Physical activity and its effect on bone in the short- and long- term perspective.

Lindén, Christian

2006

PHYSICAL ACTIVITY AND ITS EFFECT ON BONE IN THE SHORT- AND LONG-TERM PERSPECTIVE

CHRISTIAN LINDÉN

Department of Orthopaedics
Malmö University Hospital
Lund University

Malmö 2006

Physical Activity and its Effect on Bone in the Short- and Long- Term Perspective

Christian Lindén, MD
2006

Akademisk avhandling
som med vederbörligt tillstånd från Medicinska fakulteten vid Lunds universitet för avläggande av doktorsexamen i medicinsk vetenskap kommer att offentligen försvaras i Universitetsklinikernas aula, ingång 35, Universitetssjukhuset, MAS, Malmö.

Fredagen den 24 november 2006, klockan 13.00

Fakultetsopponent: Professor Olle Svensson
Norrlands Universitetssjukhus, Umeå

Handledare: Professor Magnus Karlsson
Department of Clinical Sciences, Malmö
Lund University

Organization	Document name
LUND UNIVERSITY Department of Orthopaedics Malmö University Hospital SE-205 02 Malmö	DOCTORAL DISSERTATION Date of issue: November 24, 2006 Sponsoring organization
Author(s): Christian Lindén	

Title and subtitle: Physical Activity and its Effect on Bone in the Short- and Long-Term Perspective

Abstract

Osteoporosis is a growing problem worldwide, Sweden having one of the highest fragility fracture incidences in the world. The growing years are thought to be an opportune time to build strong bones and the enhancement of peak bone mass accrual has been suggested as a prevention strategy for osteoporosis. Most exercise intervention studies in children, evaluating the accrual of aBMD, include volunteers and use specifically designed osteogenic exercise programs. The aim of the POP-study, a prospective controlled intervention study, is to evaluate a general curriculum-based exercise intervention program in a population-based cohort of children, aged 7-9 years at study start. The data presented in this thesis are the results from the first 2 years in 99 girls and 137 boys and shows that daily physical activity within the school curriculum seems to be associated with benefits in the accrual of BMC, aBMD and gain in bone width, all traits important for bone strength. The benefits are present already after 1 year of intervention. This thesis supports the view that general moderately intense physical activity could be recommended as a strategy to increase BMC, aBMD and bone size in prepubertal children.

Our study measuring aBMD in 22 active soccer players, 128 former soccer players and 138 age-matched controls and also studying the frequency of fractures in 284 former soccer players and 568 controls evaluates the relationship between exercise during growth and bone mineral density and fractures in old age. This study provides evidence that exercise during growth results in biologically important benefits in peak aBMD at weight-bearing sites. The data suggests that cessation of exercise may result in loss of the benefit in aBMD, leaving only modest residual benefits in middle age and perhaps none in old age, when fracture occurs. This study does not support the notion that vigorous exercise during growth and young adulthood reduces the risk of fracture in old age. However, we are aware of the power problem when evaluating the fracture data, and that later studies from our group, including a larger sample size, have opposed this view. Therefore, our view today is that vigorous exercise during growth and young adulthood is associated with reduced fracture risk in old age.

Key words: Osteoporosis, aBMD, BMC, peak BMD, bone size, exercise, fractures, girls, boys, prepubertal children, former athletes

Classification system and/or index termes (if any):

Supplementary bibliographical information:	Language: English
ISSN and key title: 1652-8220	ISBN: 91-85559-50-4
Recipient's notes	Number of pages: 104 Price Security classification

Distribution by (name and address) Dep. of Orthopaedics, Malmö University Hospital SE-205 02 Malmö

Signature _____ Date October 10th 2006

Physical Activity and its Effect on Bone in the Short- and Long- Term Perspective

Christian Lindén, MD
2006

LUND
UNIVERSITY

Financial support was obtained from Swedish Research Council, ALF, Research and Development Unit, Centre for Research in Sport, Herman Järnhardt's Foundation, Malmö Hospital Foundations, Österlund Foundation, Påhlson's Foundation, The Mayflower Foundation, The Royal Physiographic Society in Lund and Regional Research Grants.

Layout: Annelie Norrby
Language revised by Alan Crozier
Photographs by Christian Lindén

ISBN: 91-85559-50-4
ISSN: 1652-8220

Cover: The POP-study mascot Jumper illustrating the core values of the study; joyful and heartfelt physical activity, with nutritional food as a base.

Printed in Sweden 2006 by Medicinsk Informationsteknik
Björn Henriksson AB, Universitetssjukhuset MAS SE-205 02 Malmö

To Camilla and Robin
– the Meaning of Life

”Where there is haste, thinking is impossible”
Platon, ca 400 BC

Contents

List of papers

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

I. **Exercise during growth and bone mineral density and fractures in old age**
Karlsson M, Lindén C, Karlsson C, Johnell O, Obrant KJ, Seeman E.
The Lancet 2000; 355: 469-470.

II. **Exercise, bone mass and bone size in prepubertal boys: one-year data from the pediatric osteoporosis prevention study**
Lindén C, Alwis, G, Ahlborg H, Gardsell P, Valdimarsson Ö, Stenevi-Lundgren S, Besjakov J, Karlsson M
Scand J of Med & Sci in Sports 2006 Jun 15 [Epub ahead of print]

III. **A School Curriculum-Based Exercise Program Increases Bone Mineral Accrual and Bone Size in Prepubertal Girls: Two-Year Data From the Pediatric Osteoporosis Prevention (POP) Study**
Lindén C, Ahlborg H, Besjakov J, Gardsell P, Karlsson M.
Journal of Bone and Mineral Research 2006; 21 (6): 829-835.

IV. **A School-Curriculum-Based Exercise Program Increases Bone Mineral Accrual and Bone Size in Pre-pubertal Boys – Two-Year Prospective Data from the Pediatric Osteoporosis Prevention (POP) Study**
Lindén C, Alwis, G, Stenevi-Lundgren S, Ahlborg H, Besjakov J, Gardsell P, Karlsson M.
Submitted to Journal of Bone and Mineral Research.

V. **Appendix: Paper I** – full text version

Abbreviations

aBMD	Areal bone mineral density, g/cm^2
ADHD	Attention deficit hyperactivity disorder
BA	Bone area, cm^2
BMAD	Bone mineral apparent density, g/cm^3, equal to vBMD
BMC	Bone mineral content, g
BMD	Bone mineral density, g/cm^2, equal to aBMD
BMI	Body mass index, kg/m^2
BMU	Basic multicellular unit
CSA	Cross-sectional area, cm^2
CSMI	Cross-section moment of inertia, cm^4
DXA	Dual-energy x-ray absorptiometry
FM	Femoral midshaft
FN	Femoral neck
GH	Growth hormone
HSA	Hip strength analysis
IGF	Insulin like growth factor
LS	Lumbar spine
L3	Third lumbar vertebrae
MPA	Medical products agency
PF	Proximal femur
pQCT	Peripheral quantitative computed tomography
POP	Pediatric osteoporosis prevention (study)
QCT	Quantitative computed tomography
QUS	Quantitative ultrasound
RCT	Randomized controlled trials
SD	Standard deviation
SEM	Standard error of the mean
TB	Total body
Tr	Femoral trochanter
vBMD	Volumetric bone mineral density, g/cm^3
WHO	World Health Organization
Z	Section modulus, cm^3

Extended abstract

Osteoporosis is a growing problem worldwide where Sweden has one of the highest fragility fracture incidences in the world. The growing years are thought to be an opportune time to build strong bones and the enhancement of peak bone mass (PBM) has been suggested as a prevention strategy for osteoporosis. Most exercise intervention studies in children, evaluating the accrual of areal bone mineral density (aBMD), include volunteers and use specifically designed osteogenic exercise programs. The aim of the prospective controlled paediatric osteoporosis prevention (POP) study is to evaluate a daily general curriculum-based exercise intervention program of 40 min/school day in a population-based cohort of children, aged 7-9 years at study start. All children in grades 1 and 2 in the intervention school were invited and 93% agreed to participate. Age-matched children from 3 nearby schools assigned to the ordinary Swedish school curriculum of 60 minutes per week served as controls. Bone mineral content (BMC; g) and aBMD (g/cm^2) were measured with DXA at the total body, lumbar spine and the hip. Bone size and volumetric bone mineral density (vBMD; g/cm^3) of the femoral neck and the third lumbar vertebrae (L3) were calculated from the dual energy X- ray absorbtiometry (DXA) scans. A questionnaire previously used in several studies but modified for children evaluated lifestyle factors. All participants remained in Tanner stage I during the study period.

The data presented in this thesis are the results from the first 2 years in 99 girls and 137 boys. For both boys and girls there were no differences between the intervention group and the controls at baseline in anthropometrics, bone parameters or lifestyle factors such as dietary habits, chronic diseases, ongoing medication, fractures, smoking and alcohol intake. The only difference found was that the girls in the control group exercised more during leisure time (0.7 ± 0.7 vs. 1.3 ± 1.6 h/week, $p=0.02$). After the intervention was initiated, the intervention group spent more time on physical activity both in school and in total compared with the controls. In both boys and girls the mean annual gain in bone mineral accrual and bone width in the lumbar spine was greater in the intervention group than in the controls. In addition, in girls there was also a difference between the groups in the annual gain in total body bone mineral accrual and femoral neck width. When the data for all individuals (exercise and intervention) within each gender were pooled, the total duration of exercise including both school-based and spare-time organized physical activity correlated with L3 BMC, L3 BMD and L3 width. No such correlations were found for the femoral neck parameters.

In summary, the data from the POP-study show that daily physical activity within the school curriculum seems to be associated with benefits in the accrual of BMC, aBMD and gain in bone width, all traits important for bone strength, in both boys and girls. These benefits were detected after the first year of the intervention and remained after 2 years. This thesis supports the view that general moderately intense physical activity could be recommended as a strategy to increase BMC, aBMD and bone size in prepubertal children.

If exercise during growth is to be recommended as a prevention strategy for osteoporosis, benefits in aBMD must be maintained in old age when fragility fractures occur. Our study in 22 active soccer players, 128 former soccer players and 138 age-matched controls, evaluates the relationship between exercise during growth and bone mineral density and fractures in old age. Bone mass was measured by DXA and the frequency of fractures was

obtained in 284 former soccer players and in 568 controls identified from the computerized city files of Malmö. Relative to controls, the aBMD in the leg was almost 12% higher in the active soccer players. During the more than 35 years following retirement the diminution in leg aBMD, estimated from the regression line, was 0.33% per year in the former soccer players compared with 0.21% in the controls. A greater proportion of former soccer players than controls had fractures when they were active and below 35 years of age, but the proportion of fragility fractures in old age was no different between the groups. This study provides evidence that exercise during growth results in biologically important benefits in peak aBMD at weight-bearing sites. The data suggests that cessation of exercise may result in loss of the benefit in aBMD, leaving only modest residual benefits in middle age and perhaps none in old age, when fracture occurs. This study does not support the notion that vigorous exercise during growth and young adulthood reduces the risk of fracture in old age. However, we are aware of the power problem when evaluating the fracture data, and that later studies from our group, including a larger sample size, have opposed this view. Therefore, our view today is that vigorous exercise during growth and young adulthood is associated with reduced fracture risk in old age.

Introduction

Osteoporosis has today become one of the most common diseases in modern human beings and due to its financial burden it is also a huge problem for society. The term osteoporosis was first coined in French in the early 1820s as a description of a pathologic state of bone[159]. Senile osteoporosis was properly described in 1926 by Alwens et al.[5] and postmenopausal osteoporosis in 1941 by Albright et al [4]. In the modern definition of osteoporosis it is often described as a "disease characterized by low bone mass and microarchitectural deterioration of bone tissue leading to enhanced bone fragility and a consequent increase in bone fracture risk"[6]. But, it is imperative to realize that osteoporosis is only one risk factor for fractures, while it is the clinical aspect of osteoporosis, fractures, that cause problems for the individual patient as well as society. Furthermore, during the latter part of the 20th century the incidence of fracture increased worldwide, and nowadays Sweden has one of the highest fragility fracture incidences in the world[28,84,88,96-98,130,146]. Recent studies have indicated a diminishing rate of hip fractures in certain regions[78,140,154], yet the lifetime risk of a Swedish middle-aged woman suffering from one or more fragility fractures is still 50% and the corresponding figure in men is 25%[78,92,93,140,154]. As a result of the fractures, increased morbidity, mortality and costs associated with a fragility fracture have now grown into a major health problem[24,27,80]. In younger age groups the consequences of a low-energy fracture are often limited, but with ageing the fractures often lead to permanent disability, impaired quality of life and sometimes death[23,86]. The fracture-related costs for society are huge. In Sweden 70 000 fractures associated with bone fragility occur every year, 18 000 of them being hip fractures[93], for which the annual financial burden of osteoporosis in Sweden is estimated at SEK 4.6 billion (2004)[17].

