from a healthy control cohort of the same ages [16–18]. All participants were Caucasian, without any disease or medication known to affect bone metabolism. No follow-up measurements were originally planned, but several decades later we designed the present study and conducted follow-up measurements by inviting all participants originally included. Of the original 296 participants 214 were remeasured with the same SPA apparatus, a mean 28 years (range 25–29) later, then at a mean age of 37 years (range 28–44). Among the nonparticipants, 5 men and 2 women had died, 13 men and 9 women had relocated, 19 men and 15 women could not be located, 9 men and 8 women declined further participation, and 2 men were unable to attend due to illness. This corresponds to an overall participation rate of 72 %, equally distributed in both genders. Seventy-four of the original 90 individuals (82 %) were remeasured in the fracture cohort, 56/75 (75 %) in the premature birth cohort, and 84/131 (64 %) in the control cohort. Age, height, weight, body mass index (BMI), gender distribution, and lifestyle factors were similar in the three cohorts, as well as in those individuals who attended the follow-up exam and those who did not (data not shown).

Bone traits were measured in the distal forearm 6 cm proximal to the ulnar styloid on both occasions. The scanning technique has previously been described in detail [2, 16]. We scanned both arms and used the mean value except in individuals with a history of upper extremity fracture (11 on the right side and 17 on the left side), where we used only the result from the nonfractured arm. The coefficient of variation was 2 % with a standardized phantom and 4 % after repeated measurements in 14 subjects after repositioning. The long-term drift, evaluated by a standardized phantom was 0.1 %/year at baseline and follow-up measurements (95 % confidence interval [CI] −0.2 to 0.4; [2]). Because of the nonsignificant drift, there were no corrections of data. One technician performed all baseline measurements, another all follow-up measurements, and one of the authors analyzed all plots. Body weight and height were measured with standard equipment. Lifestyle factors, diseases, and medications were evaluated by questionnaires at both baseline [16, 17] and follow-up [6].

Statistical Evaluation

The study was approved by the Ethics Committee of Lund University. We used SPSS® version 20.0 (SPSS, Chicago, IL) for statistical calculations. Group differences were evaluated by the χ^2 test, Student's t test, or analysis of covariance with adjustment for age, height, and weight. As there were no existing reference data at baseline, individual and age-specific Z scores (the number of standard deviations [SDs] above or below the age-predicted mean) were gender-specifically derived by linear regression at baseline and follow-up, respectively, using the baseline control cohort as the reference population. Tracking (i.e., correlation) of the Z scores between baseline (age 4–16) and follow-up (age 28–44) was evaluated by Pearson's correlation coefficient, and partial correlation was used to adjust for height and weight. We also stratified the Z scores of each bone trait in quartiles and (1) examined the proportion of individuals who left their original quartile during the study period, (2) estimated the sensitivity of a pediatric bone scan with a result in the lowest quartile to predict an adult result in the same quartile, and (3) estimated the specificity for a scan outside the lowest quartile to predict an adult result outside the lowest quartile. Data are presented as numbers (n), means ± SDs, means with 95 % CIs, or proportions (%).

Results

Children Aged 3–17 Years at Baseline (All, $n = 214$)

Anthropometry, bone traits, and lifestyle data are presented in Table 1. There were correlations between Z scores in childhood and adulthood for BMC ($r = 0.56$, $p < 0.001$), BMD

Table 1 Age, anthropometry, BMI, distal forearm BMC, BMD, and bone width in 120 boys and 94 girls with a mean age of 10.3 years (range 3–17) at baseline and mean 28 years (range 25–29) later at a mean age of 37 years (range 28–44)

	Women ($n = 94$)		Men ($n = 120$)	
	Baseline	Follow-up	Baseline	Follow-up
Age (years)	10.7 ± 3.9		37.4 ± 4.1	
Height (cm)	142.4 ± 20.3		166.7 ± 6.4	
Weight (kg)	37.0 ± 13.9	72.7 ± 15.9	9.9 ± 4.0	36.6 ± 4.0
BMI (kg/m^2)	17.4 ± 2.5	26.1 ± 5.3	140.3 ± 23.5	179.4 ± 7.6
BMC (g)	0.47 ± 0.2	0.72 ± 0.1	35.7 ± 17.4	86.3 ± 14.3
BMD (g/cm^2)	0.42 ± 0.1	0.54 ± 0.1	17.2 ± 3.0	26.8 ± 4.0
Bone width (cm)	2.1 ± 0.31	2.65 ± 0.24	0.47 ± 0.2	1.03 ± 0.1
Age at menarche (years)	12.7 ± 1.3	12.7 ± 1.3	0.42 ± 0.1	0.67 ± 0.1
Smokers[a] (%)	–	35.1	2.17 ± 0.4	3.09 ± 0.23
Alcohol[b] (%)	–	3.2	–	–
Chronic disease[c] (n)	–	1	–	33.1
Food intolerance (n)	–	4	–	12.5

Data are shown as unadjusted mean ± standard deviation (SD), as proportions (%) or as numbers (n)

BMI body mass index, *BMC* bone mineral content, *BMD* bone mineral density

[a] Proportion of individuals with a smoking history of at least 5 years

[b] Proportion of risk for consumers of alcoholic beverages as defined by the National Board of Health and Welfare in Sweden (>9 units of alcohol/week for women and >14 units for men)

[c] Number of individuals with chronic disease on medication (men: hypertension and Mb Crohn, a type of inflammatory bowel disease resulting in swelling and dysfunction of the intestinal tract, women: hypothyroidism)

($r = 0.42$, $p < 0.0001$), and bone width ($r = 0.58$, $p < 0.001$), evident also in gender-specific analyses (Table 2). Adjustment for differences in height and weight at baseline did not change the results (data not shown). Correlations between Z scores were also found in subgroup analyses of children with a history of fracture (BMC: $r = 0.51$, BMD: $r = 0.32$, and bone width $r = 0.64$; all $p < 0.01$), children with premature birth (BMC: $r = 0.65$, BMD: $r = 0.48$, and bone width $r = 0.56$; all $p < 0.0001$), and children from the former control cohort (BMC: $r = 0.53$, BMD: $r = 0.44$, and bone width $r = 0.55$; all $p < 0.0001$).

The sensitivity and specificity of a childhood measurement in the lowest quartile of Z scores to predict an adult value in the same quartile of Z scores are shown in Table 3. The low correlations (Table 2) and low sensitivity (Table 3) indicate that a large proportion of participants moved from one quartile of Z scores to another (Figs. 2, 3, 4). The proportion of participants who left the lowest quartile of Z scores (for higher quartiles) during growth was 58 % for BMD (Fig. 2), 47 % for BMC (Fig. 1), and 53 % for bone width (Fig. 3).

As expected, there was some correlation between Z scores of the accrued amount of mineral (BMC) and gain in bone size ($r = 0.43$, $p < 0.001$), although 93/211 (44 %) of the participants had a proportionally higher accrual of BMC Z scores than gain in bone size Z scores (points above the dotted line in Fig. 4) and 118/211 (56 %) had a proportionally higher gain in bone size Z scores than accrual of BMC Z scores (points below the dotted line in Fig. 4). This heterogeneity was more evident in those who left the lowest quartile of BMD Z scores during the study period ($n = 31$) as we in this group found a higher accrual of bone mineral (BMC, ΔZ score 0.54, 95 % CI 0.19–0.89) and a trend for a lower gain in bone size (ΔZ score −0.31, 95 % CI −0.65 to 0.02; Fig. 5). In contrast, those who left the highest quartile of BMD Z scores during the study period ($n = 26$) had a lower accrual of bone mineral (BMC, ΔZ score −1.10, 95 % CI −1.44 to −0.76) but also a trend for a higher gain in bone size (ΔZ score 0.24, 95 % CI −0.08 to 0.56; Fig. 5).

Children 10 Years or Older at Baseline ($n = 110$)

In children ≥10 years at baseline we found Z-score correlations between bone traits in childhood and adulthood for BMC ($r = 0.64$, $p < 0.001$), BMD ($r = 0.51$, $p < 0.0001$), and bone width ($r = 0.64$, $p < 0.0001$), evident also in gender-specific analyses (Table 2). Adjustment for differences in height and weight at baseline did not change the results (data not shown). The sensitivity and specificity (as described above) of a bone mass measurement in children aged ≥10 years to predict the adult values

Table 2 Correlations between baseline and follow-up Z scores of distal forearm BMC, BMD, and bone width measured by SPA in 214 children with a mean age of 10.3 years (range 3–17) at baseline and mean 28 years (range 25–29) later at a mean age of 37 years (range 28–44)

	BMC (g)			BMD (g/cm^2)			Bone width (cm)		
	All children	Girls	Boys	All children	Girls	Boys	All children	Girls	Boys
Children 3–17 years at baseline	0.56 ($n = 214$)	0.64 ($n = 94$)	0.48 ($n = 120$)	0.42 ($n = 214$)	0.50 ($n = 94$)	0.35 ($n = 120$)	0.58 ($n = 214$)	0.65 ($n = 94$)	0.51 ($n = 120$)
Children ≥10 years at baseline	0.64 ($n = 110$)	0.73 ($n = 51$)	0.52 ($n = 59$)	0.51 ($n = 110$)	0.58 ($n = 51$)	0.43 ($n = 59$)	0.64 ($n = 110$)	0.73 ($n = 51$)	0.55 ($n = 59$)
Children <10 years at baseline	0.47 ($n = 104$)	0.52 ($n = 43$)	0.44 ($n = 61$)	0.31* ($n = 104$)	0.40** ($n = 43$)	0.26* ($n = 61$)	0.50 ($n = 104$)	0.52 ($n = 43$)	0.50 ($n = 61$)

Data are reported as Pearson's correlation coefficient (r) with the number of individuals in the analyses in parentheses

BMC bone mineral content, *BMD* bone mineral density, *SPA* single photon absorptiometry

* $p < 0.05$; ** $p < 0.01$; all other analyses were significant at a level of $p < 0.0001$

are shown in Table 4. Due to the small sample size, we did not estimate gender-specific sensitivity and specificity.

Children Below Age 10 at Baseline ($n = 104$)

In children <10 years at baseline we also found *Z*-score correlations between bone traits in childhood and adulthood for BMC ($r = 0.47$, $p < 0.001$), BMD ($r = 0.31$, $p < 0.05$), and bone width ($r = 0.50$, $p < 0.001$), evident also in gender-specific analyses (Table 2). Adjustment for differences in height and weight at baseline did not change the results (data not shown). The sensitivity and specificity (as described above) of a bone mass measurement in children <10 years to predict the adult values are shown in Table 4. Due to the small sample size, we did not estimate gender-specific sensitivity and specificity.

Discussion

This study shows that a pediatric BMD scan has poor ability to predict adult BMD and that childhood BMD was only able to explain 12 % of the variance in adult BMD for men and 25 % for women. The sensitivity of a pediatric BMD scan in the lowest quartile to predict an adult result in the same quartile was also low. The variance for BMC was 23 % in men and 41 % in women. The higher correlation for BMC than BMD is supported by previous reports [11, 12, 19]. This could reflect the fact that BMC, although associated with skeletal size, only estimates the amount of mineral while BMD reflects two separate estimates, the amount of bone mineral and areal bone size. This hypothesis is supported by the greater change in bone size in those who changed quartile of BMD during growth (Fig. 5). It must, however, be emphasized that there were children in our study with BMD below −2.5 SD who ended with a higher than average BMD in adulthood (Fig. 2).