The inevitable question then arises: can we capture individuals with low skeletal strength before the fracture event to initiate prophylactic treatment? The World Health Organization (WHO) has defined operational guidelines (Table 1) to diagnose osteoporosis according to measurements of areal bone mineral density (aBMD) using dual-energy

Table 1. The four diagnostic osteoporosis categories recommended for women by the World Health Organisation and the International Osteoporosis Foundation in 1994.

Diagnostic category	Definition	BMD T-score
Normal bone mass	BMD above 1 standard deviation below the average young adult value	>-1
Osteopenia	BMD between 1 and 2.5 standard deviations below the average young adult value	-1 to -2.5
Osteporosis	BMD more than 2.5 standard deviations below the average young adult value	<-2.5
Established osteoporosis	BMD more than 2.5 standard deviations below the average young adult value and at least one osteoporotic fracture	<-2.5

X-ray absorptiometry (DXA), expressed in standard deviations (SD) discrepancy compared to the mean of young individuals of the same gender (T-score)[192]. Yet this definition is based solely on DXA data in women, whereas the "microarchitectural deterioration" in the definition has not yet achieved clinical application[162]. However, only half of all women sustaining a fragility fracture have a bone mineral density value that fulfils the diagnosis criterion for osteoporosis[158]. While a single bone mineral density measurement can predict fracture risk, it cannot specifically identify individuals who will actually sustain a fracture[126].

Another question of importance is to explain the pathophysiology of osteoporosis. Osteoporosis is a multifactorial disease that depends on both environmental and genetic factors. Genetic factors are estimated to be responsible for about 70% of the variance in bone mass[47,90,165,168]. Primary osteoporosis occurs in both sexes at all ages, but becomes more frequent after menopause in women[151] and occurs in higher age groups in men[142]. Secondary osteoporosis is the result of underlying diseases, for example intestinal bowel disease, chronic renal failure or hypogonadism but can also be a result of medications such as glucocorticoids or lifestyle factors such as alcoholism, smoking and inactivity[6,91]. Risk factors for osteoporosis and fragility fractures are divided into those that are possible to influence and those that are not (Table 2). Changes in modifiable risk factors can be used to prevent future fractures, while unmodifiable risk factors can be used to predict the risk of future fractures, when deciding whether prevention strategies should be initiated.

Table 2. Risk factors for osteoporosis. Adapted from SBU 2003.

Risk factors that are impossible to influence	Risk factors that are possible to influence
• Age	• Physical inactivity
• Previous facture	• Low body weight/low BMI
• Female sex	• Glucocorticoid therapy
• Premature menopause	• Low bone mineral density
• Family history of factures	• Susceptibility to falls
• Ethnic origin	• Cigarette smoking
• Height	• Excessive alcohol consumption
	• Poor exposure to sunlight
	• Poor visual acuity

Bone

The human skeleton is composed of 206 different bones and has evolved into a specialized connective tissue to reflect a balance between its primary functions. It keeps us upright and, together with the muscular system, provides mechanical integrity for efficient locomotion. It also protects vulnerable inner organs and serves as a reservoir for minerals, mainly calcium and phosphorus, and finally it hosts the red bone marrow where new bone cells can be produced. These apparently simple tasks require bone to have unique features in order not to make transportation a metabolic burden – it must serve the contradictory needs of stiffness yet flexibility and lightness yet strength.

The skeleton is made of two types of bone, cortical and trabecular, with 75–80% of the total skeleton made up of cortical bone and 20–25% of trabecular bone. Cortical bone is a densely compacted tissue, providing a protective outer layer predominantly in the long bone shafts of the appendicular skeleton. Trabecular bone is found mainly in the metaphyseal regions and in the vertebrae, featuring an inner network of thin calcified trabeculae. Trabecular bone has approximately twenty times greater surface area to volume ratio than cortical bone and is hence more metabolically active. The turnover rate of trabecular bone is 20–25% per year, whereas the turnover in cortical bone is only 3–5% per year. Therefore, trabecular bone is more sensitive to conditions or diseases affecting bone cells, with a more rapid response to stimuli than that of the cortical bone.

The bone consists of an organic component (25%), an inorganic component (70%) and water (5%). The inorganic component of bone consists mainly of calcium and phosphate in the form of platelike hydroxyapatite crystals. Ninety-eight percent of the organic matrix consists of type I collagen and non-collagenous proteins, while the remaining 2% consists of bone cells – osteoblasts, osteoclasts and osteocytes. The differentiation of these cells makes bone a highly dynamic structure, undergoing a remodelling process exchanging around 10% of the skeleton annually. Remodelling is a stereotyped, organized bone cell activity that is a locally coordinated, sequential activity of osteoclasts, osteoblasts and their precursors. This activated site is called the basic multicellular unit (BMU)[144]. Osteoclasts are large multinucleated bone-resorbing cells with a ruffled cell membrane in order to increase the contact surface with the bone. The mineralized bone is dissolved and the organic substance degraded by proteinases and enzymes[36]. When the osteoclasts have created a groove in the bone, the bone-forming cells, osteoblasts, enter the scene and produce new bone in the cavity. Osteoclastic resorption lasts about three weeks and is followed by 3–4 months of osteoblastic bone formation[50]. When osteoblasts stop synthesizing new bone they differentiate into osteocytes embedded in newly synthesized bone matrix. Osteocytes are connected to each other through gap junctions on filamentous cell projections that pass through a system of fluid-filled canaliculi. These canaliculi are thought to be responsible for the response of bone to mechanical stimuli[167]. Bone formation and bone resorption are closely linked during bone remodelling, and imbalance in the system can lead to skeletal disease, e.g. osteoporosis. The remodelling process continues throughout life in order to maintain adequate structure, repair damaged bone and control the calcium haemostasis.

In contrast to the delicate balance of remodelling, the term bone modelling refers to an organized bone cell activity that improves bone strength by adding mass and expanding the periosteal and endocortical diameters of the skeleton. In the growing skeleton modelling is the dominant mode while in the adult skeleton remodelling is the dominant process.

In modelling, osteoblasts continue to function at one site for a number of years, while during remodelling they only operate at one location for a relatively short period of time[145].

Biochemical markers

During the process of bone formation and resorption, biochemical markers are released into the circulation. These markers can be analysed to obtain a more dynamic picture of the remodelling sequence, which cannot be captured by the more static aBMD measurement. Measurements of biochemical markers are relatively inexpensive, generally available and can measure changes in bone turnover over a short period of time. On the other hand, they can neither distinguish between remodelling of the cortical or the trabecular bone nor identify the geographic locations where the remodelling is taking place. Also, there are concerns about their clinical utility because of considerable variability in the assessments due to technical as well as biologic factors, and the magnitude of the error can range from 5–47%[117]. A list of commercially available markers is provided in Table 3.

Calcium and Vitamin D

A newly delivered child is born with 25 grams of calcium, an amount which increases to approximately 1000–1200 mg by adulthood[179]. Of the total amount of calcium in the body, 98% exists in bone while 2% exists as plasma calcium, either as free calcium or bound to albumin. That is, the skeleton serves as a large reservoir for the central pool of calcium. The reserves are designed to be used in times of need, normally only during a temporary period. Sustained deficits deplete the reserves and thereby reduce the bone strength. The central pool of calcium is adjusted via a negative feedback mechanism that involves the alimentary tract, the kidneys and the skeleton. Bone resorptive activity is controlled systemically by the parathyroid hormone (PTH), which in turn reacts to extracellular fluid calcium ion homeostasis. Whenever calcium levels are insufficient to meet bodily demands, the resorption will be stimulated and the bone mass will be reduced. When adequate calcium is absorbed, PTH-stimulated remodelling decreases immediately[190]. The reduction in remodelling rate accounts for the increase that occurs during the first year of treatment with calcium. Daily recommendations of calcium intake according to the Swedish Medical Products Agency (MPA) for 2003: women: 800 mg (900 mg if < 20 years of age); pregnancy: 900 mg; breast feeding: 1200 mg; and men 800 mg.

Table 3. Currently available bone biochemical markers. Adapted from ASBMR Primer, 6th edition.

Bone Formation Markers	Bone Resorption Markers
• Bone-specific alkaline phoshatase (BSALP)	• Free and total pyridinolines (Pyd)
• Osteocalcin (OC)	• Free and total deoxypyridinolines (Dpd)
• Carboxyterminal propeptide of type I collagen (PICP)	• N-telopeptide of collagen cross-links (NTx)
• Aminoterminal propeptide of type I collagen (PINP)	• C-telopeptide of collagen cross-links (CTx)
	• Cross-linked C-telopeptide of type I collagen (ICTP)
	• Tartrate-resistant acid phosphatase 5b (TRACP5b)

Vitamin D is a hormone that influences calcium metabolism at a number of levels including calcium transport, renal calcium resorption, intestinal calcium resorption and mobilization of calcium from bone. Vitamin D is acquired from the diet and from skin synthesis on exposure to ultraviolet (UV) light. Vitamin D undergoes two key metabolic conversions, first in the liver, then in the kidney, to become the most biologically active metabolite 1, 25-dihydroxivitamin D. There is less efficient skin synthesis of vitamin D with ageing, while the intestinal absorption seems unchanged[74,125.] As older individuals usually also spend less time outdoors, this explains the high need for vitamin D supplemented diets for the elderly, especially in countries where sunlight exposure is limited. Vitamin D also influences muscle strength and muscle performance through mechanisms largely unknown, but they likely involve vitamin D receptors known to be present in muscle. This is important for the fall risk and also for the fracture risk.

The relationship between calcium, vitamin D and bone health is not as straightforward as one might think. Studies investigating this often have compliance problems and also, humans seem to be able to adapt to large changes in dietary intake of calcium, most pronounced in childhood and adolescence[7]. Whether exercise and calcium intake have a synergistic effect is unclear. The only published study trying to answer this question found no difference between the supplemented and the non-supplemented group[57]. Increased calcium intake among postmenopausal women is probably associated with a small reduction in fracture risk[30,166]. The combination of calcium and vitamin D has been shown to reduce fracture rates in older populations[25,30,35,118]. A recent meta-analysis of randomized controlled trials revealed that supplementation with vitamin D at adequate levels (800 IU/day) lowers the risk of hip fractures by 26% and any non-vertebral fracture by 23%[13]. But, there is no evidence that individuals with an adequate diet and a low risk of fracture would benefit from calcium or vitamin D supplementation.

Bone mass measurements

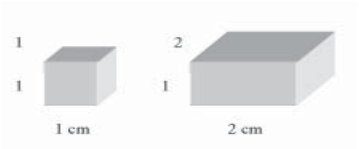
Several non-invasive methods have been developed to measure bone mass. The most common methods used are dual energy X-ray absorptiometry (DXA), quantitative ultrasound (OUS) and quantitative computerized tomography (QCT).

DXA

Dual energy X-ray absorptiometry, introduced in 1987 to estimate bone mass, is a low-dose radiation technique that utilizes the principle that calcium absorbs much more radiation than proteins and soft tissue. It uses an X-ray generator as a radiation source and a filter to achieve two energy levels[128]. This eliminates the need of constant thickness of soft tissue, necessary with older techniques, and allows measurements of all parts of the body. The amount of ionizing radiation that is absorbed by calcium in a defined section of bone reflects the bone mineral content (BMC), which is presented in grams. Areal bone mineral density (aBMD) is defined as the average amount of mineral per unit area (g/cm^2) in a section of bone. It is not a measure of true density but an estimate of the thickness of mineral per surface of bone facing the detector and is affected by the size and shape of the bone. If a large and a small bone have the same "true" bone mineral density (g/cm^3), the larger bone will still appear to have a higher areal bone mineral density. Thus aBMD tends to underestimate BMD in small bones and overestimate BMD in larger bones[53,160] (Figure 1). The inability of DXA to measure in three dimensions is especially troublesome when measuring children because of the constant change in shape, size and spatial distribution in the skeleton during growth. There is still no consensus on the most appropriate way to overcome these problems, but attempts have been made to achieve mathematical approximations of volumetric bone mineral density (vBMD; g/cm^3) in the third lumbar vertebrae, introduced by Carter[21], and the femoral neck, assuming the femoral neck to be cylindrical[175]. Again, the calculations of vBMD from DXA scans are based on assumptions and must be viewed with caution.

Despite its limitations, DXA is considered the gold standard in bone mass assessment because it is safe, accurate (precision 0.5–

Figure 1. Effect of bone size on measured bone mineral parameters in a section of bone 1 cm long. Both bone samples have identical volumetric densities (true bone mineral density); however, the bone mineral density (BMD) of the larger sample is twice that of the smaller sample. Adapted from Carter et. al. 1992.



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

2.0%[73,143], accuracy 10%[135]) and widely spread. More sophisticated methods to characterize bone accurately are nonetheless desirable[15,51]. Hip strength analysis (HSA) is one attempt to estimate the three-dimensional structure of the hip. This is a software program designed by Dr. Tom Beck at Johns Hopkins University (Baltimore, MD, USA) permitting calculations of bone geometry and bone distribution within the femoral neck from a planar scan performed by a Lunar DPX densitometer[11]. It has been developed because of the inability of the standard software of the DXA to measure bone mass in three dimensions and is described in detail in the Methods section of this thesis.

QCT

Quantitative computed tomography provides a measure of true volumetric bone density (vBMD) and can also discriminate between trabecular and cortical bone. The precision of the bone mass measurements of the QCT is 2–3%[189]. The method has so far not been shown to be superior to DXA measurements in the prediction of fragility fractures[155,162]. The downside of QCT is a relatively high radiation dose, a high cost and inaccessibility. Therefore, cheaper and smaller peripheral QCT has therefore gained in popularity, especially for use in young populations[66]. pQCT has later been added to the protocol in the POP study but is outside the scope of this thesis.

QUS

Quantitative ultrasound uses the absorption of a sound signal expressed as broadband ultrasound attenuation (BUA, dB/MHz), often described as reflecting the density of the bone whereas the speed of sound (SOS, m/s) is often described as reflecting the architecture and elasticity of the bone. The calcaneus is the bone most often used for these measurements, as only skeletal parts not covered by extensive soft tissue can be used. The in vivo precision of OUS is 0.3%–1.5%. The advantage of this method is that it is radiation-free and cheap, but one disadvantage is that it does not discriminate between trabecular and cortical bone[160,188].

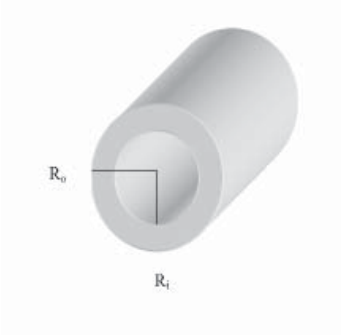
Figure 2. Dual energy X-ray absorptiometri (DXA).

Bone strength

Bone strength, however, is not only decided by the amount of bone mineral alone, but also depends on structural characteristics such as size, shape and three-dimensional architecture. The mechanical properties of the bones are subject to the same principles as a man-made load-bearing structure. When a load is applied to a bone it will cause a deformation of the bone, and after the load is released the bone will return to its original shape. This deformation is elastic and allows storage of energy needed during impact loading and muscle contraction, and represents the stiffness or rigidity of the bone. If the load applied exceeds the elastic limit, the yield point is reached and fracture occurs. The relationship between stress (force per unit area) and strain (relative deformation) before the yield point decides the stiffness, or Young's modulus. The degradation of Young's modulus and strength with time is called fatigue. The fatigue strength of bone is far less than its static strength, allowing failure to occur at loads well below those that would normally cause fracture, a phenomenon giving rise to stress fractures.

The mechanical properties of bone also depend on the orientation of the applied forces, and stress can be divided into three basic types: compressive, tensile or shear. An axial load will produce a compressive stress and the bone becomes shorter. Tensile stresses are developed when the bone is stretched and shear stresses are generated when torque is applied. A bone that is subjected to bending will experience tension on one side and compression on the other side.

The cross-section moment of inertia (CSMI) is a key biomechanical parameter that describes the ability of the skeleton to withstand bending forces. A hollow structure provides the least mass and the greatest strength during bending and torsional loading, and maximal CSMI is achieved when cross-sectional bone area is as far from the neutral axis as possible. Small changes in the outer dimensions of the bone translate into relatively large changes in biomechanical parameters, as these are proportional to the fourth power of the radius (Figure 3).



Cross-sectional moment of inertia = $(\pi/4)\ (Ro^4 - Ri^4)$

Polar moment of inertia = $(\pi/2)\ (Ro^4 - Ri^4)$

Section modulus = $((\pi/4)\ (Ro^4 - Ri^4))\ /\ Ro$

Figure 3. Depiction of cross-sectional moment of inertia, polar moment of inertia and section modulus for a diaphyseal bone section assuming the bone to be cylindrical. R0 represents the outer radius (half the periosteal diameter), and Ri represents the inner radius (half the medullary diameter). Note that small changes in the outer dimensions of the bone translate into fairly large changes in the biomechanical parameters, as these are proportional to the fourth power of the radius. The estimation of moments of inertia and the section modulus represents the geometrical contribution of bone strength and is independent of the material properties of the bone tissue. Figure presented by courtesy of Henrik Ahlborg.

The concept can easily be perceived when bending a ruler in different directions.The bone's resistance to torsion is also dependent on the distribution of the material in relation to its neutral axis, its axis of twist, and can be described as the polar moment of inertia. The section modulus describes the resistance to bending and is calculated as the ratio of CSMI to half the periosteal width. The estimation of moments of inertia and the section modulus represents the geometrical contribution of bone strength and is independent of the material properties of the bone tissue[180].

Figure 4. Bone strength is not only decided by the amount of bone mineral alone, but also depends on structural characteristics such as size, shape and three-dimensional architecture.

Bone development

Childhood and adolescence are periods of pronounced changes in the size and shape of bones during longitudinal growth. At birth, skeletal mass is approximately 70–95g, which increases to 2400–4000g in young adulthood, in the literature referred to as "peak bone mass" (PBM)[133]. In pre-puberty (Tanner stage I) about 85% of adult height and 50% of peak BMC is reached and the total body BMC and aBMD increase linearly with age[132]. During pre-puberty, bone mineral accrual is largely GH-dependent, but IGF-I is also an important regulator[8,9]. Puberty is a time of spectacular changes in bone mineral accrual, with a dramatic increase in GH and IGF-I, augmented by the increasing levels of sex steroids[34]. The appendicular skeleton is relatively more dependent on GH, while the axial skeleton is relatively more dependent on sex steroids and hence limp growth is completed before the growth of the axial skeleton[177]. The relative dominance of androgens in boys results in more bone being added on the periosteal surface, while girls, mainly by the action of estrogens, either add bone on the endocortical surface, or diminish the endocortical removal of bone[63,145,65]. It has been suggested that the maintenance of this mechanically less favourable distribution of bone in females during puberty is to create a reservoir of calcium for future pregnancy and lactation[145,22,60]. With the rise in sex- and growth-hormones, there is a rapid gain in bone mass, with approximately 26% being acquired during the 2 years surrounding peak height velocity[8], and as much bone mineral will be laid down during these years as most people will lose during their entire adult life. Bone mineral accrual is preceded by growth in bone size, resulting in transient bone fragility during adolescence[20].

Peak bone mass (PBM), the highest achieved amount of bone mass during life, seems to be an important determinant for future fracture risk[31,62,76,87]. Bone mineral accrual continues after the cessation of longitudinal growth, but the exact age at which PBM is reached is debated and varies with the skeletal region, sex and measuring technique[83]. In the axial skeleton PBM may be achieved by the end of the second decade, while the timing of PBM in the appendicular skeleton in different reports varies between age 17 and age 35[16,127,178].

The accrual of bone mass during puberty is a major determinant of PBM and is therefore a key factor for osteoporosis prevention. Small changes in PBM could produce important reductions in fracture rates, since changes of 1 SD (about 10%) in aBMD can reduce vertebral fractures by 50%[32, 46, 76,85]. Boys have a greater PBM than girls largely due to two factors: boys have 2 more years of prepubertal growth because of a later onset of puberty (age 14 rather than age 12 in girls) and their growth spurt lasts for 4 years rather than the 3 years in girls[20]. Many factors influence the accumulation of bone mineral during growth. Genetics are estimated to account for 60–80% of the variance in bone mass, while the remaining part is modulated by environmental factors, such as diet, low body weight, hormonal status, smoking, alcohol consumption, glucocorticoid therapy and last but not least, physical activity.

Exercise and bone

Physical activity is one important regulator of bone mineral density. Furthermore, to understand the mechanism behind the effect of physical activity on bone, one must look into the theories on impact of mechanical loading on bone.

From animal models, predominantly rats and birds, it is known that the important stimulus in osteogenesis is bone deformation or *strain*, which is imposed on bone by dynamic muscle contractions or dynamic ground reaction forces[152,156,181]. Strain (Δl/l) is defined as the degree of deformation (Δl) in relation to the original dimensions (l) and is normally expressed in microstrains. One microstrain equals a deformation of 0.0001%. Activities with a high strain magnitude, a high strain rate and where the strain is applied at different angles give the most effective load to increase aBMD and are referred to as osteogenic activities. The duration of the activity seems to be of minor importance and a sufficient amount of rest periods between stimuli will increase the osteogenic response, seemingly due to a decreased stimulus accommodation [115,116,141,150,155,156,157].

When external forces act upon the skeleton, inducing bone strain, they create a hydrostatic pressure gradient within the canalicular network connecting the osteocytes, generating shear stress on the bone cells. The bone cells are highly sensitive to shear stress and respond by initiating a cascade of cellular events. Briefly, they include elevation of intracellular calcium, paracrine/autocrine secretion, expression of growth factors and ultimately bone matrix production[183,184]. This mechanism by which the mechanical stimulus on bone is transformed into biologically active signals is called mechanotransduction. The mechanism behind mechanotransduction is poorly understood but is believed to include four phases[182]:

1) Mechanocoupling: transduction of external force into a local mechanical signal that is perceived by a sensor cell.
2) Biochemical coupling: transduction of a local mechanical signal into a biochemical signal that alters gene expression and/or protein activation
3) Cell-to-cell communication: transmission of a signal from sensor cell to effector cell
4) The effector cell response: production or removal of bone tissue.

Wolff's law stated, more than a 100 years ago, that changes in bone function are followed by changes in internal architecture and external conformation[194]. In 1983 Harold Frost introduced the more modern Mechanostat theory, based on the same principals, describing a model by which bone adapts to external loading or disuse under influence of hormonal and biochemical substances[55, 58,59,61,181]. According to this theory, there are two thresholds ranges for strain below or above which bone adaptation will be turned on. The lower range is called the minimum effective strain for remodelling (MESr) and below this, there is an inadequate stimulus and resorption will be the dominant process, resulting in loss of bone. The upper range is called the minimum effective strain for modelling (MESm), and above this threshold, the modelling is stimulated to add more bone. The strain between these two threshold ranges is called the physiological loading zone, and in this zone remodelling is in a kind of steady state, adjusted by sufficient strain stimuli where there is more or less no change in bone mass. The skeleton's sensitivity to mechanical loading differs during different periods in life. The underlying mechanism for this is not yet fully understood, but may be related to the fact that during growth, the bone surfaces are covered with a greater proportion of active osteoblast than after puberty[184].

The growing skeleton

The most compelling evidence for the beneficial effect of exercise on aBMD is during growth, and one of the few "facts" in the bone field is that vigorous competitive loading exercise during growth produces an anabolic response. This view is based on studies first published over 25 years ago, comparing bone mass and structure of the playing and non-playing arms of racket sports. Professional tennis players had 25–35% thicker cortices and lifetime tennis players had 4–7% higher aBMD in the dominant arm than in the non-dominant arm[75,89]. This is an excellent methodological approach, as the region-specific differences in playing versus non-playing arm cannot be the result of sampling bias or genetic factors. In addition, these first results have been repeated several times[70,72,94]. Moreover, studies have shown that the starting age is crucial for the accrual of bone mineral, and Kannus et al. reported in a cross-sectional study of female tennis and racket players that the bone mass benefits of mechanical loading were about two to four times greater if women started playing at or before menarche rather than after it[94]. Prospective controlled intervention studies verify that the pre- and peripubertal period is an opportune time to achieve an exercise-induced increase in aBMD. Hitherto, seven elegantly performed intervention studies in children have been published, the longest having a follow-up of 20 months (Table 4). A similar training regimen during the peri- and postpubertal period showed no difference between cases and controls[14].

Table 4. The skeletal response to exercise in intervention studies in children.

Author	Year	Participants´	Age in years	Exercise 3 times a week	Duration in months	Increase in Cases versus Controls
Morris et al.	1997	71 girls	9-10	High impact	10	BMC: TB, LS, FN, PF aBMD: TB, LS, FN, PF BMAD: LS, FN
Bradney et al.	1998	40 boys	8.4-11.8	Weight bearing	8	aBMD: TB, LS, legs vBMD: FM
McKay et al.	2000	144 children	6.9-10.2	High impact	8	aBMD: Tr
Fuchs et al.	2001	99 children	5.9-9.8	High impact jumping	7	BMC: FN, LS aBMD: LS BA: FN
MacKelvie et al.	2002	64 boys	8.8-12.1	High impact	20	BMC: FN
MacKelvie et al.	2003	75 girls	8.8-11.7	High impact	20	BMC: FN, LS
Valdimarsson et al.	2005	103 girls	6.5-8.9	Moderate impact	12	BMC: LS aBMD: LS, L3 width

BMC=bone mineral content, aBMD=areal bone mineral density, BA=bone area, vBMD=volumetric bone mineral-density, BMAD=bone mineral apparent density, FN=femoral neck, LS=lumbar spine, TB=total body,FM= femoral midshaft, Tr=femoral trochanter, PF= proximal femur.

The mature skeleton

The effect of physical activity on the mature skeleton seems to be more modest, and the data paint a picture that is more blurred. In randomized controlled intervention studies, both aerobic and weight-bearing exercise have, at best, shown increments of aBMD in the order of 2–5% in the intervention gro ups[18,33,41,54,57,68,105,121,153,170,171]. The studies have produced inconsistent findings, perhaps due to insufficient loading regimens and small cohorts with a dropout frequency of up to 30% and a participation rate of no more than 50% .