There are prospective studies that have followed bone mass in the short-term perspective during growth [11–14, 19–22]. The only longitudinal study with distal forearm SPA data, by Magarey et al. [19], utilized measurements every second year during a 6-year period in 108 children aged 11 years at baseline. They reported that up to 88 % of the variance in bone mass at age 17 years could be explained by the bone mass at age 11 years and that 80–90 % of those in the top or bottom quintile at baseline remained in the same quintile 6 years later [19]. Kalkwarf et al. [22] followed 1,554 children aged 6–16 at baseline for 3 years with dual-energy X-ray absorptiometry (DXA) for total body, spine, hip, and radius and reported that 58–76 % of the variance in bone mass at follow-up was explained by baseline values and that 72–87 % of children

Table 3 Sensitivity and specificity of a pediatric bone mass scan to predict adult bone mass: BMC, BMD, and bone width (cm) were measured by SPA in the distal forearm in 214 children with mean age of 10.3 years (range 3–17) at baseline and mean 28 years (range 25–29) later at a mean age of 37 years (range 28–44)

	BMC (g)			BMD (g/cm^2)			Bone width (cm)		
	All children	Girls	Boys	All children	Girls	Boys	All children	Girls	Boys
Sensitivity (%)	52 (38–66)	48 (27–69)	55 (36–74)	48 (27–69)	65 (43–84)	38 (21–57)	50 (36–65)	55 (32–76)	46 (28–66)
Specificity (%)	75 (69–81)	76 (66–84)	75 (66–83)	76 (66–84)	89 (79–95)	79 (69–87)	83 (77–89)	85 (74–92)	82 (73–89)

BMC, BMD and bone width *Z* scores in childhood and adulthood were calculated for each individual using all 214 individuals as controls. Individuals were stratified in quartiles based on *Z* scores at baseline and follow-up. Data are shown as means with 95 % confidence intervals in parentheses

Sensitivity (%) the probability of a pediatric bone scan in the lowest quartile to predict an adult result in the same quartile, *Specificity* (%) the probability of a pediatric bone mass scan in the three highest quartiles to predict an adult result outside the lowest quartile, *BMC* bone mineral content, *BMD* bone mineral density, *SPA* single photon absorptiometry

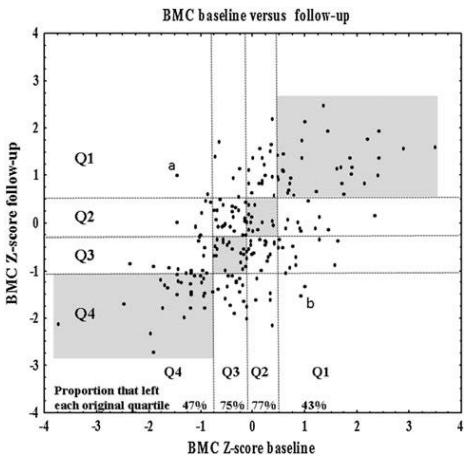

Fig. 1 *Z* scores for bone mineral content (BMC) in childhood and adulthood in each participant. Data points within the *shaded squares* represent individuals who remained in their baseline quartile of BMC at follow-up. Among the participants we found individuals who moved from the lowest quartile of BMC at baseline to the highest at follow-up (*a*) and others who moved from the highest to the lowest quartiles of BMC (*b*)

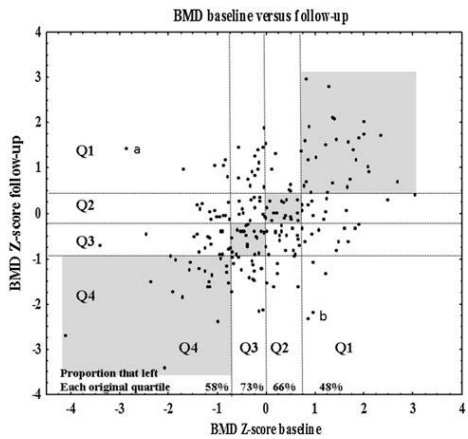

Fig. 2 *Z* scores for bone mineral density (BMD) in childhood and adulthood in each participant. Data points within the *shaded squares* represent individuals who remained in their baseline quartile of BMD at follow-up. Among the participants we found individuals who moved from the lowest quartile of BMD at baseline to the highest at follow-up (*a*) and others who moved from the highest to the lowest quartiles of BMD (*b*)

with a bone mass below −1.5 SD had a value lower than −1.0 SD at follow-up. Another longitudinal (8.5-year follow-up) study in 125 prepubertal girls by Ferrari et al. [11] reported that a pediatric BMC scan explained 29–66 % of the variance in postpubertal BMC, Foley et al. [14] reported that a prepubertal scan explained 24–79 % of the variance in postpubertal bone mass in 183 children followed from age 8 to 16 years, Budek et al. [23] inferred that 25–66 % of the BMC at age 17 years could be explained by the level of BMC at age 11 years, while Fujita et al. [24], following 225 children from age 9 to 12 years for a 6-year period, inferred that 42 % of the variance in BMD in older boys and 58 % in older girls could be explained by the baseline BMD. It is, however, unlikely that peak bone mass was captured in any of these studies as they all ended before termination of growth and peak bone mass, usually regarded to occur around age 20 in the hip [15] and after age 30 in the distal radius [1]. The long observation period in our study, however, covers this period and probably also explains our lower correlations.

The inclusion of pre-, peri-, and postpubertal children could influence our inferences since bone properties change rapidly at puberty [1]. As girls in the study

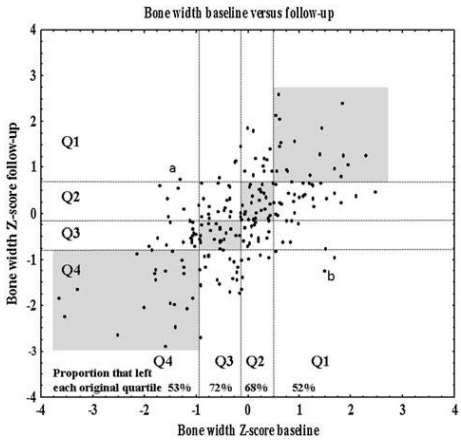

Fig. 3 *Z* scores for bone width in childhood and adulthood in each participant. Data points within the *shaded squares* represent individuals who remained in their baseline quartile of bone width at follow-up. Among the participants we found individuals who moved from the lowest quartile of bone width at baseline to the highest at follow-up (*a*) and others who moved from the highest to the lowest quartiles of bone width (*b*)

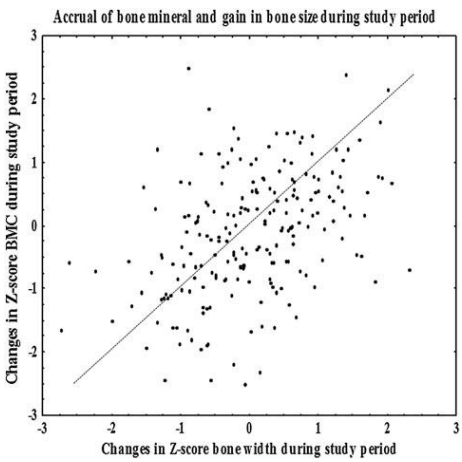

Fig. 4 Individual bone growth (ΔZ score of bone width) and bone mineral accrual (ΔZ score of bone mineral content, BMC). Of the participants 93/211 (44 %) had a proportionally larger accrual of BMC than gain in bone size (markers above the *dotted line*) and 118/211 (56 %) a proportional higher gain in bone size than accrual of BMC (marker below the *dotted line*)

experienced menarche at a mean age of 12.7 years (range 10–18) and boys are known to reach puberty approximately 1.5 years later [25], we stratified children below and above

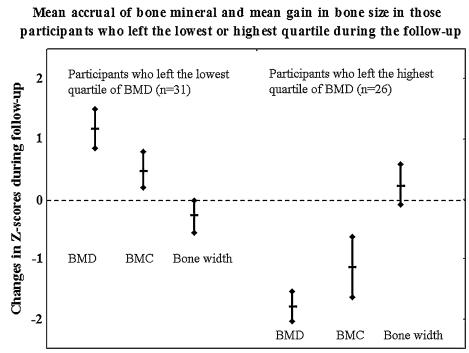

Fig. 5 Changes in *Z* score in participants who left the lowest quartile of BMD during the study period ($n = 31$) and those who left the highest quartile ($n = 26$). Those who left the lowest quartile of BMD had a significantly higher accrual of bone mineral and a trend for a lower gain in bone size. Those who left the highest quartile of BMD had a significantly lower accrual of bone mineral and a trend for a larger gain in bone size. Data are presented as means with 95 % confidence intervals

age 10 years. We hence predominantly included children before they reached the fast prepubertal growth spurt [25] in the strata of children <10 years. This enabled us to confirm our hypothesis of higher tracking in older than younger children, probably due to the longer remaining growth period in young children. The lower correlation in boys than girls of the same chronological age probably reflects the later onset of puberty in boys and their longer remaining growth period [19, 22].

About 44 % of the participants in our study had a more pronounced accrual of bone mineral than expected (markers positioned above the dotted line in Fig. 4) and 56 % a more pronounced gain in bone width (markers positioned below the dotted line in Fig. 5). If the accrual of bone mineral (BMC) and the gain in bone size had been proportional, BMD would remain the same as BMD is an estimate that combines the amount of bone mineral (BMC) and the bone size. We found, however, low correlation between BMD in childhood and adulthood and that a large proportion of participants also changed BMD quartile during growth. Actually, we could identify individuals who had a childhood BMD *Z* score of −2.7 and an adult *Z* score of 1.5 (marked with "a" in Fig. 2). The different accrual of bone mineral and gain in bone size during growth are supported by our data, in which individuals who improved from the lowest quartiles of BMD had a higher accrual of bone mineral (BMC) but also a trend for a smaller gain in bone size (Fig. 5). In contrast, those deteriorating from the highest quartile of BMD had a smaller accrual of bone mineral but also a trend of greater

Table 4 Sensitivity and specificity of a pediatric bone mass scan to predict adult bone mass: BMC, BMD, and bone width were measured by SPA in distal forearm in 214 children with mean age 10.3 years (range 3–17) at baseline and mean 28 years (range 25–29) later at a mean age of 37 years (range 28–44) years

	BMC (g)		BMD (g/cm^2)		Bone width (cm)	
	Children <10 years at baseline ($n = 104$)	Children ≥10 years at baseline ($n = 110$)	Children <10 years at baseline ($n = 104$)	Children ≥10 years at baseline ($n = 110$)	Children <10 years at baseline ($n = 104$)	Children ≥10 years at baseline ($n = 110$)
Sensitivity (%)	52 (31–72)	50 (30–71)	40 (21–66)	46 (27–67)	35 (15–59)	60 (41–77)
Specificity (%)	85 (75–92)	84 (75–91)	81 (71–89)	83 (73–91)	88 (79–94)	78 (21–57)

BMC, BMD, and bone width Z scores in childhood and adulthood were calculated for each individual using all 214 individuals as controls. Children were stratified by age <10 or ≥10 years and thereafter by quartile of baseline and follow-up Z score. Due to the sample size, no gender-specific analyses were done. Data are presented as means with 95 % confidence intervals in parentheses

Sensitivity (%) the probability of a pediatric bone scan in the lowest quartile to predict an adult result in the same quartile, *Specificity* (%) the probability of a pediatric bone mass scan in the three highest quartiles to predict adult result outside the lowest quartile, *BMC* bone mineral content, *BMD* bone mineral density, *SPA* single photon absorptiometry

gain in bone size (Fig. 5). The heterogeneity of bone mineral accrual and gain in bone size could also explain why a pediatric bone mass scan could explain 31 % of the variance in adult BMC (which only evaluates the amount of mineral) but only 18 % of the variance in adult BMD (which, in addition to the amount of both mineral, reflects bone size).