The ageing skeleton

Exercise during adulthood may reduce the risk and severity of falls, but evidence in randomized controlled trials (RCT) that this translates into fewer fractures is lacking[99]. Physical activity may diminish the rate of loss of bone mineral in the spine and possibly also in the hip, and it seems to have a weak positive effect on aBMD at sites that are exposed to mechanical strain with a limited effect of around 1–2% if any[19,33,134,148,149]. The studies executed were mostly limited in duration, had a low number of participants and the exercise regimens were very diverse. One out of three studies did not show any difference and, as with the other age groups, corresponding studies in men are lacking[104].

Cessation of exercise

The Achilles heel of exercise is its cessation. Most cross-sectional studies suggest that aBMD benefits of 0.5–1 SD are retained for one to two decades of retirement in previous athletes, a benefit no more than half that observed in active athletes. But after three or four decades, when the incidence of fragility fractures rises dramatically, no benefits in bone mass seem to be retained[100-103]. Data are inconclusive as studies report both that aBMD benefits are maintained[108-110,112] and that they are lost[33,69,81,131,193,195] after cessation of activity. Data exist to suggest that aBMD benefits in athletes are retained after active career if they continue to be active at a lower level[75,109.] The need to shed light on this matter is obvious, as before exercise during adolescence can be recommended as a prevention strategy for osteoporosis, data must be presented to corroborate that the beneficial effects of exercise are retained with reduced activity level, as virtually all individuals reduce their activity level with advancing age.

The prospective controlled pediatric osteoporosis prevention (POP) study

Papers II–IV are based on the POP study (in Sweden often called the Bunkeflo project, derived from the name of the suburb in Malmö where the intervention was done). The overall aim of the project was to evaluate whether non-drug-related interventions could be initiated so as to reduce the burden of low bone mass and fragility fractures in society. Several criteria must be fulfilled to achieve success with such a project. The intervention must be efficacious, safe, accessible to all, easily carried out and inexpensive to implement[161]. Of all the modifiable lifestyle factors that influence the skeleton it is probably exercise that has the potential to best fulfil all these criteria. The answer to the question whether exercise during growth is the answer to the public health burden of fractures will never be based on the highest level of experimental evidence. It will obviously never be possible to perform a double-blind trial of exercise. A prospective study evaluating whether exercise during growth and adolescence protects against fragility fractures in old age would be virtually impossible to perform because of compliance problems and funding issues over the more than six decades such a study would span. Therefore we have to use a lower level of evidence in the evidence-based hierarchy when trying to draw inferences as regards the effect of exercise during growth.

Ever since our late Professor Bo Nilsson constructed one of the first bone scanners in the early sixties[136,137], research in the field of osteoporosis in general and bone mass measurements in particular has been a major issue at the Department of Orthopaedics in Malmö. In 1995 an exercise intervention study known as the Sösdala study was initiated. Children in grades 6 and 7 (age 12–13) in the small rural town of Sösdala were invited to participate and the physical education within the school curriculum was increased for 3 to 4 years from 100 min/week to 160 min/week. The background was the increasing problem of osteoporosis and inactivity throughout the world. Every new invention is spawned by the desire to move a little less, stairs are regarded as emergency exits, football is replaced by computer games and every second journey by car goes no further than 5 kilometres. The importance of physical activity is also a well-known fact, as 90% of the population know that exercise is good for health. In spite of this, 75% are not active enough to reach recommended level of physical activity and 1 out of 4 of these are regarded as totally inactive[176]. The problem is most extensive in young girls, as 1 out of 5 girls never participate in any physical education and 1 out of 4 do not participate in any physical activity in their spare time[172]. While these reports keep coming, physical education classes as part of the school curriculum in Sweden have decreased from 20% to 7% over a period of 60 years[49]. In other words, something has to be done!

The results from the Sösdala study showed that increased physical activity in the school curriculum during the ages of 12 to 16 conferred positive skeletal effects in boys but not in girls. The author, Martin Sundberg, speculated in his thesis[173] that the intervention might have been too weak and that it should have been implemented earlier when the children were prepubertal, if the purpose was to increase the accrual of bone mineral. The idea of the Pediatric Osteoporosis Prevention (POP) study – a prospective controlled intervention study with increased physical activity within the school curriculum, but now following children from school start – was born. After thorough preparations the POP study was launched at the school of Ängslätt in the suburb of Bunkeflostrand in 1999, under the

supervision of the driving spirit, Per Gärdsell, our former head of staff at the Department of Orthopaedics in Malmö. All children in grades 1 and 2 were invited and time spent in physical education classes was increased from 1–2 lessons a week to one lesson every school day. Yearly measurements of bone mass, bone size, muscle strength, balance etc. were performed. The study was initiated from the Orthopaedics Department in Malmö but other departments were invited and agreed to participate. The Unit of Clinical Physiology and Nuclear Medicine at The Department of Clinical Sciences, Malmö, investigate the association between physical activity and important health parameters, such as body fat and aerobic fitness[37-40]. The Department of Paediatric Psychiatry examined the relationship between physical activity and the problem of attention deficit hyperactivity disorder (ADHD). The Department of Paediatrics studied the problem of asthma, and in 2003 Ingegerd Ericsson from the School of Education presented her thesis on motor skills, attention and academic achievements[49]. The author concluded that the intervention resulted in enhancement of motor skills and academic achievements. Finally the School of Dentistry evaluated whether physical activity has a positive effect on the dental status of young individuals.

The ideas behind the POP study have spread and over a thousand Swedish schools have now joined our network aiming to create health-related schools (www.bunkeflomodellen.com). Collaboration with other research groups in Australia, Denmark and Norway, also evaluating the effect of physical activity in children, has flourished. Our work has also received awards from several national and international institutions in the bone field. Following the inception of our study, daily physical education classes have been introduced in Norwegian schools and a similar resolution has been passed by the Swedish government. And the work continues.

Figure 5. All children in grades 1 and 2 were invited and time spent in physical education classes was increased from 1–2 lessons a week to one lesson every school day.

Aims

The overall aim of this thesis was to study physical activity and its effect on bone parameters important for bone strength in the short- and long-term perspective.

The specific aims were to evaluate whether:

- active soccer players and retired soccer players have higher aBMD than gender- and age-matched controls.
- the aBMD benefit in former soccer players is less pronounced than in active soccer players.
- former soccer players have fewer fractures than predicted in old age when fragility fractures occur.
- the duration of physical activity during childhood is directly associated with the accrual of bone mineral and the gain in bone size.
- a moderately intense general exercise intervention programme for one to two years within the school curriculum in prepubertal children aged 7-9 years at baseline, results in measurable skeletal benefits in bone mineral accrual and gain in bone size.

Material and methods

Paper I

Study population
aBMD was measured using DXA (Lunar DPX-L, Lunar, Madison, WI, USA) in an all-Caucasian population of 22 active male premiere league soccer players, 128 former national or international soccer players and 138 healthy controls. A questionnaire was used to document present and past exercise (hours per week), years since retirement, total years as a top athlete, occupation, alcohol and tobacco use, diseases and medications by use of self-estimation. To evaluate the prevalence of fractures, 284 former soccer players living in Malmö during growth and adolescence and still resident in this city were identified. Using the computerized city files, 568 controls were identified by matching for date of birth, birth in Malmö and current residence in Malmö. All radiographs and referrals have been archived at the University Hospital of Malmö since 1900, and since it is the only hospital in the city virtually all cases of fractures are treated in this institution. If a resident of the city suffered a fracture elsewhere, the follow up at the Orthopaedics Department was archived to ensure case ascertainment.

Statistics
Student's t-test was used to compare aBMD in athletes and controls according to decades. Z- scores, the number of standard deviations above or below the age-predicted mean, were derived by linear regression using data in the controls. Linear regression was used to examine the association between aBMD and age, and soft tissue composition and age. Comparison of the slopes (of aBMD versus age, and soft tissue versus age) in the former soccer players and controls was examined by analysis of covariance. Pearson's correlation coefficient and multiple regression were used to correlate aBMD with present activity, past activity, years since retirement, years as a top athlete and age. The significance of the proportions of cases and controls with fractures was assessed using a Chi-square test. Given the observed prevalence of fractures in the controls, the study had 80% power at $p < 0.05$ to detect a difference of 30% between the groups. The results are presented as mean ± SEM, or mean and range, unless otherwise stated.

Papers II–IV

Study population
Papers II to IV originate from the Malmö Pediatric Osteoporosis Prevention (POP) study, a prospective controlled exercise intervention study following skeletal development in children from school start. Four neighbouring elementary schools in a middle-class area in Malmö, Sweden, were invited to participate in the study. The schools had the same socioeconomic background and they were all government-funded with the compulsory standard Swedish curriculum including 60 minutes of physical education every week. All children were allocated to their school according to their residential address. All schools invited accepted participation; thus the cohort could be regarded as a cluster of convenience, the schools being the clusters and the convenience being that they are from the same neighbourhood. One of the schools was invited to participate as the intervention school, that is, no randomization was done. We did not choose a school with an already high level of physical activity and the school accepted, even though they had to modify their curriculum.

All children in grades 1 and 2 in the intervention school were invited to attend. Of 150 children 139 agreed to participate, an attendance rate of 93%. 132 age- and sex-matched controls

(attendance rate 40%) were collected from three neighbouring schools. All except one boy from Colombia were Caucasian without any medication known to influence bone accrual. To further evaluate the study sample, the height and weight of all invited children were retrieved from the first acquired general school health examination, registered by the school nurses at the first medical school check in grade 1, in order to evaluate if selection bias had occurred at baseline. However, no differences between the groups were found.

Informed consent was obtained from parents or guardians of participants before the study start. The study was approved by the Ethics Committee of Lund University and the Radiographic Committee at Malmö University Hospital, Malmö, Sweden. The Swedish Data Inspection Board approved the data collection and the set-up of the database.

Intervention

The intervention, which began at school start just after the baseline measurement was performed, consisted of the ordinary physical activity used within the Swedish school curriculum, now increased from 60 minutes a week to 40 minutes/day (200 minutes/week). The physical education classes were supervised by ordinary teachers so that no extra resources increasing costs were needed to conduct the intervention. The physical education classes did not consist of any programmes specifically designed to be osteogenic. The classes included both indoor and outdoor general physical activities used in the Swedish physical education, such as ball games, running, jumping and climbing. The teachers aimed to conduct a variety of physical activities, in order not to bore the children with repeated standardized activities. This was done with the aim of minimizing the dropouts in the long term, as is reported to occur frequently in other exercise intervention studies[10]. Thus the only modification of the curriculum was the increased duration of physical activity. The same type of physical activities were used in the control schools but at the level decided by the Swedish school curriculum. No other regular health-related modifications were performed, but during the study period we also provided a few irregular health-related activities, such as health education and health information, for the pupils, the parents and the teachers. The same education was given in all four schools.

Bone measurements

Bone mass and bone size were estimated by DXA (DPX-L version 1.3z; Lunar, Madison, WI, USA). Pediatric software, provided by the manufacturer, was used for children with a weight < 35 kg. BMC and aBMD were evaluated annually for the total body, the lumbar spine, the third lumbar vertebrae, and the femoral neck. The width of the L3 vertebrae, estimated as the distance from one edge of the vertebrae to the other, and the width of the FN, calculated as the FN area divided by the scan length of the measured area, was evaluated by the DXA scan, as previously described in the literature[43,164]. Volumetric bone mineral density (vBMD, g/cm^3) was calculated for L3 using the algorithm introduced by Carter[21], and for the FN using the formula vBMD = BMC/estimated FN volume (π x r^2 x FN length) where r = FN mid-diameter/2, assuming the FN to be cylindrical[29]. The children were evaluated dressed in light clothes with no shoes. During the measurement of the lumbar spine, the child was supine, and the physiological lumbar lordosis was flattened by elevation of the knees. The precision, evaluated by duplicate measurements in 13 healthy children aged 7–15 years (mean age 10 years) was for BMC 1.4%–3.7%, aBMD 1.6%–2.8%, L3 width 2.2%, FN periosteal diameter 1.5%, FN CSA 2.2%, FN CSMI 6.2%, total body fat mass 3.7% and total body lean mass 1.5%. Daily calibration of the machine was executed with the Lunar phantom. The technicians in our research group performed all the measurements and the software analyses. Total lean mass and

total fat mass were estimated from the DXA total body scan, body weight was measured with an electric scale to the nearest 0.1 kg and body height by a wall-tapered height meter to the nearest 0.5 cm.

Baseline measurements in the intervention group were performed in August and September, just after school start and before the exercise intervention started. To avoid seasonal variations in aBMD, the annual follow-up evaluations were carried out during the same months. The same principle was used in the controls and they had their measurements done during the months of November and December. From these data we calculated the annual changes in the evaluated traits (changes per 365 days). During the study period there were 9-week summer breaks annually without any school classes, in both the intervention and the control group.

All standard image files of the proximal femur were reanalysed by one technician, using the hip strength analysis (HSA) software provided by Lunar Instruments Corporation (Madison, WI). With this software, the X-ray absorption data of the proximal femur are extracted from the output image data file and the amount of bone mineral and its distribution within the femoral neck (FN) are calculated. First the operator has to manually define the centre of the femoral head and place the FN axis as accurately as possible along the FN. Then the FN region of interest is placed in the proximal part of the FN and finally, the femoral shaft axis is defined centrally along the shaft. The software will then iteratively assess all cross-sections in the FN region of interest and identify the plane with the least cross-sectional moment of inertia (CSMI, cm^4). CSMI is an estimate of the ability of the FN to withstand bending forces and was calculated using the mass distribution of the absorption curve.[196] The CSMI estimated with DXA has been found to be highly correlated with the CSMI measured directly on cadaver specimens (r^2=0.96)[180]. The automatic identification of the weakest cross-section of the FN is the central part of the hip strength analysis software and this cross-section level is then used for the subsequent calculations of section modulus (Z, cm^3) and cross-sectional area (CSA, cm^2). The section modulus is also an estimate of the ability of the FN to withstand bending forces, and was calculated as CSMI divided by half of the width of the FN. CSA, a measure of the resistance of the bone to axial forces, represents the area of mineral packed together in the defined cross-section of the FN and is in principle proportional to the bone mineral content (BMC).

Questionnaire

A questionnaire previously used in several studies but modified to suit prepubertal children[45,174], evaluated lifestyle factors such as socioeconomic and ethnic background, diseases and medications, fractures, dairy products, exclusion of food ingredients, coffee consumption and physical activity in school and during leisure time. The total time spent in physical activity was calculated as the duration of activity in the school and at leisure time at baseline (after the intervention was started) and at follow-up divided by two. The questionnaire was answered together with the parents in order to minimize errors, with the knowledge that there are difficulties estimating an accurate level of physical activity in young children. The maturity of the children was assessed by Tanner staging[177], conducted by our research nurses.

Statistics

Statistical calculations were performed with Statistica®, version 6.1 (StatWin®). Data are presented as mean ± SD. Student's t-test was used between means and Fisher's exact test for group comparisons. Analyses of variance (ANCOVAs) were used to adjust for chronological age and increments in height and weight in the follow-up evaluations if necessary. The annual change for the traits,

expressed in standard deviations (SD), was calculated as the annual absolute changes divided by the SD at baseline. Pearson's correlation test was used to correlate the total mean physical activity, calculated as the mean of the total physical activity at baseline and at follow-up, with changes in the bone parameters during the study period. A p-value of < 0.05 was considered as a statistically significant difference.

Figure 6. Participants enjoying intervention activities.

Summary of papers

Paper I

Exercise during growth and bone mineral density and fractures in old age

Introduction: If exercise during growth is to be recommended as a prevention strategy for osteoporosis, benefits in aBMD must be maintained in old age when fragility fractures occur. In order to try and answer this question we measured aBMD, using DXA, in 22 active soccer players, 128 former soccer players and 138 controls matched for age. The frequency of fractures was obtained in 284 former soccer players of mean age 64 years and in 568 controls identified from the computerized city files of Malmö.

Results: The active soccer players exercised 12 h per week (range 8–12) at elite level in the premier team for 5 years (1–15). The former soccer players had exercised 8 h per week (4–21) during 11 years of their active career (1–30) and had been retired for 23 years (1–65); 52% were sedentary compared to 61% of the controls. High alcohol consumption was more frequent (4.6% vs. 0.8%) and coffee consumption less frequent (93.5% vs. 98.6%) in former soccer players than in controls (both $p<0.05$). Relative to controls, leg aBMD was 11.6 ± 1.2% higher in the 22 active soccer players ($p<0.0001$), 10.3 ± 1.4% higher in 25 soccer players retired for 5 years ($p=0.0005$), 5.1 ± 1.6% higher in 29 players retired for 16 years ($p=0.01$), and 2.8 ± 1.4% higher in 23 players retired for 25 years ($p=0.03$), but no higher in the 51 players retired for over 35 years. The diminution in leg aBMD across age, estimated from the regression line, was 0.33% per year in the former soccer players compared with 0.21% per year in the controls ($p<0.01$). Former soccer players aged 70 years or older had 6.5% higher leg aBMD than controls after adjustment for current activity and body composition ($p=0.04$). A greater proportion of former soccer players than of controls had fractures when they were active and under 35 years of age (23% vs. 16%, $p<0.05$). The proportion was no different during the years of retirement (20% vs. 21%, ns), and similarly, the proportion of former soccer players with fragility fractures was no less than in controls (2.1% vs. 3.7%, ns).

Conclusion: This study, in concordance with the literature, provides evidence that exercise during growth results in biologically important benefits in peak aBMD at weight-bearing sites. However, cessation of exercise may result in loss of the benefit in aBMD, leaving only modest residual benefits in middle age and perhaps none in old age, when fracture occurs. This study does not support the notion that vigorous exercise during growth and young adulthood reduces the risk of fracture in old age.

Papers II–IV

A school curriculum based exercise programme confers benefits in bone mineral accrual and influence structural changes in boys and girls during the early school years

Introduction: Cross-sectional and prospective exercise trials report that weight-bearing physical activity is associated with a high aBMD. It is also well known that exercise ought to include high-impact activities if the purpose is to enhance bone strength. Less is known about whether a general exercise intervention programme on a moderate level increases bone mineral accrual, and perhaps also bone size, another trait contributing to bone strength. Our non-randomized prospective controlled intervention study,

also known as the Pediatric Osteoporosis Prevention (POP) Study, evaluates a daily school-based exercise intervention programme of 40 min/school day. All children in grades 1 and 2 in the intervention school were invited and 93% agreed to participate. Age-matched children from 3 nearby schools assigned to the ordinary Swedish school curriculum of 60 minutes per week served as controls. BMC (g) and aBMD (g/cm^2) were measured with DXA at the total body, lumbar spine and the hip. Bone size and vBMD of the femoral neck and the L3 vertebrae were calculated from the DXA scans. A questionnaire previously used in several studies but modified for children evaluated lifestyle factors. All participants remained in Tanner stage 1 during the study period.

Paper II

1-year follow-up in boys

Results: In 81 boys (age 7–9) in the intervention group compared to 57 age-matched boys in the control group there were no differences at baseline in lifestyle factors such as dietary habits, chronic diseases, ongoing medication, fractures, smoking and alcohol intake. Before the intervention was initiated there was no difference in the duration of physical activity performed but after the intervention began the intervention group spent more time on physical activity both in school and in total (4.9 ±1.2 vs. 2.4 ± 1.4 h/week, p<0.001) compared to controls. In addition, there was no difference at baseline in anthropometrics or bone parameters when cases and controls were compared. The mean annual gain in L3 BMC was 5.9 percentage points higher (p<0.001), L3 aBMD a mean 2.1 percentage points higher (p=0.01) and L3 width a mean 2.3 percentage points higher (p=0.001) in the cases than in the controls. When all individuals were included in one cohort, the total duration of exercise including both school-based and spare-time organized physical activity correlated with L3 BMC (r=0.26, p=0.003), L3 aBMD (r=0.18, p=0.04) and L3 width (r=0.24, p=0.006).
No such correlations were found for FN BMC, aBMD or width. There was no difference in the vBMD gain between cases and controls, and the same was true for biomechanical calculations using the HSA software on DXA scans of the hip.

Conclusion: The data support previous reports of a positive relationship between the duration of exercise and the accrual of bone mineral and gain in bone size in prepubertal boys aged 7–9 years, at least in the L3 vertebrae. The data also show that that a 1-year moderately intense school-based exercise intervention programme in a population-based cohort of prepubertal boys confers skeletal benefits.

Paper III

2-year follow-up in girls

Results: In 49 girls (age 7–9) in the intervention group compared to 50 age-matched girls in the control group, there were no differences at baseline in lifestyle factors such as dietary habits, chronic diseases, ongoing medication, fractures, smoking and alcohol intake. The girls in the control group exercised more during leisure time (0.7 ± 0.7 vs. 1.3 ± 1.6 h/week, p=0.02). After the intervention was initiated, the intervention group spent more time on physical activity both in school and in total compared with the controls. There was no difference at baseline in anthropometrics or bone parameters between the intervention group and the controls. The annual gain in BMC was higher in the intervention group than in the control group: L2–L4, a mean difference of 0.21 SD (p=0.007); L3 vertebrae, a mean difference of 0.29 SD (p<0.001). In addition, the annual gain in aBMD was higher in the intervention group; total body, a mean difference of 0.14 SD (p=0.006); L2–L4, a mean difference of 0.10 SD (p=0.02); L3 vertebrae, a mean difference of 0.15 SD

(p=0.007). The annual increase in bone width was also higher in the intervention group than in the control group: L3 vertebrae a mean difference of 019 SD (p<0.001); FN, a mean difference of 0.03 SD (p=0.02). There was also a discrepancy in the changes in FN vBMD gain, where there was a higher gain in the control group, with a mean difference of 0.24 SD (p=0.002). When all girls were included, the total duration of physical activity correlated with the annual changes in the third lumbar vertebrae in BMC (r=0.33, p=0.001), aBMD (r=0.37, p=0.002), and width (r=0.22, p=0.03). No such correlations were found for FN.

Conclusion: This study was able to conclude that a school-based exercise intervention programme for 2 years during the first school years in prepubertal girls seems to influence the accrual of BMC and aBMD and the gain in bone size in a positive way. This study supports the view that general moderately intense physical activity could be recommended as a strategy to increase BMC, aBMD and bone size in prepubertal children.

Paper IV

2-year follow-up in boys

Results: In 80 boys (age 7–9) in the intervention group compared to 57 age-matched boys in the control group there were no differences at baseline in lifestyle factors such as dietary habits, chronic diseases, ongoing medication, fractures, smoking and alcohol intake. Before the intervention was initiated there was no difference in the duration of physical activity performed, but after the intervention began the intervention group spent more time on physical activity both in school and in total (4.9 ±1.3 vs. 2.4 ± 1.4 h/week, p<0.001) compared to controls. In addition, there was no difference at baseline in anthropometrics or bone parameters when cases and controls were compared. The mean annual gain in L3 BMC was 3.0 percentage points (p<0.01) greater and the mean annual gain in L3 bone width 1.3 percentage points (p<0.01) greater in the intervention group than in controls. The total duration of physical activity during the study period correlated with annual changes in the L3 BMC (r=0.25, p=0.005) and L3 width (r=0.20, p=0.02). No such correlations were found for FN BMC or FN width. No differences between the groups were found in biomechanical strength parameters using the HSA software on the DXA scans of the hip.

Conclusion: This population-based prospective controlled intervention study, with the so far longest follow-up period published, supports the view that a two-year moderately intense school-based intervention programme in a cohort of prepubertal boys is associated with measurable skeletal benefits. In addition, the duration of physical activity is directly associated with the accrual of bone mineral and bone size.

Discussion

This thesis deals with the problem of osteoporosis and seeks to find ways to prevent the disease already at an early age. Osteoporosis is a multi-factorial, chronic condition that progresses silently for decades until the final clinical outcome – a fragility fracture – occurs. The magnitude of the problem has increased in recent decades [28,44,84,88,96,98,130,146] and the suffering of those afflicted as well as the financial burden on modern society is considerable[24,27,80]. There are different ways to try and tackle the problem: by diminishing age-related loss, restoring bone already lost or by increasing peak bone mass – the highest amount of bone achieved some time after cessation of growth. There are indications that the prerequisites for the disease develop already in the early stages of life and that if the accrual of bone mass during growth is impaired, individuals will not reach the predisposed PBM and will be at risk of developing osteoporosis and subsequent fragility fractures[77,107].

Perhaps 70% of PBM is decided by genetic factors[80,163,165,168] but a substantial part is susceptible to lifestyle factors, with physical activity being one important contributing factor[79,106,169]. The idea that vigorous competitive loading exercise during growth is good for bone, is based on studies comparing bone mass and structure of the playing and non-playing arms of racket sports. Professional tennis players had 25–35% thicker cortices and lifetime tennis players had 4–7% higher aBMD in the dominant arm than in the non-dominant arm[75,89]. The pre- and peripubertal years have been suggested to be an opportune time to build strong bones with the help of exercise, because during this period in life, there is a higher gain in BMC than the amount that is lost during the rest of the lifespan[8]. A small deficit in the accrual of BMC during these crucial years may therefore result in a substantially lower PBM, a difference that could confer a higher fracture risk, as a 1 SD lower aBMD is estimated to double the fracture risk[32].