Study strengths are the prospective design, the long follow-up spanning the period of peak bone mass, measurements by the same scanner at the same skeletal site, and continuous validation of the apparatus by a phantom during the entire study. The fact that all measurements at each occasion were performed by one technician and all graphical analyses by one author is also advantageous. An attendance rate of 72 % after 28 years is superior to previous prospective studies [11, 13, 14, 19, 22, 26, 27], and the fact that dropout analyses revealed similar anthropometrics, bone trait, and lifestyle factors in participants and nonparticipants further strengthens the quality of the data. Limitations include few individuals in the subgroups, resulting in the risk of a Type II error, forcing us to refrain from gender-specific evaluations in separate age strata. SPA was the only available scanning technique in 1979, and it would have been advantageous to use modern scanning techniques as well as evaluation of other anatomical regions, especially the hip and spine, commonly used for clinical evaluation of osteoporosis [28]. As growth occurred and ended during the study period, this could hypothetically influence the location of the position of measurement and influence the acquired absolute bone mass value. To take this into account, we used *Z* scores and estimated tracking between *Z* scores instead of absolute values. A registration of pubertal maturity to stratify the children by true pubertal status would have been preferred as well as individual registration of menopause, which would have given reasonable estimates of individuals at risk of postmenopausal bone loss. As the oldest woman in our cohort was 44 years, the mean age at menopause in Scandinavia is 51 (95 % CI 45–55) years [29], and bone loss in the cortical region of the distal forearm is initiated after age 40 years [30], there is a low risk of any significant age-related bone loss in our data. Finally, it would have been advantageous to have serial measurements to pinpoint the exact time of peak bone mass.

The association between childhood and adult BMD was in our study low. The data further indicate that a childhood BMD scan is of limited use for prediction of adult BMD, at least in healthy children. Further long-term longitudinal studies, preferably with modern measuring techniques (DXA and pQCT) and sites (spine and hip) as well as inclusion of children with BMD below −2.5 SD, are advocated before any definite clinical inferences can be drawn regarding the use of childhood BMD measurements.

We conclude that the correlation of distal forearm bone mass from childhood to adulthood is low and that a pediatric bone mass scan has poor ability to predict adult BMD. This seems attributable mainly to heterogeneity of bone mineral accrual and gain in bone size during growth.

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Paper III

A Pediatric Bone Mass Scan Has Poor Ability to Predict Peak Bone Mass – An 11-Year Prospective Study in 121 Children

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Key words: Adult, BMD, BMC, Bone mass, Child, Growth, Peak Bone Mass

Running title: Bone mass from childhood to young adulthood

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Abstract

Purpose: This 11-year prospective longitudinal study examined how a pre-pubertal pediatric bone mass scan predicts peak bone mass.

Methods: We measured bone mineral content (BMC; g), bone mineral density (BMD; g/cm^2) and bone area (cm^2) in femoral neck, total body and lumbar spine by dual energy X-ray absorptiometry (DXA) in a population based cohort including 65 boys and 56 girls. At baseline all participants were pre-pubertal with a mean age of 8 years (range 6-9), they were re-measured at a mean 11 years (range 10-12) later. The participants were then mean 19 years (range 18-19), an age range that corresponds to peak bone mass in femoral neck in our population. We calculated individual BMC, BMD and bone size Z-scores, using all participants at each measurement as reference and evaluated correlations between the two measurements. Individual Z-scores were also stratified in quartiles to register movements between quartiles from pre-pubertal age to peak bone mass.

Results: The correlation coefficients (r) between pre-pubertal and young adulthood measurements for femoral neck BMC, BMD and bone area varied between 0.37-0.65. The reached BMC value at age 8 years explained 42% of the variance in the BMC peak value, the corresponding values for BMD was 31% and bone area 14%. Among the participants with femoral neck BMD in the lowest childhood quartile, 52% had left this quartile at peak bone mass. A pediatric bone scan with a femoral neck BMD value in the lowest quartile had a sensitivity of 47% (95% CI 28, 66) and a specificity of 82% (95% CI 72, 89) to identify individuals who would remain in the lowest quartile at peak bone mass.

Conclusions: The pre-pubertal femoral neck BMD explained only 31% of the variance in femoral neck peak bone mass. A pre-pubertal BMD scan in a population based sample has poor ability to predict individuals who are at risk of low peak bone mass.

Introduction

Peak bone mass is reached in early adulthood and is a major determinant for fracture risk later in life and is thought to explain 50% of the variance of bone mineral density (BMD) at age 65 years[1-3]. The accrual of BMD during growth has therefore attracted interest and there is an ongoing debate on whether a low BMD in childhood predicts a low BMD in adulthood. Cross-sectional studies have inferred that a childhood excess [4-5] or deficit[6-7] in BMD remains in adulthood and recent prospective studies have inferred a partial tracking of BMD during growth[8-12]. Making prediction on adult BMD from childhood measurements is however difficult as bone properties change

rapidly during growth[3]. It is therefore important to perform serial measurements that cover both the pre-pubertal and post-pubertal phases including the time of peak bone mass to make accurate predictions. Only few have followed children with dual energy X-ray (DXA) for more than 6 years[10, 12-14] and none a period exceeding 10 years. We have previously in a 28-year prospective study shown that childhood forearm BMD measured with single photon absorptiometry (SPA) explained 17% of the variance in adult BMD and therefore concluded that a pediatric distal forearm BMD scan had poor ability to predict adult bone mass[15]. BMD was however only followed in an appendicular unloaded site with an older technique and studies with modern measuring technique (DXA) evaluating axial skeletal sites, preferably the femoral neck which is the gold standard region in clinical work are necessary.

We therefore conducted this 11-year prospective study to answer the following questions: (i) Does axial BMC and BMD track from childhood to peak bone mass? (ii) Is tracking more evident in older than younger children? (iii) Is there a gender discrepancy? (iv) What proportion of individuals in the lowest quartile of BMD in childhood remains in this quartile at peak bone mass? (v) What is the sensitivity and specificity of a pediatric bone scan to predict low peak bone mass? (vi) Is movement from one BMD quartile in childhood to another at peak bone mass due to different accrual of bone mineral or gain in bone size?

Material

Femoral neck, total body and lumbar spine bone mineral content (BMC; g) and bone area (cm^2) were measured at study start by dual-energy X-ray absorptiometry (DXA) and based on these measurements, bone mineral density (BMD, g/cm^2) was estimated by standard software in a population based cohort that included 65 boys with a mean age of 8 years (range 6–9) and 56 girls with a mean age of 8 years (range 6–9). The children were included from the Malmo Pediatric Osteoporosis Prevention (POP) study, a population-based, prospective, controlled, exercise intervention study, were the intervention children received daily school physical education (PE) while the controls received the Swedish standard 1 to 2 PE lessons per week, during the nine compulsory school years. The study has the main objective to follow skeletal development in children aged 6- to 9-years at study start until peak bone mass and has been described in detail previously [16-19]. The study was approved by the Ethics Committee of Lund University.

All participants were Caucasians, without any diseases or medications known to affect bone metabolism. The study protocol included yearly bone scanning until age 15 years, then an additional follow-up at age 19 years (range 18-19), thus rendering a mean follow-up period of 11 years (range 10-12). To be included in this report, participants

had to participate in the first and the last of the serial measurements. Out of the 338 children at baseline, 121 went through the last measurement. Among the drop outs, 1 boy and 1 girl had died, 20 boys and 15 girls had relocated, 44 boys and 24 girls could not be located, 58 boys and 51 girls had during the study period declined further participation, and 1 boy and 2 girls were excluded due to low age or growth hormone medication. 78 of the original 207 individuals (38%) from the intervention school with daily school physical education attended the last follow-up and 43 out of the original 131 individuals (33%) in the control school. Age, height, weight and body mass index (BMI), bone traits, gender distribution and lifestyle factors were at baseline similar in the intervention and the control schools. Furthermore, at baseline these traits were also similar in children who attended the follow-up and those who did not (data not shown). Finally, the descriptive data showed that anthropometry and bone traits were similar when comparing the intervention and control group both at baseline and follow-up (the only statistically significant difference was found in girls in total body BMC at follow-up ($p=0.03$) and since we found no statistically significant difference between the childhood-young adulthood correlations in the intervention and control cohorts (data not shown), all data were pooled.

Bone mineral content (BMC, g) and bone area (cm^2) were measured by dual X-ray absorptiometry based on these measurements, bone mineral density (BMD, g/cm^2) was estimated by standard software (DXA; DPX-L version 1.3z; Lunar, Madison, WI) in the femoral neck (FN) by a standard hip scan, total body (TB) by a total body scan and first to fourth lumber vertebra (L1-4) by a standard lumbar spine scan respectively. For the last measurement we shifted scanner from DPX-L to a Lunar Prodigy, Compaq DP, cross calibration was performed and the last measurements were corrected accordingly. Since we used Z-scores in the analyses, this did not affect our inferences. Our research technicians calibrated the machine daily with a lunar phantom and performed all measurements and all software analyses. The coefficients of variation (CV %), evaluated by duplicate measurements in 13 healthy children, were in the measured regions for BMD 1.4–3.8 %, BMC 1.3–3.2%, area 1.1- 2.2 %.

Body weight and height were measured with standard equipment. Lifestyle factors, diseases, and medications were evaluated by questionnaires at both baseline and follow-up[17].

The maturity of the children was assessed by Tanner staging[20], conducted by our research nurses at baseline and by self-report at follow-up.

Baseline measurements were done at a mean age of 8 years (range 6-9) when all children were pre pubertal in Tanner stage I. First follow-up in this study was done mean 5 years (range 4.8-5.2) later when the children were mean 13 years (range 12-13) and 5% were in Tanner stage I, 49.5% in Tanner stage II-III and 42.9% in Tanner stage IV-V and second follow-up mean 11 years (range 10-12) later when the participants were mean 19 years (range 18-19) and all in Tanner stage V. The last follow-up included the age

we have previously shown to correspond to peak bone mass in femoral neck in our population[21]. The FN measurements at follow up are therefore considered as a peak bone mass estimation, while peak bone mass in TB and L1-4 occur at higher ages[21].

Statistical evaluation

We used SPSS® version 20.0 for statistical calculations. Group differences were evaluated by chi-square test, Student's t-test or ANCOVA with adjustment for age. Individual Z-scores (the number of standard deviation above or below the age predicted mean) were derived by linear regression using all 121 individuals as reference population. Tracking (i.e. correlation) of the Z-scores between the baseline measurements at mean age 8 years and follow-up measurement at mean age 19 years and between 13 and 19 years using Pearson's correlation coefficient, partial correlation was used to adjust for height and weight at age 8 and 13 years respectively. We also stratified the Z-scores of each bone trait in quartiles and (i) examined the proportion of individuals that left their original quartile during the study period, (ii) estimated the sensitivity of a pediatric bone scan with a result in the lowest quartile to predict a follow-up measurement in the same quartile, and (iii) the specificity for a scan outside the lowest quartile to predict an adult result outside the lowest quartile. Data are presented as numbers (n), means ± standard deviations (SD), means with 95% confidence intervals (95% CI) or as proportions (%).

Results

Anthropometry, bone traits, and lifestyle data are presented in table 1. There were statistically significant correlations (all $p<0.001$) for femoral neck BMC ($r=0.65$), BMD ($r=0.56$) and bone area ($r=0.37$) between the first (mean age 8 years) and last measurement (mean age 19 years). The correlations for total body and lumbar spine between the same ages, were also statistically significant, with higher absolute r-values; for BMC ($r=0.79$ respectively $r= 0.79$), BMD ($r=0.73$ respectively $r=0.79$) and area ($r=0.73$ respectively $r=0.76$). The correlations for femoral neck BMC, BMD and bone area were statistically significantly lower than for total body and lumbar spine BMC, BMD and bone area (all $p<0.05$). In contrast correlations were similar for bone traits in total body and lumbar spine. Adjustment for differences in height and weight at baseline did not change the results (data not shown). The gender specific correlations are shown in Table 3. The gender specific correlations were similar in boys and girls (Table 3) as well as in all children from age 8 to age 19 and from age 13 to age 19 years (Table 4). We however found in our cohort a non-significantly lower FN correlation between the ages 8-19 years than between the age 13-19 years in all evaluated traits.

The sensitivity and specificity of a childhood measurement in the lowest quartile to predict an adult value in the same quartile are shown for separate anatomical regions in table 5. Although moderate to high correlations, (Table 3), the low sensitivity (table 5) indicates that a large proportion of participants moved from one quartile to another (shown for femoral neck separately in figures 1-3).

The proportion of participants who during growth left the lowest quartile for higher quartiles was for femoral neck BMC 31% (figure 1), for total body BMC 32% and for lumbar spine BMC 24%. The same proportions for femoral neck BMD was 52% (figure 2) for total body BMD 43% and for lumbar spine BMD 53% and for femoral neck area 54% (figure 3) for total body area 29% and for lumbar spine area 50%.

There were correlations between the accrued amount of mineral (BMC) and gain in bone size in femoral neck ($r=0.55$, $p<0.001$) (figure 4), total body ($r=0.84$, $p<0.001$) and lumbar spine ($r=0.82$, $p<0.001$). In femoral neck 57/114 (50%) of the participants had a proportionally higher accrual of BMC than gain in bone size (points above the dotted line in figure 4) and 57/114 (50%) a proportionally higher gain in bone size than accrual of BMC (points below the dotted line in figure 4). In total body 48/115 (42%) of the participants had a proportionally higher accrual of BMC than gain in bone size and 67/115 (58%) a proportionally higher gain in bone size than accrual of BMC. In lumbar spine 67/117 (57%) of the participants had a proportionally higher accrual of BMC than gain in bone size and 50/117 (43%) a proportionally higher gain in bone size than accrual of BMC.

In sub analysis of participants who improved from the lowest quartile of femoral neck BMD during the study period (n=15), we found a statistically significantly higher accrual of bone mineral (BMC) (ΔZ-score 0.39; 95% CI 0.09, 0.70) than the average participant but no significant change in bone size (ΔZ-score –0.21, 95% CI –0.78, 0.36) (figure 5). In contrast participants deteriorating from the highest quartile of femoral neck BMD during the study period (n=11) had a statistically significantly lower accrual of bone mineral (BMC) (ΔZ-score –1.01; 95% CI –1.45, –0.58) than the average participant but no significant change in bone size (ΔZ-score -0.34, 95% CI –1.14, 0.46) (figure5).

Discussion

This study shows that even if there is a correlation for both BMC and BMD between childhood and peak bone mass, the sensitivity of a childhood bone mass scan to predict peak bone mass is in a normal population low since a large proportion of individuals move between the quartiles of BMC as well as BMD during growth. That is, the

prediction of peak bone mass by a childhood scan in a normal healthy pediatric population in one specific individual is of low clinical value.

The age for peak bone mass has been thoroughly discussed, most estimates indicate that it is reached at different ages in different anatomical regions [3, 22-23]. Many studies infer that BMD does not significantly increase after the third decade of life[3, 22]. However at some regions such as the distal radius reports have shown that peak bone mass may be reached as late as age 40 years[23]. Studies that have specifically evaluated peak bone mass in femoral neck have suggested that peak bone mass is reached between 16-18 years of age in girls and 18-20 years in boys[3, 22, 24]. This has also been confirmed in a large normative pediatric bone mass study from our region where peak BMC and BMD were reached around 18 years of age in both girls and boys[21]. This study also showed that peak total skeletal BMD and peak lumbar spine BMD are expected to peak 5-10 years later than in femoral neck[21]. Due to this, we have in the current study used femoral neck as the region of interest when evaluating tracking until peak bone mass but we also present data for total body and lumbar spine for completeness, with the knowledge that these regions have substantial remaining growth after our last follow-up evaluation. We found a statistically significantly lower correlation of FN compared to TB and LS this supports our hypothesis that correlations between childhood and young adulthood measurements in regions that had reached peak value would be lower compared to regions with remaining growth potential. A plausible explanation could be that a region in which the peak value has been reached has undergone larger changes in BMC and BMD from baseline than a region with remaining growth [25-26].Another plausible explanation is that the femoral neck undergoes more changes in structure than the spine and whole body during growth

Previous reports have suggested that the correlation of BMD from childhood to young adulthood is higher in girls than boys [8, 12, 27], and higher in older children than younger[28]. In our study correlations were similar in boys and girls and from 8 to19 years of age and from 13 to 19 years. However, since the sample size in the subgroup analyses was small, and since we found trends for differences, we can not rule out a type II error.

There were a large proportion of individuals moving from the lowest quartile from baseline to follow-up measurements. This resulted in a low sensitivity for a childhood scan in the lower quartile to predict a peak bone mass value in the lowest quartile. We also found a larger proportion moving between quartiles and a lower sensitivity for BMD compared to BMC, supporting the hypothesis that growth related changes in the combined traits of bone mineral and bone size (which are both included in the BMD estimate), will result in lower correlations than in BMC which only estimates this single trait (bone mineral).

We must highlight that our study included only children within normal ranges of BMC, BMD and bone size, we can therefore not state if there is a higher correlation or

better prediction with higher sensitivity and specificity to predict peak bone mass in children with more extreme deficits in pediatric bone traits. Wren et al for example reported that almost all children with a baseline Z-score of below -1.5 had a final adult Z-score below average, with the majority remaining lower than -1.0[12]. As we had only 5 children with a Z-score below -1.5 we were not able to follow this line of investigation further and future studies including children with lower BMD are necessary.

Femoral neck and lumbar spine area measurements were included since we try to evaluate prediction of both the amount of bone minerals and bone size and since the clinical used measure of bone mass in adulthood (BMD) is dependent on both the amount of mineral and the bone size. There was moderate correlation between the accrued amount of bone mineral (BMC) and gain in bone size in femoral neck ($r=0.55$, $p<0.001$), where half of the participants had a proportionally higher accrual in BMC than gain in bone size (figure 4). However in sub analysis of the participants who improved from the lowest quartile of femoral neck BMD during the study period, we found a statistically significantly higher accrual of bone mineral (BMC) than the average participant but with no difference in the gain of bone size (figure 5). In contrast the participants who deteriorated from the highest quartile of femoral neck BMD during the study period had a statistically significantly lower accrual of bone mineral (BMC) than the average participant but no difference in the gain of bone size (figure5). Since there is heterogeneity of bone mineral accrual and gain in bone size, the correlations from childhood to young adulthood of BMC as well as bone size ought to be greater than for BMD (which in addition to the amount of mineral also reflects bone size). This hypothesis is supported by previous studies[15] and by our determination coefficients (r^2) in that a pediatric bone mass scan explains 42% of the variance in adult peak BMC but only 31% of the variance in peak BMD also supported in previous studies.[9-10, 29]

Study strengths include the prospective study design, the use of DXA, the clinically most used technique to estimate bone properties, the inclusion of appendicular data, the long follow-up period that spans femoral neck peak bone mass, serial measurements by the same technique and conducted by the same technicians at the same skeletal regions and with continuous validation of the apparatus by a phantom during the entire study period. An attendance rate of 35% is comparable to other studies with shorter follow-up period[12], but must still also be considered a limitation. The fact that dropout analyses revealed similar anthropometrics, bone trait and lifestyle factors in participants and non participants however strengthens our results. Limitations also include the small sample size in the sub-group analyses which increases the risk of conducting a type II error, and precludes gender-specific evaluations per age strata. Tanner stage classification by self assessments in the follow-up evaluation rather than expert classification must also be regarded as a study weakness. It would have been advantageous to have annual measurements also in the higher ages and to follow the

children also after age 19 years to more exactly pinpoint peak bone mass not only in the femoral neck but also in total body and the lumbar spine. The proximal femur region, commonly assessed by DXA in adults, is more challenging to evaluate in children. Skeletal landmarks, which guide proper positioning, may not be well developed in young children, which can lead to errors in positioning and placement of the region of interest (ROI) using standard software. However, data from the Bone Mineral Density in Childhood Study (BMDCS) suggest that age-related precision of the total hip and femoral neck is comparable to both that of the spine and TBLH [30]. Further there are limitations to DXA measurements in children, as size and developmental status must be considered before interpretation on BMD. Growth in size will influence the BMD value as the BMD estimate is a function of BMC and bone area, which changes non-linearly during growth. The situation is therefore not the same as in adults. This is one of the reasons why we used the current standard recommended by ISCD 2013[30] for reporting DXA results, BMD Z-score, which provides an estimate of the SD(s) away from the mean for chronologic age and sex.To have a cohort without any intra-curricular physical activity would be ideal, but as the exercise cohort and the controls were similar in anthropometry and bone traits at study start as well as in bone traits at follow up (the only statistically significant difference was found in girls in total body BMC at follow-up (p=0.03)), we allowed pooling all data.

We conclude that although there is a correlation of bone traits from childhood to peak values, the clinical value of a pediatric bone mass scan in a specific individual within a healthy cohort of children with non extreme values of BMC or BMD to predict peak bone mass is low. If there is better prediction in children with specific diseases or very low BMC or BMD must be evaluated in future studies.

Conflict of interests

Karlsson Caroline, Dencker Magnus, Nilsson Jan-Åke, Karlsson K. Magnus have no potential conflicts of interest to report.

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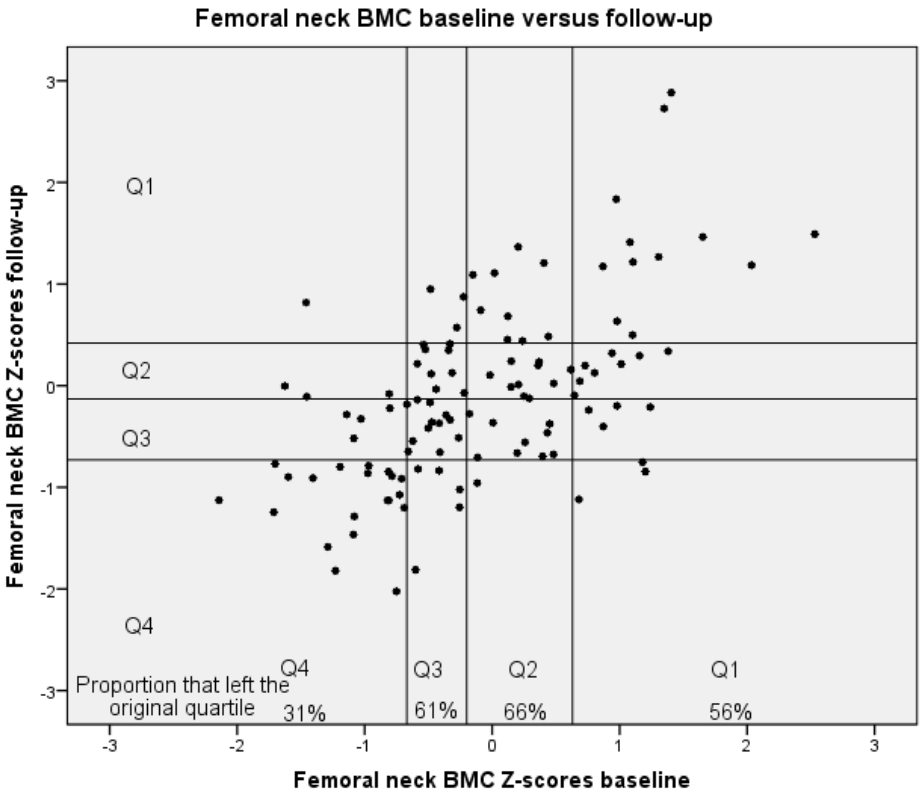

Figure 1.
Z-scores for femoral neck bone mineral content (BMC) at mean age of 8 years and at peak bone mass. Data points within the squares represent individuals that remained in their baseline quartile of BMC at follow-up.