A number of cross-sectional and observational studies suggest that physical activity in growing children is associated with a high aBMD[70,94,95]. In contrast, only seven performed prospective controlled exercise intervention trials have been published (Table 4). Apart from Papers III and IV in this thesis, MacKelvie et al. have the longest follow-up to date evaluating an exercise intervention programme (osteogenic activities for 10 min, 3 times/week) in 10-year-old girls and found that the intervention group (n=32) had 4% higher gain in femoral neck and lumbar spine BMC than in the control group (n=43)[123]. In 64 boys involved in the same programme there was a difference in the BMC of the femoral neck only but of the same magnitude[124]. The rest of the studies have a short follow-up time with a maximum of 12 months, and all but two of the studies include volunteers, children with a special interest in sports, a fact that increases the risk of selection bias. In addition, most studies use specifically designed osteogenic intervention programmes, such as jumping up and down a small height, programmes that it is probably difficult to motivate children to continue with for a long period, a hypothesis supported in previous reports[10]. Less is known about whether a general moderately intense exercise intervention programme increases BMC, and if so, at what age or which Tanner stage the training should be initiated. Also, the effect on bone size should be evaluated, as bone size irrespective of bone mass contributes to bone strength[3].

With this in mind we designed a prospective controlled exercise intervention study, a study with the hitherto longest follow-up period

published, evaluating a moderately intense general exercise intervention programme in a population-based cohort of boys and girls in Tanner stage 1. Our hypothesis was that the intervention programme would enhance the accrual of BMC and bone width. Our aim was not to show once more that a specifically designed high-impact exercise programme in the short term influences bone mass accrual but to see whether there is an effect on the group of children as a whole, also including children without a specific interest in physical activity. Swedish reports have shown that, despite the knowledge regarding the importance of physical activity, time spent in physical education classes has more than halved during the last 60 years and while more children today are active in organized sports activities, the size of the group of children classified as totally inactive has risen dramatically (25% in boys and 40% in girls)[48]. Our intervention of 200 minutes per week takes us back to "the good old days" when 20% of the time spent in school was time spent in motion.

The 2-year results from the POP study, so far the longest follow-up trial with a similar study design published to date, prospectively evaluating an exercise intervention programme in children, suggests that by merely increasing the time spent in normal moderate physical education classes there is a possibility to increase the accrual of BMC and aBMD in prepubertal children. As expected, the most obvious effects are found in weight-loaded regions with predominantly trabecular bone, as this entity of bone is more metabolically active, and thus responds faster to changes in mechanical load. Another important finding is that our moderate exercise intervention programme also seems to influence bone size. Bone strength is not only dependent on aBMD but also on skeletal geometry, architecture and bone size[43,164]. For example, women with spine fractures have smaller lumbar spine vertebrae but normal FN size, whereas women with hip fractures have a smaller FN size but normal vertebral body size compared with controls[42]. Similarly, a recent study of 6213 children, aged 12 years at baseline and followed for 2 years, showed that children with a fracture tend to have a smaller skeleton relative to their overall body size[26]. Also, mechanical calculations used in construction reveal the importance of bone size for resistance to fracture, as bone strength increases by the fourth power of the radius of a tubular structure. Previous cross-sectional data indicate that highly intense exercise increases bone size[71,111]. Now the POP study further improves our understanding of exercise-induced effects by suggesting that even moderate physical activity in boys and girls at Tanner stage 1 enhances the periosteal apposition.

The finding of a dose-response relationship, even if weak, between the total duration of physical activity and the gain in BMC, aBMD and bone size, supports the view that there is an association between the duration of physical activity and the accrual of bone parameters. Before getting too disappointed by the relative weakness of the relationship, one must bear in mind that physical activity is only one of many factors affecting bone mineral accrual.

One strength of our study is the high attendance rate in the intervention school, which indicates that our sample could be regarded as representative of Swedish children. Because of the lower attendance rate in the control group we also conducted a dropout analysis based on school record data, revealing that there were no differences at baseline in height, weight or BMI between children who participated in the study and those who did not. When evaluating physical activity, selection bias is always a risk, as exercise may be preferred by individuals with larger muscle mass accompanied by a higher aBMD due to shared genetic regulation or more advanced maturation. The minimal risk of selection bias in our study is also supported by the baseline data which show that there were no differences

between the groups regarding anthropometrics or bone parameters, minimizing the risk of selection bias. The school is a perfect forum to reach all children, not only children interested in sports but also those with a minimal interest and minimal participation in other physical activities, perhaps those who will benefit most from an intervention programme.

Earlier studies often use specifically designed osteogenic intervention programmes. One study reported that exercises including jumping up and down a small step 30 minutes per day, three times per week, increased the accrual of aBMD in the greater trochanter by 1.4% over a period of 8 months[129]. These repetitive kind of exercises have been shown to be effective in the short term but involve the risk of boring the children, leading to high dropout frequencies[10]. This is a major drawback, as continuous physical activity seems to be essential to increase PBM[139]. This is why we chose to use the normal physical education classes with a variety of different activities and sports, with possibilities for the children to influence the choice of activity and thus include the element of joy in the intervention. In addition, by doing so we did not have to add extra costs to the intervention, making it easy for all schools to copy our strategy, which further increases the value of our report and enhances our inferences that physical activity could be a cost-effective strategy to increase bone strength.

There are of course some limitations to our study. The trial is not a study randomized by each individual. However, randomization was not possible, because the principals, the teachers, the parents and the pupils made it clear that the school could not accept that some children were sent to physical activity during compulsory school hours while others were not. Randomization within classes would, through time, have led to enormous problems with children crossing over between groups. Therefore, we accepted choosing one school as the intervention school, and then choosing 3 schools with the same curriculum before the intervention and with the same socio-economic background as control schools.

Quantifying physical activity is known to be difficult, especially in children. We chose the level of organized physical activity evaluated through a questionnaire used in earlier studies, but modified for children[45,175]. It can definitely be argued whether this is the best way to quantify physical activity because with the same duration of activity, different types of activities may have a different osteogenic response, as could different intensities of the same type of exercise performed during the same period. However the main purpose of our study was not to define exactly the duration of physical activity in each child or define the osteogenic index for each type of physical activity. The main aim was to evaluate whether an exercise intervention programme could enhance the accrual of aBMD and bone size on a group level. Accelerometers have been used in other studies to evaluate the physical activity in more detail[82] and have also been added to our protocol, but the data have not yet been analysed.

DXA measurements for clinical use in adults often use the hip measurement, while the lumbar spine measurement is used in children because there are known limitations when measuring the hip in the youngest individuals[1]. At the start of our study no normative data for children existed and we were obliged to use a special paediatric software for individuals under 35 kg of weight. This could possibly have influenced the data, but our finding of changes with a similar magnitude in those who were measured with paediatric software on both occasions and those who were measured with paediatric software at baseline but with adult software at follow-up contradicts this view. To further secure our method, we evaluated the precision of the DXA measurements by duplicate measurements in 13 healthy children

aged 7–15 years (mean age 10 years) and the result was for BMC 1.4%–3.7%, aBMD 1.6%–2.8%, L3 width 2.2%, FN periosteal diameter 1.5%, FN CSA 2.2%, FN CSMI 6.2%, total body fat mass 3.7% and total body lean mass 1.5%. Finally, the estimation of vBMD using the two-dimensional DXA technique is known to be associated with errors[197]. The difference in the annual changes in FN vBMD between cases and controls could possibly be erroneously estimated due to methodological problems. However, there are also reports inferring that there is a preferential adaptation in bone geometry over bone density with increased physical activity especially in the young skeleton[2,119,185,191]. The hypothesis presented is that a modest increase in vBMD would confer a less significant increase in bone strength than would improvement in bone geometry. There are even studies using pQCT that infer exercise to be associated not only with a larger skeleton but also with a lower vBMD of the cortical bone[111], the net result being a light skeleton with a high bone strength.

The DXA technique is not designed to measure bone geometric properties. To further evaluate the DXA hip measurements we applied the Hip Strength Analysis (HSA) software to the measurements in boys. From the two dimensional projection the HSA software transfer the data to an estimation of the three-dimensional structure. A miss-classified positioning of the limb and a small deviation of the placement of the region of interest and the reference points will inevitable lead to remarkable errors in the HSA data, especially the width data. This is the reason why several authors infer that prospective studies in children should exclude outliers in the calculations, as proposed by Beck et al [12]. Furthermore, the estimates of the cortical thickness and endosteal diameter are drawn after making assumptions of a homogenous porosity in the cortical shell and a cross-sectional shape and relative fixed distribution of trabecular and cortical bone within the femoral neck, assumptions that have not been assessed in children. Furthermore, the estimation of the periosteal dimension is only made in two dimensions, but the skeleton could expand in other directions, then not captured by HSA analyses. Thus, small increases in the periosteal diameter may not have been detected because of methodological, rather than biological reasons. Also, a small error in the measurement of the bone width may results in large errors in the estimate of bone strength, CSMI and section modulus, as CSMI is proportional to the fourth power of the radius of a tubular bone[147]. In spite of these mentioned technical limitations and possible errors of the method, HSA should be regarded as the first attempt to estimate the structure of the FN, an acceptable method before we can present structural data based on true three dimensional imaging techniques such as CT or MRI.

The exercise-induced beneficial effects in boys seem to be less obvious than in girls. Several plausible explanations can be pointed out. Several studies infer that the pre- or early peri-pubertal period is the time when the skeleton shows the highest responsiveness to mechanical load, and as girls at the same chronological age are closer to puberty than boys, this difference in pubertal maturation could possibly explain the discrepancy. Another possible explanation is that the boys in the POP study had a higher level of leisure-time activity than the girls, that is, the intervention programme made a bigger difference for the total skeletal load in the girls than in the boys. Also, when analysing data further into the POP study outside the scope of this thesis, a small difference was found in pubertal maturation between the intervention group and the controls, the control group reaching pubertal maturation earlier than the intervention group and this can possibly conceal more pronounced exercise-induced benefits.

Due to lack of resources in our research laboratory, the controls were not re-measured until after 2 years. This is a clear limitation in Paper II, where the intervention group was followed for 1 year and the controls for 2 years. But this study design comparing the annual changes in bone mass accrual must be regarded as acceptable, as extensive literature consistently suggests that there is a linear increase in the accrual of bone mineral and bone size during the years spanned by this study (Tanner stage I) and also that the increase in bone traits is first seen in Tanner stage II or later [8,9,16,56,67,113,114,120,122,178]. Swedish data show that growth rate is linear from age 6 to peak height velocity and that virtually none of the boys had reached peak height velocity at age 10[120]. Peak height velocity is usually reached much later, in boys at mean age 13.4 years in Tanner stage III, while peak bone mineral accrual occurs at mean age 14.0 years and in Tanner stage IV[122]. A study from Australia further supports this view, reporting that peak bone mass accrual and peak height velocity occur from Tanner stage II or later, whereas the growth and bone mineral accrual are linear in Tanner stage I and during the years covered by our study[9]. Finally, the results in boys from the 2-year follow-up (Paper IV) confirm the results from the 1-year follow-up, suggesting that our line of argument in Paper II was correct.

In Paper III the girls in the intervention group had a greater annual gain in fat mass and lean mass than the controls, a discrepancy difficult to explain, but a discrepancy that could influence the inferences. Increased duration of exercise could enhance the participants' appetite and result in a higher food intake that hypothetically could affect the bone accrual. An alternate explanation could be that the increased weight and muscle gain could be the direct result of the exercise programme, benefits that could influence the accrual of bone mass and bone size. However, because there was no dose-response relationship between the duration of activity and gain in fat mass, this suggests that the discrepancy in fat gain was the result of other influences than the activity. There is also a possibility that exercise influences the skeleton through changes in soft-tissue composition, and this is the reason why we only adjusted for differences in growth, i.e. chronological age and changes in height and weight.

We conclude that a 2-year moderately intense school-based exercise intervention programme in a population-based cohort of prepubertal boys and girls seems to be associated with skeletal benefits. Then the next clinically important question arises: will the benefits remain with years of intervention, and even more importantly, will they remain through life into old age when fragility fractures occur? As mentioned earlier, the Achilles heel of exercise is its cessation. Cross-sectional and prospective controlled and uncontrolled trials do suggest that high physical activity is associated with a high peak bone mass[94,95,139,187]. But decades later, when the incidence of fragility fractures rises dramatically, no benefits in bone mass seem to be retained[102,103]. Data in the literature are somewhat inconclusive. Kontulainen et al. have shown in a series of fine studies that benefits are maintained in the short term perspective after cessation of activity[108-111] although questions have been raised that the cohort "retired during the study period" included highly active individuals[186]. Other groups have failed to show any maintained benefits after cessation of physical activity[33, 69,81,138,139]. For example Michel, et al. reported a clear decrease in spine aBMD in male ex runners compared to no loss among the still active runners[131]. Also, Valdimarsson et al. found in their 8-year controlled follow-up study of 66 Swedish soccer players that players who retired during the follow-up period lost aBMD in the femoral neck, whereas the controls did not[187]. Furthermore, Nordstrom et al. found that reduced activity is followed by an aBMD loss within 3 years of cessation of sports in

43 male ice-hockey players, while there was no change in bone size.[138] The mechanism of the purported rapid bone loss that follows cessation of exercise is unknown. Data in the literature imply that aBMD benefits in athletes are retained after active career if they continue to be active on a lower level[75,109].