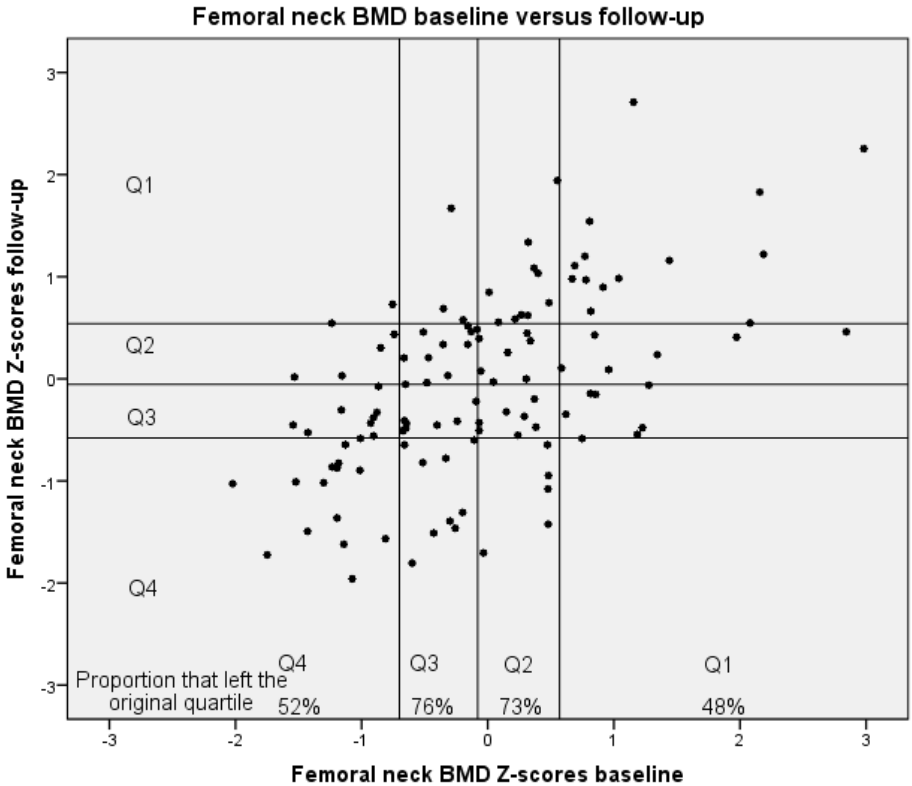

Figure 2.
Z-scores for femoral neck bone mineral density (BMD) at mean age of 8 years and at peak bone mass. Data points within the squares represent individuals that remained in their **baseline quartile of BMD at follow-up.**

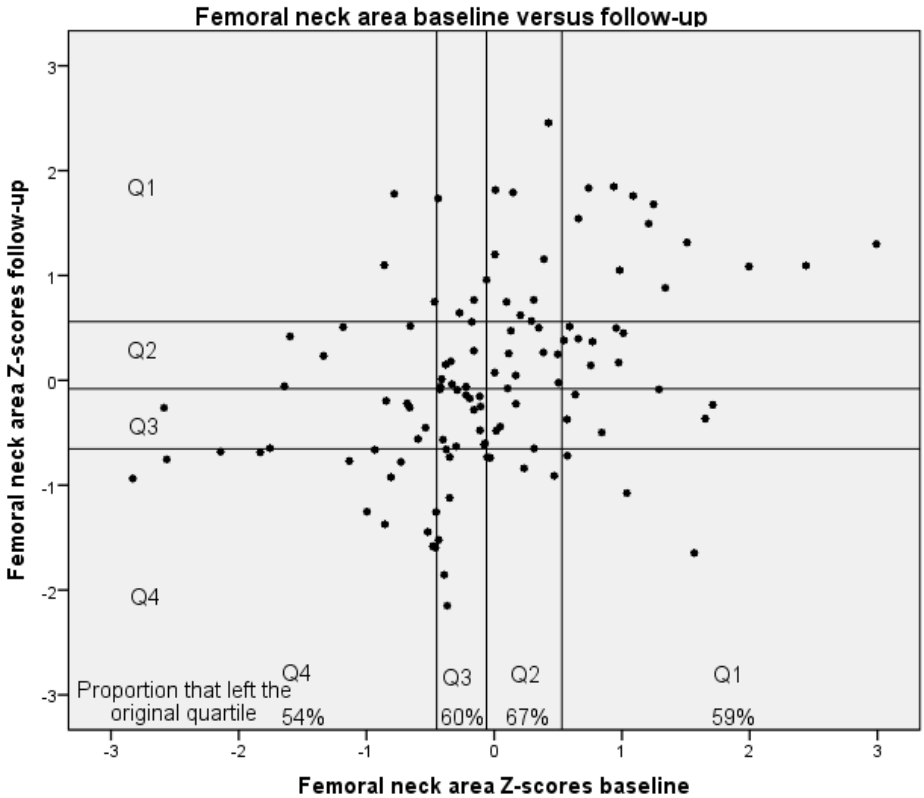

Figure 3.
Z-scores for femoral neck area at mean age of 8 years and at peak bone mass. Data points within the squares represent individuals that remained in their baseline quartile of femoral neck area at follow-up.

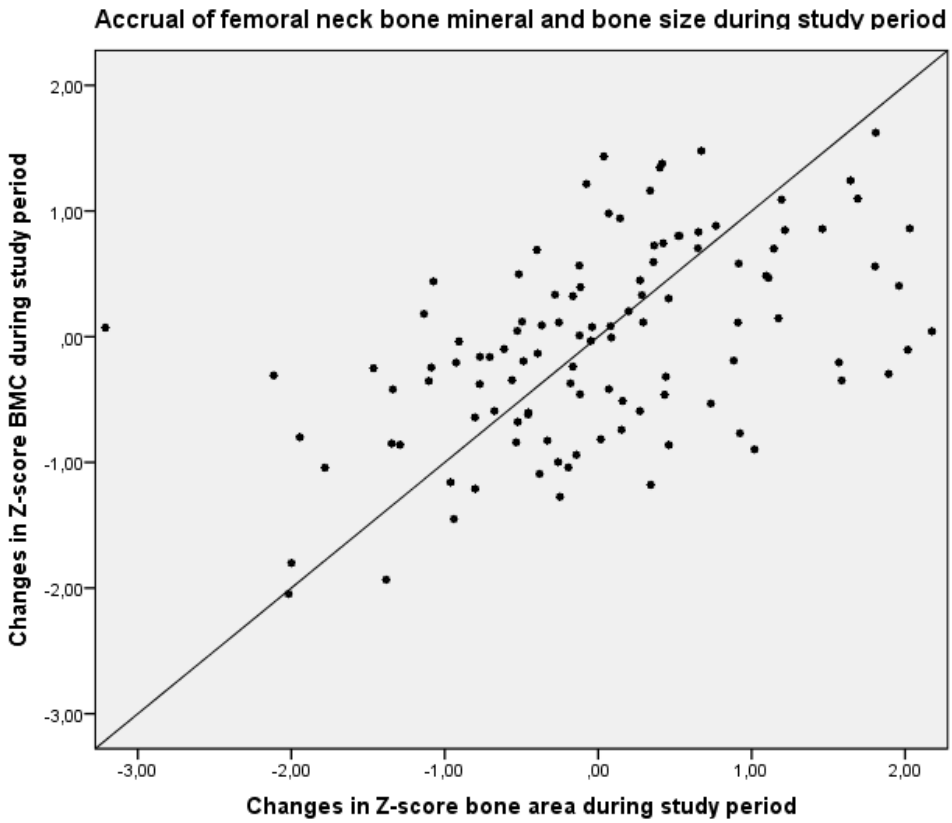

Figure 4.
Individual femoral neck bone growth (delta Z-score of bone width) and bone mineral accrual (delta Z-score of bone mineral content; BMC). 57/114 (50%) of the participants had a proportional larger accrual of BMC than gain in bone size (markers above the dotted line) and 57/114 (50%) a proportional higher gain in bone size than accrual of BMC (marker below the dotted line).

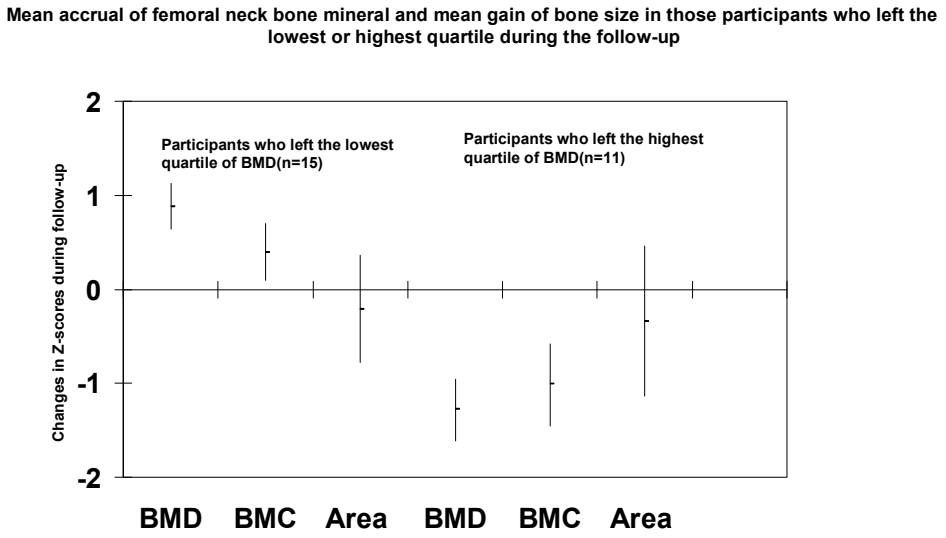

Figure 5.
Changes in femoral neck Z-score in participants who left the lowest quartile of BMD during the study period (n=15) and those who left the highest quartile (n=11). Those who left the lowest quartile of BMD had a significantly higher accrual of bone mineral and no significant gain in bone size. Those who left the highest quartile of BMD had a significantly lower accrual of bone mineral and no gain in bone size. Data are presented as means with 95% confidence intervals (95% CI).

Table 1.
Age, anthropometry, body mass index (BMI; kg/m^2), total body, lumbar spine and femoral neck bone mineral content (BMC; g), bone mineral density (BMD; g/cm^2) and bone area (cm^2) in 121 children with a mean age of 7.7 years (range 6-9) at baseline and mean 11.1 years (range 10-12) later at a mean age of 18.8 years (range 18-19). Data are shown as unadjusted means ± standard deviation (SD) or as numbers (n).

	Cases			
	Baseline	Follow-up	Baseline	Follow-up
	Girls		**Boys**	
	n=56		n=65	
Age (year)	7.7±0.6	18.8±0.3	7.7±0.6	18.8±0.3
Height (cm)	127.6±6.5	167.4±5.4	129.0±7.3	180.6±6.8
Weight (kg)	27.0±5.5	62.3±9.6	27.7±5.2	75.2±12.8
BMI (kg/m^2)	16.1±0.3	22.2±3.0	16.4±2.0	23.1±3.8
Total Body				
BMC (g)	921±161	1242±180	983±184	1568±261
BMD (g/cm^2)	0.84±0.04	1.14±0.09	0.85±0.05	1.22±1.10
Lumbar Spine				
BMC (g)	19.1±4.1	62.5±10.5	19.9±4.1	75.3±15.5
BMD (g/cm^2)	0.69±0.08	1.19±0.14	0.68±0.08	1.21±0.14
Area (cm^2)	27.6±3.4	52.4±4.2	29.0±3.7	61.9±7.0
Femoral Neck				
BMC (g)	2.5±0.5	5.1±0.8	2.8±0.6	6.3±1.2
BMD (g/cm^2)	0.71±0.10	1.07±0.13	0.78±0.12	1.15±0.16
Area (cm^2)	3.6±0.5	4.7±0.3	3.6±0.3	5.5±0.4

Table 2.
Age, anthropometry, body mass index (BMI; kg/m^2), total body, lumbar spine and femoral neck bone mineral content (BMC; g) and bone mineral density (BMD; g/cm^2)) in 34 girls and 44 boys in the intervention school and 22 girls and 21 boys in the control school with a mean age of 7.7 years (range 6-9) at baseline and mean 11.1 years (range 10-12) later at a mean age of 18.8 years (range 18-19). Data are shown as unadjusted means ± standard deviation (SD) or as numbers (n).

	Cases					
	Baseline			Follow-up		
	Girls					
	Intervention	Controls	p-value	Intervention	Controls	p-value
	n=34	n=22		n=34	n=22	
Age (year)	7.5±0.5	7.9±0.6	**0.02**	18.8±0.4	18.7±0.3	0.69
Height (cm)	127.2±5.9	128.2±7.6	0.57	168.1±5.2	166.4±5.5	0.24
Weight (kg)	26.9±5.1	27.0±6.0	0.95	64.2±10.2	59.2±7.8	0.06
BMI (kg/m^2)	16.1±1.7	16.2±2.1	0.77	22.4±2.9	21.9±3.2	0.59
Total Body						
BMC (g)	921±161	918±181	0.92	1284±187	1177±151	**0.03**
BMD (g/cm^2)	0.84±0.04	0.84±0.06	0.84	1.15±0.09	1.12±0.07	0.20
Lumbar Spine						
BMC (g)	19.1±4.1	18.9±4.3	0.75	64.6±10.9	59.5±9.2	0.08
BMD (g/cm^2)	0.70±0.08	0.67±0.08	0.32	1.22±0.15	1.15±0.13	0.08
Femoral Neck						
BMC (g)	2.52±0.54	2.45±0.41	0.61	5.2±0.85	4.84±0.69	0.36
BMD (g/cm^2)	0.71±0.10	0.71±0.10	0.86	1.09±0.13	1.04±0.13	0.054

Table 2 continued.

	Baseline			Follow-up		
	Boys					
	Intervention	Controls	p-value	Intervention	Controls	p-value
	n=44	n=21		n=44	n=21	
Age (year)	7.6±0.6	8.0±0.7	**0.01**	18.8±0.3	18.8±0.3	0.56
Height (cm)	128.5±7.4	130.0±7.0	0.46	180.3±7.3	181.5±5.8	0.52
Weight (kg)	27.8±5.5	27.4±4.5	0.77	75.5±13.4	74.4±11.7	0.73
BMI (kg/m^2)	16.6±2.3	16.1±1.5	0.43	23.2±3.9	22.9±3.6	0.77
Total Body						
BMC (g)	981±197	987±159	0.91	1575±281	1553±208	0.77
BMD (g/cm^2)	0.86±0.05	0.85±0.05	0.47	1.22±0.11	1.21±0.10	0.68
Lumbar Spine						
BMC (g)	19.7±4.3	20.2±3.8	0.70	75.5 ±16.2	75.1±14.3	0.93
BMD (g/cm^2)	0.68±0.08	0.68±0.09	0.95	1.21±0.15	1.20±0.12	0.87
Femoral Neck						
BMC (g)	2.79±0.66	2.83±0.52	0.81	6.40±1.3	6.16±0.82	0.45
BMD (g/cm^2)	0.78±0.12	0.78±0.13	0.98	1.15±0.17	1.15±0.15	0.98

Table 3.
Correlations between baseline and follow-up Z-scores of bone mineral content (BMC; g), bone mineral density (BMD; g/cm^2) and bone area (cm^2) measured by Dual-energy X-ray Absorptiometry (DXA) in Total body, Lumbar spine and femoral neck in 121 children with mean age of 8 years (range 6-9) at baseline and mean 11.1 years (range 10-12) later at a mean age of 18.8 years (range 18-19). Data are reported as the Pearson's correlation coefficient (r) with the 95% confidence interval (CI 95%) within brackets. *p<0.02, **p<0.01, all other analyses were significant at a level of p< 0.0001.

	Bone mineral content (BMC)			**Bone mineral density (BMD)**			**Bone area**	
	Total Body	Lumbar spine	Femoral Neck	Total Body	Lumbar spine	Femoral Neck	Lumbar spine	Femoral Neck
All (n=121)	0.79 (0.71, 0.85)	0.79 (0.71, 0.85)	0.65 (0.53, 0.74)	0.73 (0.64, 0.80)	0.79 (0.71, 0.85)	0.56 (0.44, 0.68)	0.76 (0.67, 0.83)	0.37 (0.21, 0.51)
Girls (n=56)	0.80 (0.68, 0.88)	0.78 (0.65, 0.87)	0.68 (0.51, 0.80)	0.76 (0.62, 0.86)	0.81 (0.70, 0.89)	0.63 (0.44, 0.77)	0.70 (0.53, 0.82)	0.33* (0.07, 0.55)
Boys (n=65)	0.78 (0.66, 0.86)	0.80 (0.69, 0.87)	0.62 (0.44, 0.75)	0.70 (0.56, 0.81)	0.77 (0.65, 0.86)	0.49 (0.29, 0.66)	0.81 (0.70, 0.88)	0.41** (0.18, 0.60)

Table 4.
Correlations for bone trait Z-scores between age 8 and age 19 years (n=121) and between age 13 and 19 years (n=119). Bone mineral content (BMC; g), bone mineral density (BMD; g/cm^2) and bone area (cm^2) were measured by Dual-energy X-ray Absorptiometry (DXA) in Total body, Lumbar spine and Femoral neck. Data are reported as the Pearson's correlation coefficient (r) with 95% confidence interval (95% CI) within brackets. All analyses were significant at a level of $p < 0.0001$.

	Bone mineral content (BMC)			**Bone mineral density (BMD)**			**Bone area**	
	Total Body	Lumbar spine	Femoral Neck	Total Body	Lumbar spine	Femoral Neck	Lumbar spine	Femoral Neck
Age 8 to 19 (n=121)	0.79 (0.71, 0.85)	0.79 (0.71, 0.85)	0.65 (0.53, 0.74)	0.73 (0.64, 0.80)	0.79 (0.71, 0.85)	0.56 (0.44, 0.68)	0.76 (0.67, 0.83)	0.37 (0.21, 0.51)
Age 13 to 19 (n=119)	0.72 (0.62, 0.80)	0.61 (0.48, 0.71)	0.66 (0.55, 0.75)	0.77 (0.69, 0.83)	0.68 (0.57, 0.77)	0.65 (0.53, 0.74)	0.59 (0.46, 0.70)	0.49 (0.34, 0.62)

Table 5.
Sensitivity* and specificity** of a pediatric bone mass scan to predict adult bone mass. Bone mineral content (BMC; g), bone mineral density (BMD; g/cm^2) and bone area were measured by Dual-energy X-ray Absorptiometry (DXA) in Total body, Lumbar spine and femoral neck in 121 children with mean age of 7.7 (range 6-9) at baseline and mean 11.1 years (range 10-12) later at a mean age of 18.8 years (range 18-19). BMC, BMD and bone area Z-scores in childhood and adulthood were calculated for each individual using all 121 individuals as controls. The individuals were stratified in quartiles based on Z-scores at baseline and follow-up. Data are shown as means with 95% confidence interval (95% CI) within brackets.

	Bone mineral content (BMC)			**Bone mineral density (BMD)**			**Bone area**	
	Total Body	Lumbar spine	Femoral Neck	Total Body	Lumbar spine	Femoral Neck	Lumbar spine	Femoral Neck
Sensitivity(%)	64 (44, 81)	71 (51, 87)	69 (49, 85)	54 (34, 72)	64 (44, 88)	47 (28, 66)	59 (39, 77)	52 (32, 71)
Specificity(%)	89 (80, 94)	90 (82, 95)	90 (81, 95)	85 (76, 92)	88 (79, 94)	82 (72, 89)	85 (76, 92)	83 (74, 90)

*Sensitivity (%): The probability of a pediatric bone scan in the lowest quartile to predict an adult result in the same quartile.
**Specificity (%): The probability of a pediatric bone mass scan in the three highest quartiles to predict an adult result outside the lowest quartile.

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Paper IV

Preterm Children Born Small for Gestational Age Are at Risk for Low Adult Bone Mass

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Short title: SGA children at risk for low adult bone mass

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What's Known on This Subject: Cross sectional studies suggest that premature birth and low birth weight may both be associated to low peak bone mass (PBM). But data is conflicting and since no prospective studies are available from childhood to adulthood.

What This Study Adds: We followed bone traits in preterm children and controls for 27 years and also examined the effects of birth weight relative to gestational age (stratified as small for gestational age (SGA) or appropriate for gestational (AGA)) on adult BMD.

Abstract

Background: Cross sectional studies suggest that premature birth and low birth weight may both be associated to low peak bone mass (PBM). We followed bone traits in preterm children and controls for 27 years and examined the effects of birth weight relative to gestational age (stratified as small for gestational age (SGA) or appropriate for gestational (AGA)) on adult BMD.

Methods: We measured distal forearm BMC (g/cm) and BMD (g/cm2) with single photon absorptiometry (SPA) in 46 preterm children (31 AGA and 15 SGA) at mean age 10.1 years (range 4-16) and in 84 healthy age matched children. The measurements were repeated 27 years later with the same SPA apparatus but then also with dual energy absorptiometry (DXA) and peripheral computed tomography (pQCT).

Results: At baseline, preterm children were shorter and thinner ($p<0.05$) but had similar BMC and BMD compared to controls. At follow-up, preterm individuals were still shorter ($p=0.03$) and had lower femoral neck (FN) BMC, FN BMD, tibial cortical BMD, tibial cross sectional area and SSI than controls (all p-value 0.001 to <0.05). The deficits were driven by lower bone traits in preterm SGA individuals while no differences were seen in preterm AGA individuals compared to controls. The gain in forearm BMC from childhood to adulthood were also lower in preterm SGA individuals than controls ($p=0.005$).

Conclusion: Preterm SGA individuals are at increased risk for reaching low adult BMD. In our cohort we were unable to find increased risk for obtaining low BMD in preterm AGA individuals.

Introduction

One standard deviation lower peak bone mass (PBM) is associated with a doubled fracture risk later in life[1]. PBM is, depending on the specific anatomical region, reached during the second to fourth decade in life [2-4], both benefits [5-6] and deficits [7-8] in bone mineral density (BMD) at birth and during growth may influence PBM. The variance in PBM is thus regulated by both genetic and environmental factors [2] and as a consequence, the variance of PBM may be influenced by pre- and post-natal growth[9].

A most important period for the skeletal development is the intra-uterine third trimester as 80% of the bone mass formation in a newly born infant is acquired during this period. While there is some evidence that preterm birth and low birth weight are associated with low bone mass in adulthood [9-10] other studies have found that preterm birth, regardless of birth weight, does not influence bone mass in adulthood[11-12]. Recent research has therefore focused on the contribution birth weight relative to the

gestational age has for the variance in BMD at growth and PBM [12-15]. The skeletal development during both infancy[16-18] and the overall growth period[11, 19-22] has been in focus, especially the pubertal growth spurt, as 36% of the total amount of the adult BMD is acquired during the four peri-pubertal years, similar to the total amount of loss during adult life[23].