In Paper I we investigated whether benefits in peak aBMD derived from exercise during growth are present in old age, and whether former soccer players have fewer fractures. The study supports the notion that exercise during growth results in biologically important benefits in peak aBMD at weight-bearing sites such as the proximal femur, the site of fracture associated with high morbidity and mortality in old age. The finding that the benefits attained by exercise during adolescence attenuated with increasing number of years since retirement so that those aged 70–85 had no residual benefit was in accordance with earlier findings. If you don't use it you lose it. Similarly, the finding that the fracture prevalence in former soccer players was higher during active career than in controls was hardly surprising to those of us involved in competitive sports. We failed to identify a protective effect of exercise against fractures in old age in former soccer players. This may have been due to a power problem, even though our sample size was sufficient to detect a difference of 30% or more in the relative proportion of persons with fractures among each group. When we studied the absolute values in fragility fractures in each group we found a small difference between the groups, although not statistically significant. This made us speculate whether we were suffering from a lack of statistical power. To further investigate this, the research group later – in one publication not included in this thesis – extended the study to involve 400 former soccer players and 800 controls, and this boost in power enabled us to detect a significant difference between the former active soccer players and the controls (2.0% vs. 4.2%, $p<0.05$)[138]. Again, bone strength is not dependent on aBMD alone. The higher loss in aBMD in former athletes occurs at the endosteal surface[71], that is, a higher bone loss in former athletes would finally lead to a skeleton with a similar aBMD to controls, yet larger in size. This would render a skeleton with higher bone strength, as small changes in bone size are of greater importance for bone strength than small changes in aBMD[3].

In summary, a 2-year moderately intense school-based exercise intervention programme in a population-based cohort of prepubertal boys and girls seems to be associated with benefits in the accrual of BMC, aBMD and gain in bone width, all traits important for bone strength, and the benefits are present already after 1 year of intervention. Whether a school-based exercise programme exceeding 2 years could be beneficial for bone health, and whether the intervention may be used as a prevention strategy for osteoporosis, remains to be evaluated in future studies. The POP study is planned to continue until peak bone mass is reached to try and answer these question. God and our brave children willing, the researcher's dream would be to follow this cohort until they are 80 years of age and subjected to fragility fractures. I leave that task to my younger peers.

Conclusions

- Vigorous exercise during growth seems to be associated with biologically important benefits in peak aBMD at weight-bearing sites.

- Cessation of exercise seems to be associated with a loss of the benefit in aBMD, leaving only modest residual benefits in middle age and probably none in old age, the years when fracture occurs.

- Soccer players seem to have more fractures during their active career under the age of 35 than do sedentary controls.

- Based on data presented in this thesis, vigorous exercise during growth and young adulthood seems not to be associated with reduced fracture risk in old age. However, we are aware of the power problem when evaluating the fracture data in paper I, and that later studies from our group[138], including a larger sample size, have opposed this view. Therefore, our view today is that vigorous exercise during growth and young adulthood is associated with reduced fracture risk in old age.

- The duration of physical activity seems to be associated with the accrual of bone mineral and gain in bone size in prepubertal boys and girls.

- A 2-year moderately intense school-based exercise intervention programme in a population-based cohort of prepubertal boys and girls seems to be associated with benefits in the accrual of BMC, aBMD and gain in bone width, benefits present already after 1 year of intervention.

- This thesis supports the view that general moderately intense physical activity could be recommended as a strategy to increase BMC, aBMD and bone size in prepubertal children.

Summary in Swedish

Fysisk aktivitet och dess effekter på skelettet i det korta och långa perspektivet

Benskörhet (osteoporos), är ett komplext, multifaktoriellt tillstånd som utvecklas i det tysta fram till den dag man drabbas av en fraktur. De senaste 40 åren har antalet benskörhetsfrakturer ökat och i dag beräknas varannan kvinna och var fjärde man någon gång drabbas av en eller flera osteoporosrelaterade frakturer. Exempel på sådana benbrott är frakturer i kota, höft, axel eller handled. Som en orsak till ökningen av antalet frakturer har nämnts att vår medellivslängd det senaste seklet har ökat med 25 år. Med ökande ålder försämras nämligen skelettets kvalitet och låg benmassa är en av de största riskfaktorerna för att råka ut för en fraktur. Ekvationen är svårlöst och innebär att 2000-talets människa blivit ett hållfasthetsproblem. I Sverige inträffar nu årligen ca 70 000 benskörhetsfrakturer där det, förutom stort lidande för den drabbade, dessutom uppkommer enorma kostnader för samhället. Exempelvis beräknas den årliga sjukvårdskostnaden för enbart höftfrakturer till dryga 3 miljarder kronor. När dessutom Sverige i dag har den tveksamma förmånen att tillhöra världseliten gällande den proportionella förekomsten av benskörhetsfrakturer förstår man att denna utveckling måste stoppas.

Ungefär 70 % av benmassan regleras av genetiska faktorer medan återstående 30% regleras av påverkbara yttre faktorer, som exempelvis fysisk aktivitet. Det finns även andra kända riskfaktorer för benskörhet såsom hög ålder, låg kroppsvikt, rökning, lågt kalciumintag, kortisonmedicinering och vissa sjukdomstillstånd, alla faktorer som påverkar skelettets utveckling.

En av de viktigaste livsstilsfaktorerna som reglerar benmassan är fysisk aktivitet. Denna faktor har utvecklats till en bristvara i den moderna människans vardagsliv. En sannolik förklaring till detta är att vi i övergången från industrisamhälle till kunskapssamhälle rationaliserat bort stora delar av den fysiska aktiviteten från vårt vardagsliv. Trappor har förvandlats till nödutgångar, bollspel bytts mot TV-spel och varannan bilresa når aldrig över 5 km. Larmrapporterna från Statens folkhälsoinstitut duggar tätt. 90 % av befolkningen vet att motion är viktigt för hälsan, samtidigt som 75 % inte motionerar tillräckligt ur hälsosynpunkt. Ännu mer alarmerande är att 25 % av dessa individer är i det närmaste helt inaktiva. Värst utsatta är unga flickor där undersökningar visar att var femte flicka sällan eller aldrig deltar i skolgymnastiken samt att var fjärde flicka inte tränar eller motionerar alls på fritiden. Andelen överviktiga barn har 6-dubblats sedan mitten av 80-talet och man har också noterat att barn drabbas av vällevnadssjukdomar relaterade till fysisk inaktivitet man normalt associerar med ett betydligt äldre klientel. Listan på negativa effekter som uppkommer genom en mer fysiskt inaktiv livsstil kan göras betydligt längre. Trots detta har skolgymnastikens del av skoldagen under de gångna decennierna mer än halverats.

För att finna individer med hög risk att drabbas av en fraktur har man börjat mäta individens benmassa. Benmassa är nämligen en av de viktigaste faktorerna som påverkar skelettets hållfasthet. Benmassan kan dessutom mätas med flera olika metoder. Den mest förekommande metodiken är en lågdosröntgenteknik kallad DXA. Men inte bara benmassan påverkar skelettets hållfasthet. Även skelettets storlek och tredimensionella struktur är viktiga faktorer för skelettets förmåga att motstå en fraktur.

Den största mängd benmassa en individ uppnår i livet, sannolikt strax efter tillväxtens avslutning, benämns peak bone mass (PBM).

PBM är också relaterad till den framtida risken att drabbas av fraktur. En ökning av PBM på ca 10 % har kopplats till en halvering av risken för kotfraktur. PBM har dessutom visats kunna påverkas av graden av fysisk aktivitet under uppväxten där studier har indikerat att tiden strax före och under puberteten sannolikt är den tidsperiod där fysisk aktivitet har sin största möjlighet att påverka skelettet. De flesta studier med högt bevisvärde som har tittat på hur träning påverkar skelettet har använt speciella övningar som man vet påverkar skelettet, övningar med hög belastning, snabbt insättande belastning och belastning i olika riktningar. Många studier har dessutom upprepat dessa övningar gång på gång, vilket har medfört att barnen har tröttnat på denna träning och därför slutat träna strax efter det att studien avslutats. Deltagandet har dessutom ofta baserats på de barn som självmant har anmält sig till studien vilket troligen har resulterat i att studien har inkluderat ett selekterat material med individer specialintresserade av fysisk aktivitet. För att kringgå dessa bekymmer har studierna i denna avhandling inkluderat alla individer i en åldersgrupp, alltså även barn med lägre grad av intresse i idrott, detta för att vi skall kunna utvärdera om måttlig fysisk aktivitet kan leda till gynnsamma effekter på skelettet när barn med alla olika intressegrader i fysisk aktivitet inkluderas.

Detta var bakgrunden som fick oss att starta våra studier som utvärderar om träning kan påverka skelettets tillväxt. Den första studien, den s.k. Sösdalastudien, utvärderade om barn mellan åldrarna 12 till 16 år som fick skolgymnastik 4 gånger per vecka förbättrade hållfastheten av skelettet mer än barn som hade gymnastik en till två gånger per vecka. I denna studie såg vi att ökad fysisk aktivitet påverkade benmassan i gynnsam riktning bland pojkarna men inte hos flickorna. Som en förklaring framlades tanken att man sannolikt borde inleda träningen än tidigare hos flickorna, under en tid där de fortfarande hade stor kvarvarande tillväxtpotential, och möjligen borde träningen även intensifieras.

Som ett logiskt steg startades därför Bunkefloprojektet 1999 – på engelska kallad POP-studien. I detta projekt följs pojkar och flickor årligen redan från första årskurserna i grundskolan. Hälften av barnen får skolgymnastik dagligen och den andra hälften en till två gånger per vecka enligt standardschemat i svenska skolor. Årliga mätningar utförs av bl.a. benmassa och skelettstorlek, muskelstyrka, balans och koordination.

Artiklarna II-IV i denna avhandling redovisar resultaten från Bunkefloprojektets första två år. I artikel II redovisas 1-årsresultaten hos 81 pojkar (7-9 år gamla) jämfört med 57 åldersmatchade kontroller. Ingen skillnad kunde påvisas mellan grupperna vid studiestart när det gällde längd, vikt, benparametrar eller s.k. bakgrundsfaktorer såsom matvanor, kroniska sjukdomar, medicinering, frakturer, rökning eller alkohol. Före interventionen var det heller ingen skillnad i tid i organiserad fysisk aktivitet mellan grupperna, men när barnen i interventionsgruppen fick daglig fysisk aktivitet på schemat blev det en klar och statistiskt verifierad skillnad i nivån av fysisk aktivitet jämfört med kontrollbarnen. Vi fann att den årliga inlagringen av benmassa i ländryggen var högre i interventionsgruppen och på samma sätt sågs även en skillnad i vidden av ländryggens tredje kota mellan grupperna, till interventionsgruppens fördel. När alla barnen studerades som en grupp visade det sig att tiden spenderad i någon form av organiserad fysisk aktivitet, såväl inom som utom skolans ramar, var positivt relaterad till mängden benmassa och även till skelettstorleken i ländryggen. Några skillnader mellan grupperna kunde inte ses vid utvärdering av skelettutveckling i höftleden. De funna resultaten efter 1 år visade sig kvarstå även efter 2 års intervention, vilket redovisas i artikel IV.

I artikel III redovisas resultaten efter 2 års intervention hos 49 flickor (7-9 år gamla) jämfört med 50 åldersmatchade kontroller. Precis som hos pojkarna var grupperna väldigt lika vid studiestart och den enda skillnad vi fann då var att kontrollgruppens flickor var mer aktiva i organiserad idrott utanför skoltid (1.3 jämfört med 0.7 timmar per vecka). Efter interventionen var barnen i interventionsgruppen emellertid betydligt mer aktiva (4.0 jämfört med 2.3 timmar per vecka). I likhet med fynden hos pojkar fann vi att den årliga inlagringen av benmassa i ländryggen var högre i interventionsgruppen jämfört med kontrollgruppen. Dessutom var den årliga ökningen i vidden av tredje ländryggskotan samt vidden av lårbenshalsen också större i interventionsgruppen jämfört med kontrollerna. När alla barnen studerades som en grupp visade det sig även hos flickor att tiden spenderad i någon form av organiserad fysisk aktivitet, såväl inom som utom skolans ramar, var positivt relaterad till mängden benmassa och skelettstorlek i ländryggen. I höften fann vi däremot inga liknande idrottsbetingade effekter. Några sådana förändringar förelåg inte i höften.

Sammanfattningsvis visar de tre studierna från Bunkefloprojektet entydigt att måttlig fysisk aktivitet är associerad med en ökad benmassa och ett större skelett hos både prepubertala flickor och pojkar. Dessa effekter kan ses redan efter ett års ökad träning. Dessa data motsäger inte att fysisk aktivitet kan rekommenderas som ett sätt att öka benmassan och skellettstorleken hos prepubertala barn. Först när vi följt grupperna upp i vuxen ålder kan vi närmare uttala oss om träning under ungdomsåren kan påverka PBM och användas som en strategi för att förebygga osteoporos.