Prospective reports have found that infants born small for gestational age (SGA) have a lower bone accretion during the first six post-partum months than children born appropriate for gestational age (AGA) [12]. Data from cross-sectional studies have further indicated that individuals born SGA may be at a high risk of low adult BMD[12-14]. There are however to our knowledge no prospective studies of premature born children starting in childhood and continuing to PBM.

The aim of this prospective observational case control study we therefore follow children from childhood to after completion of growth. We set out to answer: (i) if preterm children are at greater risk of low adult BMD and (ii) if there is a difference in risk between preterm SGA and AGA children? We hypothesized that premature children would reach lower adult BMD than controls.

Materials

Children at baseline

A skeletal evaluation was performed between the years 1981-1985 by single photon absorptiometry (SPA) in 75 children classified as being born premature (born before completion of the 37th gestational week) , 44 boys and 31 girls with a mean age of 10 years (range 4-17). Children were not included as a strict random sample and at baseline there were no strict exclusion criteria[19]. 45 of the preterm children were classified according to the growth charts compiled by Karlberg et al [24] as born preterm appropriate for gestational age (AGA), defined as weight ± 2SD for the gestational age and 30 were small for gestational age (SGA), defined as weight below -2SD for the gestational age[19]. A control cohort of 131 volunteers, 65 boys and 66 girls, predominantly included through asking healthy children to the medical staff, within the same age span underwent the same measurements during the same period with the same equipment. All participants were Caucasians without disease or medication known to affect bone metabolism and none had chromosomal abnormalities, The childhood comparison between the cases and the controls who are included in this report has been described earlier[19]

Follow-up evaluation

We invited all participants in the original study to participate in follow-up measurements performed at a mean 27 years (range 22–29) later. At follow-up, five men and one woman from the original study had died, 11 men and 12 women had relocated out of our region, 20 men and 15 women could not be located or declined further participation, 2 women were unable to attend the follow-up examination due to illness, 1 man had missing baseline data finally 4 men and 5 women originally classified as preterm were when we re-examining the birth cards found to be born full term (born > 37th week). Following this 46/ 75 (61%) preterm children and 84/131 (64%) control subjects were re-evaluated at a mean age of 37 years (range 28–44). Drop-out analysis revealed that children in the control cohort who did not participate at the follow-up were older than those who did (11.7 ± 4.0 versus 10.2 ± 3.6 years, p=0.04). No other statistically significant group differences were seen between participants and non-participants in the control or the preterm group.

Bone mass measurements

Bone mineral content (BMC; g) and bone mineral density (BMD; g/cm^2) were measured in the forearm both at baseline and at follow-up. At baseline on a level corresponding to 25% of the ulnar length measured from the tip of the ulnar styloid and at follow up 6 cm proximal to the ulnar styloid process by the same single-photon absorptiometry (SPA) scanner. We scanned both arms and used the mean value, a technique that has been described in detail previously[4, 25]. The coefficient of variation (CV) was 2% with a standardized phantom and 4% after repeated measurements in 14 subjects. The long-term drift, evaluated by a standardized phantom was 0.1% (95% CI –0.2, 0.4) per year[26]. One technician performed all baseline measurements and one all follow-up measurements, whereas one of the authors analyzed all plots.

At follow-up, BMC and BMD were also measured by dual X-ray absorptiometry (DXA) (Lunar® DPX-L scanner, software version 1.3z; Lunar, Madison, WI, USA) in the first to fourth lumbar vertebra (L1–4) by a lumbar spine scan and in the femoral neck by a hip scan. Daily calibration of the apparatus was done with the Lunar® phantom. The CV evaluated in 14 individuals after repositioning was 0.4–3.0% for BMD depending on the measured region. Peripheral quantitative computed tomography (pQCT) (pQCT; XCT 2000; Stratec, Pforzheim, Germany) measured trabecular and cortical BMD (g/cm^2), Cross Sectional Area (CSA; mm^2) and Stress Strain Index (SSI, mm^3) in the left radius and left tibia. We measured at the 4% and 38% level from the ankle joint and at 6% and 66% level from the wrist. Daily calibration of the apparatus was done with a standard phantom. Re-measurement of 14 individuals after repositioning resulted in a CV of 1.1-4.6% for depending on the measured region. Three research technicians performed all the DXA and PQCT measurements and analyzed all the scans.

Anthropometric measurements and registration of lifestyle factors and incident fractures

Body weight was measured to the nearest 0.1 kg with an electric scale and body height to the nearest 0.5 cm by a wall-tapered height meter. Lifestyle factors, diseases, and medications were evaluated by questionnaires at both baseline[19, 25] and follow-up[27].

Statistical evaluation

Statistical calculations were performed with SPSS® version 20.0. Data are shown as numbers (n) or as unadjusted means ± standard deviation (SD) or unadjusted means with 95% confidence interval (95% CI). Group differences were evaluated by ANCOVA with adjustment for gender, and age. Sidak were chosen for pair wise comparisons between groups to adjust for multiple comparisons.

Results

Baseline

Preterm born children were shorter with mean difference of 3.4 cm (CI 95% 0.6 to 6.1) and thinner with mean difference of 3.7 kg (CI 95% 0.7 to 6.7) than controls. Sub-group analyses, revealed that this was driven by preterm SGA children rather than preterm AGA children (Table1). Distal forearm BMC and BMD were similar in all groups (Table 2).

Follow up

Preterm born individuals were younger with mean difference of 2.2 years (CI 95% 0.7 to 3.6) and shorter with mean difference of 3.0 cm (CI 95% 0.3 to 5.8) compared to controls (Table 1). When being divided in the sub-groups, we found no significant differences in anthropometry when comparing preterm individuals born SGA or AGA (Table 1)

BMD and BMC were similar in preterm individuals and controls at follow-up but preterm SGA individuals had lower distal forearm BMC with mean difference of 0.10 (CI 95% 0.03 to 0.18), compared to controls (Table 2). Preterm SGA individuals also had lower distal forearm BMC with mean difference of 0.11 (CI 95% 0.03 to 0.19), and BMD with mean difference of 0.05 (CI 95% 0.01 to 0.10) compared to preterm AGA individuals (Table 2).

The gain in forearm BMC from childhood to adulthood was lower in preterm individuals with mean difference of 0.05 (CI 95% 0.01 to 0.08) compared to controls. In sub-group analyses, we found that this was driven by preterm SGA born individuals with mean difference of 0.09 (CI 95% 0.02 to 0.15) compared to controls but not by preterm AGA born individuals with mean difference of 0.03 (CI 95% -0.19 to 0.08) compared to controls (Table 2).

In the cross-sectional analyses at follow-up preterm individuals had statistically significantly lower FN BMC with mean difference of 0.36 (CI 95% 0.07 to 0.66), FN BMD with mean difference of 0.06 (CI 95% 0.01 to 0.11), cortical tibial BMD with mean difference of 0.07 (CI 95% 0.03 to 0.12), tibial CSA with mean difference of 23.3 mm^2 (CI 95% 5.6 to 40.9) and tibial SSI with mean difference of 217 (CI 95% 86 to 349) compared to controls (Table 3). In sub-group analyses, we found that this was driven by preterm SGA individuals, who had lower FN BMC with mean difference of 0.78 (CI 95% 0.24 to 1.31), FN BMD with mean difference of 0.12 (CI 95% 0.04 to 0.21), cortical tibial BMD with mean difference of 0.13 (CI 95% 0.05 to 0.21), tibial CSA with mean difference of 47 mm^2 (CI 95% 16 to 78), tibial SSI with mean difference of 365 (CI 95% 131 to 598), cortical radial BMD with mean difference of 0.05 (CI 95% 0.001 to 0.11) and radial SSI with mean difference of 64 (CI 95% 7 to 121) compared to controls (Table 3).

In contrast, preterm AGA individuals and controls had similar bone traits (Table 3).The preterm SGA individuals also had lower FN BMC with mean difference of 0.62 (CI 95% 0.03 to 1.21), tibial CSA with mean difference of 36 mm^2 (CI 95% 1 to 70), and cortical radius BMD with mean difference of 0.06 (CI 95% 0.01 to 0.12), than preterm AGA individuals (Table 3).

Discussion

This prospective observational cohort study shows that preterm SGA but not AGA born children, are at increased risk for reaching low adult BMD. The data also support that this is a result of a deficit in the accrual of bone mineral from childhood to adulthood.

Reports indicate that that low birth weight and low gestational age are associated with short adult stature [12-13]. This corroborate with our data that showed preterm born children to have short statute, but increase the knowledge when showing that this anthropometric feature is driven by preterm SGA individuals. A majority of cross-sectional studies also report that individuals born preterm have a deficit in bone mass in childhood and adolescence compared to individuals born full term, predominantly being driven by the fact that preterm individuals reach lower height and weight than full term born individuals[19-20, 22, 28]. This was found for

BMC in boys but not girls in the original study of our cohort in 1985[19]. Due to the smaller sample size in our current follow-up study gender specific analyses were unfortunately not possible. But there are other studies that show similar bone mass in preterm and full term born individuals in both childhood and adolescence[11, 21, 29]. To summarize, it is unclear whether or not prematurity is a risk factor for low bone mass in childhood and adolescence, and if so, if low bone mass is the result of being preterm

or being small in relation to gestational age. Low bone mass found at birth in preterm infants has been found to be transient [16, 30], which could explain the different outcomes in studies as the children have been measured at different ages.

Even if the low bone mass in early childhood can be temporarily regained, Barker et al [31] have, in line with the fetal programming hypothesis, showed that conditions during pregnancy may have long term effects such as influence on pubertal development, possibly resulting in low adult bone mass. This hypothesis has to our knowledge never been tested in preterm children.

The effect of premature birth on PBM has to date only been evaluated in cross sectional studies, that cannot distinguish deficits developing pre-, post-natal or during the pubertal growth spurt. In cross-sectional bone mass data at age 31 years in 171 neonatal survivors Dalzei et al [12] found that low birth weight and low gestational age are associated with short adult stature but that prematurity per se is of no importance for PBM. Similar results were reported by Beukhoven et al [11] in 276, young adults at mean age 21 years when measuring total body and lumbar spine BMD. Hovi et al [14] however reported in a cross-sectional study that young adults born with very low birth weight (VLBW) but appropriate for gestational age , when studied close to the age of peak bone mass, had significantly lower BMD compared to their term-born peers. Further, Laitinen et al[32] in a large cohort (n=1099) where bone mass was measured at the distal radius at a mean age of 31 years, concluded that growth retardation which takes into account both birth weight relative to gestational age and ponderal index (kg/m^3) was associated with a 2.5 risk of low adult BMC. Fewtrell et al [13] are to our knowledge the first to distinguish between preterm SGA and AGA born children in their cross sectional BMD evaluation in 202 subjects at age 20. They found that preterm children were shorter and had lower BMD than controls, and that the deficits were greatest in the preterm SGA children. To summarize it is currently unclear what effect preterm birth and being born SGA or AGA has on PBM.

We are first to present longitudinal bone mass data in preterm children that cover the period of growth from childhood to adulthood. We show that there is a deficit in bone mineral accrual during this period and that this is transferred to a lower BMD in adulthood. We also found that this deficit is only driven by preterm SGA individuals. This new knowledge could provide a possible explanation for discrepancies in previous published studies[11-12, 14, 32], since the proportion of preterm SGA and AGA children could affect the outcome.

In our cohort preterm SGA children had similar BMD to controls in childhood but lower BMD accrual during growth resulting in a lower adult BMD. The responsible factors are unknown but this finding could in part be explained by the fetal programming hypothesis[31]. This hypothesis has been found for other traits in children with a prenatal growth disturbance. Such a disturbance could during the intrauterine period result in abnormal regulatory mechanisms of puberty without affecting the pre-

pubertal period. A young child would then develop normally until puberty, where a deficit gradually develops, possibly resulting in low PBM.

Study strengths include the prospective controlled study design, with measurement performed with the same scanner and in the same skeletal region by only two technicians involved and with all analyses done by a single researcher. The long period of examination including PBM is also an advantage as PBM in some regions seems to be reached as late as the third or fourth decade of life [3-4]. The use of modern scanners and different measuring techniques, to verify the SPA data, is also beneficial. An attendance rate of 63% after almost three decades is also better than many similar studies[9, 13] and drop out analyses indicate that a representative material.

Study limitations include the small sample size which due to risk of type II error rendered gender specific sub-group analyses to be unfruitful. It would have been beneficial with prospective DXA and pQCT data but these techniques were not available at study start. Would we design the study today we would also have recorded Tanner stage at baseline and in women also age at menarche and menopause. As none of the women were older than 45 years at follow-up postmenopausal bone loss could be considered a minor confounding factor. To pinpoint PBM it would also have been advantageous with serial measurements of bone traits in early adulthood. Our study subjects were born between 1964 and 1979. The Swedish birth register did not start its registration of weight, height and gestation week until 1973 in none premature births, we could therefore not register this information in the control cohort. Through journals in local archives we have in the premature cohort been able to verify correct subgroup classification of SGA and AGA individuals. We also tried to classify the control cohort into SGA and AGA, but this could not be done since available information on either gestational week or birth weight was missing in 69/84 individuals. We consider this a limitation because it refrain us to differentiate if the bone mass deficits found in premature SGA children are the result of being born prematurely or because of pre natal growth retardation. Our aim was to evaluate the development of bone mass from childhood to adulthood in children born prematurely, either SGA or AGA. We were however not able to distinguish between the underlying reason of neither premature birth nor why the child was born prematurely SGA or AGA. In the original publication, mothers were classified as healthy during pregnancy and with no children considered to have any type of intra uterine growth retardation without further specification[19], we were not able to withdraw further information from local archives. We can therefore not distinguish if the children born SGA are constitutionally small or include a proportion that we by today's definition would classify as intra uterine growth restriction (IUGR) infants. Further, data on socio-economic position (SEP) and lifestyle factors (diet, physical activity, smoking) during the pre- and postnatal period would have been valuable as they act as confounders both to premature birth as well as to growth.

To summarize, preterm SGA individuals are at increased risk for reaching low adult BMD. In our cohort we were unable to find increased risk for obtaining low BMD in preterm AGA individuals. Our data support that this is the result of a lower accrual of bone mineral from childhood to adulthood. Future studies that evaluate bone traits should not only report data from children born preterm but stratify these into those born SGA and AGA.

Table 1

Age, anthropometry, body mass index (BMI; kg/m^2) in prematurely born children (< week 37), where 17 boys and 14 girls were born appropriate for gestational age (AGA) and 9 boys and 6 girls born small for gestational age (SGA). Comparisons are made with age matched controls consisting of 41 boys and 43 girls at childhood and mean 27 years (range 22–29) later. Data are shown as unadjusted means ± standard deviation (SD) or as numbers (n). Group comparisons are done by ANCOVA with adjustments for age and gender (for age only gender) Sidak were chosen for pair wise comparisons between groups to adjust for multiple comparisons. A p-value <0.05 was regarded as a statistical significant difference, then highlighted in bold. na = not applicable

	Absolute values		Group comparison	Absolute values		Group comparison			
	Prematures	Controls	Prematures vs Controls	AGA	SGA	AGA/SGA/controls	AGA/ controls	SGA/ controls	AGA/ SGA
	(n =46)	(n = 84)	P-value	(n =31)	(n = 15)	P-value	P-value	P-value	P-value
	Baseline								
Age (year)	10.1±4.1	10.2±3.6	0.93	10.1±4.2	10.2±4.0	0.98	na	na	na
Height (cm)	138.0±22.6	141.9±21.5	**0.02**	138.6±24.3	136.8±19.4	**0.02**	0.31	**0.04**	0.58
Weight (kg)	33.7±14.3	37.6±16.8	**0.02**	34.6±15.5	31.7±11.9	**0.02**	0.39	**0.03**	0.42
BMI (kg/m^2)	16.8±2.6	17.7±2.8	**0.04**	17.1±2.8	16.2±1.9	**0.047**	0.57	**0.051**	0.42
	Follow-up								
Age (year)	35.5±4.5	37.8±3.7	**0.003**	35.4±4.7	35.9±4.3	**0.01**	**0.02**	0.27	0.97
Height (cm)	171.8±8.7	173.9±10.4	**0.03**	172.0±8.8	171.1±8.6	0.07	na	na	na
Weight (kg)	79.2±17.6	80.5±17.5	0.35	78.9±17.1	79.4±18.9	0.65	na	na	na
BMI (kg/m^2)	26.7±5.1	26.5±4.7	0.99	26.5±4.9	27.0±5.4	0.97	na	na	ne
Gestation age	32.9±1.9	-	na	32.1±1.6	34.7±1	na	na	na	**<0.01**
Birthweigh	1593±262	-	na	1616±238	1545±348	na	na	na	0.40

Table 2

Distal forearm bone mineral content (BMC; g) and bone mineral density (BMD; g/cm^2) in prematurely born children (< week 37), where 17 boys and 14 girls were born appropriate for gestational age (AGA) and 9 boys and 6 girls born small for gestational age (SGA). Comparisons are made with age matched controls consisting of 41 boys and 43 girls at childhood and mean 27 years (range 22–29) later. Data are shown as unadjusted means with 95% confidence interval (95% CI) in brackets. Group comparisons are done by ANCOVA with adjustments for age and gender. Sidak were chosen for pair wise comparisons between groups to adjust for multiple comparisons. A p-value <0.05 was regarded as a statistical significant difference, then highlighted in bold. na = not applicable

Modality-SPA	Absolute values		Group comparison	Absolute values		Group comparison			
	Prematures (n =46)	Controls (n = 84)	Prematures vs Controls P-value	AGA (n =31)	SGA (n = 15)	AGA/SGA/controls P-value	AGA/ controls P-value	SGA/ controls P-value	AGA/ SGA P-value
Baseline									
BMC (g/cm)	0.48 (0.43, 0.54)	0.47 (0.43, 0.51)	0.32	0.50 (0.43, 0.57)	0.45 (0.36, 0.55)	0.09	na	na	na
BMD (g/cm^2)	0.44 (0.40, 0.47)	0.42 (0.40, 0.44)	0.10	0.44 (0.40, 0.48)	0.42 (0.36, 0.48)	0.051	na	na	na
Follow-up									
BMC (g/cm)	0.89 (0.83, 0.95)	0.89 (0.85, 0.94)	0.17	0.92 (0.84, 0.99)	0.83 (0.73, 0.92)	**0.003**	0.99	**0.004**	**0.006**
BMD (g/cm^2)	0.62 (0.59, 0.64)	0.61 (0.59, 0.63)	0.73	0.63 (0.59, 0.67)	0.59 (0.55, 0.63)	**0.03**	0.69	0.07	**0.02**
Delta value									
BMC (g/cm)	0.40 (0.39, 0.48)	0.42 (0.37, 0.47)	**0.007**	0.42 (0.32, 0.52)	0.37 (0.27, 0.47)	**0.005**	0.35	**0.005**	0.18
BMD (g/cm^2)	0.18 (0.14, 0.22)	0.19 (0.16, 0.22)	0.06	0.19 (0.13, 0.24)	0.17 (0.11, 0.23)	0.09	na	na	na

Table 3

Bone mineral content (BMC)and bone mineral density (BMD) measured by dual energy X-ray absorptiometry (DEXA) and BMD measured by peripheral computed tomography (pQCT) at follow up. Subjects consist of prematurely born children (< week 37), where 17 boys and 14 girls were born appropriate for gestational age (AGA) and 9 boys and 6 girls born small for gestational age (SGA). Comparisons are made with age matched controls born after week 37 consisting of 41 boys and 43 girls at childhood and mean 27 years (range 22–29) later.. Data are shown as unadjusted means with 95% confidence interval (95% CI) in brackets. Group comparisons are done by ANCOVA with adjustments for age and gender. Sidak were chosen for pair wise comparisons between groups to adjust for multiple comparisons. A p-value <0.05 was regarded as a statistical significant difference, then highlighted in bold. na = not applicable

	Absolute values		**Group comparison**	**Absolute values**		**Group comparisons**			
Modality	Prematures	Controls	Prematures vs Controls	AGA	SGA	AGA/SGA/ controls	AGA/ controls	SGA/ controls	AGA/ SGA
	(n =46)	(n = 84)		(n = 31)	(n = 15)	P-value	P-value	P-value	P-value
DEXA									
FN BMC† (g/cm)	5.36 (5.09, 5.63)	5.62 (5.41, 5.84)	**0.02**	5.54 (5.21, 5.88)	4.98 (4.51, 5.45)	**0.003**	0.72	**0.002**	**0.04**
FN BMD† (g/cm²)	1.03 (0.99, 1.07)	1.08 (1.05, 1.10)	**0.02**	1.06 (1.00, 1.11)	0.97 (0.90, 1.04)	**0.004**	0.68	**0.003**	0.06
L1-L4 BMC† (g/cm)	73.8 (69.3, 78.3)	76.8 (73.7, 79.9)	0.09	75.8 (70.2,81.3)	69.7 (61.4, 78.0)	0.066	na	na	na
L1-L4 BMD† (g/cm²)	1.23 (1.18, 1.27)	1.27 (1.24, 1.30)	0.10	1.25 (1.20, 1.31)	1.18 (1.09, 1.26)	0.057	na	na	na
pQCT									
Trabecular Tibia (4%)									
BMD (g/cm²)	1.40 (1.33, 1.47)	1.39 (1.34, 1.44)	0.76	1.43 (1.34, 1.51)	1.35 (1.21, 1.49)	0.28	na	na	na
Cortical Tibia (38%)									
BMD (g/cm²)	1.87 (1.83, 1.92)	1.93 (1.90, 1.97)	**0.002**	1.90 (1.84, 1.95)	1.83 (1.73, 1.92)	**0.001**	0.27	**0.001**	0.07
Cross Sectional Area (mm²)	295 (277, 313)	313 (299, 327)	**0.01**	305 (284, 325)	275 (238, 311)	**0.002**	0.61	**0.001**	**0.04**

SSI†† (mm^3)	1653 (1534, 1772)	1827 (1717, 1937)	**0.001**	1713 (1579, 1847)	1532 (1382, 1783)	**0.001**	0.18	**0.001**	0.11
Trabecular Radius (6%)									
BMD (g/cm^2)	0.89 (0.85, 0.93)	0.89 (0.85, 0.92)	0.46	0.91 (0.86, 0.96)	0.86 (0.79, 0.92)	0.07	na	na	na
Cortical Radius (66%)									
BMD (g/cm^2)	1.08 (1.05, 1.12)	1.08 (1.06, 1.11)	0.49	1.10 (1.06, 1.14)	1.05 (1.00, 1.10)	**0.03**	0.89	**0.05**	**0.03**
Cross Sectional Area (mm^2)	90.1 (84.9, 95.3)	89.1 (84.9, 93.4)	0.64	92.5 (85.9, 99.1)	85.2 (76.6, 93.8)	0.09	na	na	na
SSI†† (mm^3)	354 (322, 386)	363 (338, 387)	0.15	372 (331, 413)	318 (268, 367)	**0.02**	1.0	**0.02**	0.06

† FN = Femoral neck and L1-L4 = lumbar spine vertebrae 1 to 4.

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