Om träning i unga år kan påverka benmassan så uppkommer ytterligare en viktig fråga som behandlas i artikel I. Kvarstår idrottsbetingade gynnsamma skelettförändringar efter avslutad idrottskarriär och har i så fall f.d. aktiva unga människor mindre frakturer i ålderdomen i jämförelse med de individer som varit inaktiva? I artikel I undersökte vi om fotboll på elitnivå under uppväxten skyddar mot frakturer i ålderdomen. Dessutom värderade vi om fotbollsspel påverkar PBM samt om det i så fall kvarstår idrottsbetingade gynnsamma effekter på skelettet även efter avslutad idrottskarriär. Vi mätte benmassan hos 22 aktiva fotbollsspelare, 128 f.d. fotbollsspelare och 138 åldersmatchade kontroller. Frakturuppgifter hämtades från röntgenarkivet på Universitetssjukhuset MAS på 284 f.d. fotbollsspelare och 568 kontroller. Vi fann att fysisk aktivitet på hög nivå resulterar i en biologiskt betydelsefull ökning av PBM i viktbelastade delar av skelettet. Denna gynnsamma ökning av benmassan förefaller dock successivt gå förlorad under årtiondena efter det att man upphört med sin idrott. I denna studie kunde vi inte påvisa att äldre f.d. fotbollsspelare har färre frakturer, högre benmassa eller ett större skelett jämfört med individer som inte har tränat på denna nivå i ungdomen. Ett stort problem är att studien innehöll relativt få riktigt gamla idrottsmän. Detta gör att det kan vara svårt att med statistik påvisa att det finns skillnad mellan grupperna. Som exempel kan nämnas att bland de f.d. aktiva gamla idrottsmännen hade endast hälften så många drabbats av en benskörhetsrelaterad fraktur jämfört med de individer som inte hade tränat. Därför har vår forskar grupp i ett senare skede ökat studieantalet i en rapport där man nu kunde slå fast att bland de som under ungdomen hade tränat på en hög nivå, fanns det färre individer i äldre åldrar som hade drabbats av en fraktur än bland de som inte hade tränat. I dag anser vi därför att fysisk aktivitet på hög nivå i ungdomen är associerad med minskad risk för fraktur på ålderns höst.

Acknowledgement

This study was initiated and the measurements performed at the Department of Orthopaedics, Malmö University Hospital. The intervention took place at the Ängslättskolan in Bunkeflostrand while three schools in Malmö (Ribersborgsskolan, Fridhemsskolan and Mellanhedsskolan) served as controls. Understandably, a lot of people have been involved whom I am forever indebted to. I would have to write another book to thank you all, but nevertheless I want to send some extra thoughts of gratitude to a few of you:

Magnus Karlsson: My scientific guide in the amazing world of research who drafted me on my first day at work. Little did I understand then, that it was like being asked by Björn Borg if I wanted to learn how to play tennis. Thank you for your effort, patience and timely advice on any subject up for discussion: research, surgery, family matters or life in general.

Per Gärdsell: My former chief of staff and driving spirit of the Bunkeflo project from its inception. Your dedication to the project fuelled me and everybody else and sent you on a journey you still haven´t finished.

Children and parents: The core of the project without whom it would be nothing but air. Your effort and persistence will forever be engraved in the field of bone research. Future generations will benefit from your dedication.

Headmasters, teachers, health promoters and other personnel at the participating schools for your curiosity, energy and persistence throughout the years. A special thank you to **Britten Synnergren** and **Conny Lindvall** for seeing the possibilities behind the obstacles when plans for the project were drawn.

The girls at the Orthopaedic research unit, UMAS: You make the world a more beautiful place and my work as a researcher much easier.

All my co-authors and their Departments for invaluable discussions and fruitful collaborations. I send extra thoughts to **Susanna Stenevi-Lundgren** and **Karin Önnby** for your organizing skills and to **Magnus Dencker** for never ending a discussion.

All my colleagues at the Department of Orthopaedics in Malmö and Ljungby for constructive criticism, sharing valuable surgical skills and for creating a friendly environment on and off the golf course. A special thank you to **Fredrik Nyquist** who gave a sports geek the opportunity of a lifetime. **Carin Holmberg** and **Annelie Norrby** for needful secretary assistance. **Rolf Hansson** for technical assistance. **Jan-Åke Nilsson** for statistical advice.

All my friends that wondered if something serious happened to me and now hold the answer in the palm of their hands.

My family: **Christina, Björn, Karin, Fredrik, Lina, Louise** and **Aron** for their love and encouragement through the years. My sister follows her own paths in life, but fortunately every path passes the research department where she gives her brother invaluable help. **My in-laws**: **Eva** and **Lennart** for their love and support in every way.

Above all, **Camilla** and **Robin**: My orange-half and my mandarin-half - the love of my life.

References

1 Aaa. Bone mass measurements in children. Meet the professor session, ASBMR, 2006.

2 Adami S, Gatti D, Braga V, Bianchini D, Rossini M. Site-specific effects of strength training on bone structure and geometry of ultradistal radius in postmenopausal women. J Bone Miner Res 1999; 14: 120-4

3 Ahlborg HG, Johnell O, Turner CH, Rannevik G, Karlsson MK. Bone loss and bone size after menopause. N Engl J Med 2003; 349: 327-34

4 Albright F, Smith PH, Richardsson, AM. Postmenopausal osteoporosis. JAMA 1941; 116: 2465-74

5 Alwens W. Spatrachitis, Osteomalazie, Senile Osteoporosis, Hungerosteopati. Handbuch der Inneren Medizin. Berlin. Springer Verlag 1926; 584

6 Anonymous. Consensus development conference: diagnosis, prophylaxis and treatment of osteoporosis. Am J Med 1993; 94: 646-50

7 Bailey DA, Martin AD, McKay HA, Whiting S, Mirwald R. Calcium accretion in girls and boys during puberty: a longitudinal analysis. J Bone Miner Res 2000; 15: 2245-50

8 Bailey DA, McKay HA, Mirwald RL, Crocker PR, Faulkner RA. A six-year longitudinal study of the relationship of physical activity to bone mineral accrual in growing children: the university of Saskatchewan bone mineral accrual study. J Bone Miner Res 1999; 14: 1672-9

9 Bass S, Delmas PD, Pearce G, Hendrich E, Tabensky A, Seeman E. The differing tempo of growth in bone size, mass, and density in girls is region-specific. J Clin Invest 1999; 104: 795-804

10 Bass S SL, Iuliano-Burns S, Naughton G, Daly R, Nowson C, Briganti E, Austen S. Limitations of long term exercise interventions aimed at improving bone health in normally active boys. J Bone Miner Res 2003; 18: M151

11 Beck TJ, Ruff CB, Warden KE, Scott WW, Jr., Rao GU. Predicting femoral neck strength from bone mineral data. A structural approach. Invest Radiol 1990; 25: 6-18

12 Beck TJ, Oreskovic TL, Stone KL, Ruff CB, Ensrud K, Nevitt MC, Genant HK, Cummings SR. Structural adaptation to changing skeletal load in progression toward hit fragility: the study of osteoporotic fractures. JBMR 2001;16:1108-19

13 Bischoff-Ferrari HA, Dawson-Hughes B, Willett WC, Staehelin HB, Bazemore MG, Zee RY, Wong JB. Effect of Vitamin D on falls: a meta-analysis. Jama 2004; 291: 1999-2006

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

15 Bolotin HH. Inaccuracies inherent in dual-energy X-ray absorptiometry in vivo bone mineral densitometry may flaw osteopenic/osteoporotic interpretations and mislead assessment of antiresorptive therapy effectiveness. Bone 2001; 28: 548-55

16 Bonjour JP, Theintz G, Buchs B, Slosman D, Rizzoli R. Critical years and stages of puberty for spinal and femoral bone mass accumulation during adolescence. J Clin Endocrinol Metab 1991; 73: 555-63

17 Borgstrom F, Zethraeus N, Johnell O, Lidgren L, Ponzer S, Svensson O, Abdon P, Ornstein E, Lunsjo K, Thorngren KG, Sernbo I, Rehnberg C, Jonsson B. Costs and quality of life associated with osteoporosis-related fractures in Sweden. Osteoporos Int 2006; 17: 637-50

18 Bouxsein ML, Marcus R. Overview of exercise and bone mass. Rheum Dis Clin North Am 1994; 20: 787-802

19 Brooke-Wavell K, Jones PR, Hardman AE. Brisk walking reduces calcaneal bone loss in post-menopausal women. Clin Sci (Lond) 1997; 92: 75-80

20 Carrie Fassler AL, Bonjour JP. Osteoporosis as a pediatric problem. Pediatr Clin North Am 1995; 42: 811-24

21 Carter DR, Bouxsein ML, Marcus R. New approaches for interpreting projected bone densitometry data. J Bone Miner Res 1992; 7: 137-45

22 Carter DR, Van Der Meulen MC, Beaupre GS. Mechanical factors in bone growth and development. Bone 1996;18: S1:5S-10S.

23 Cauley JA, Thompson DE, Ensrud KC, Scott JC, Black D. Risk of mortality following clinical fractures. Osteoporos Int 2000; 11: 556-61
24 Center JR, Nguyen TV, Schneider D, Sambrook PN, Eisman JA. Mortality after all major types of osteoporotic fracture in men and women: an observational study. Lancet 1999; 353: 878-82
25 Chapuy MC, Arlot ME, Delmas PD, Meunier PJ. Effect of calcium and cholecalciferol treatment for three years on hip fractures in elderly women. Bmj 1994; 308: 1081-2
26 Clark EM, Ness AR, Bishop NJ, Tobias JH. Association between bone mass and fractures in children: a prospective cohort study. J Bone Miner Res 2006; 21: 1489-95
27 Cooper C, Atkinson EJ, Jacobsen SJ, O'Fallon WM, Melton LJ, 3rd. Population-based study of survival after osteoporotic fractures. Am J Epidemiol 1993; 137: 1001-5
28 Cooper C, Campion G, Melton LJ, 3rd. Hip fractures in the elderly: a world-wide projection. Osteoporos Int 1992; 2: 285-9
29 Cowell CT, Lu PW, Lloyd-Jones SA, Briody JN, Allen JR, Humphries IR, Reed E, Knight J, Howman-Giles R, Gaskin K. Volumetric bone mineral density--a potential role in paediatrics. Acta Paediatr Suppl 1995; 411: 12-6, discussion 17
30 Cumming RG, Nevitt MC. Calcium for prevention of osteoporotic fractures in postmenopausal women. J Bone Miner Res 1997; 12: 1321-9
31 Cummings SR, Black DM, Nevitt MC, Browner WS, Cauley JA, Genant HK, Mascioli SR, Scott JC, Seeley DG, Steiger P, et al. Appendicular bone density and age predict hip fracture in women. The Study of Osteoporotic Fractures Research Group. Jama 1990; 263: 665-8
32 Cummings SR, Nevitt MC, Browner WS, Stone K, Fox KM, Ensrud KE, Cauley J, Black D, Vogt TM. Risk factors for hip fracture in white women. Study of Osteoporotic Fractures Research Group. N Engl J Med 1995; 332: 767-73
33 Dalsky GP, Stocke KS, Ehsani AA, Slatopolsky E, Lee WC, Birge SJ, Jr. Weight-bearing exercise training and lumbar bone mineral content in postmenopausal women. Ann Intern Med 1988; 108: 824-8
34 Davies JH, Evans BA, Gregory JW. Bone mass acquisition in healthy children. Arch Dis Child 2005; 90: 373-8
35 Dawson-Hughes B, Harris SS, Krall EA, Dallal GE. Effect of calcium and vitamin D supplementation on bone density in men and women 65 years of age or older. N Engl J Med 1997; 337: 670-6
36 Delaisse JM, Eeckhout Y, Vaes G. In vivo and in vitro evidence for the involvement of cysteine proteinases in bone resorption. Biochem Biophys Res Commun 1984; 125: 441-7
37 Dencker M, Thorsson O, Karlsson M, Linden C, Svensson J, Wollmer P, Andersen LV. Daily physical activity in Swedish children age 8- 11 years. Scand J Med Sci Sports 2006;16:205-7.
38 Dencker M, Thorsson O, Karlsson M, Linden C, Wollmer P, Ahren B. Leptin is closely related to body fat in prepubertal children aged 8-11 years. Acta Paediatr 2006;95:975-9.
39 Dencker M, Thorsson O, Karlsson M, Linden C, Eiberg S, Wollmer P, Andersen LB. Daily physical activity related to body fat in children aged 8-11 years. J Pedriatr 2006;149:38-42.
40 Denker M, Thorsson O, Karlsson M, Linden C, Svensson J, Wollmer P, Andersen LB. Daily physical activity and its relation to aerobic fitness in children aged 8-11 years. Eur J Appl Physiol 2006;96:587-92
41 Drinkwater BL. Exercise in the prevention of osteoporosis. Osteoporos Int 1993; 3 Suppl 1: 169-71
42 Duan Y, Parfitt A, Seeman E. Vertebral bone mass, size, and volumetric density in women with spinal fractures. J Bone Miner Res 1999; 14: 1796-802
43 Duan Y, Seeman E, Turner CH. The biomechanical basis of vertebral body fragility in men and women. J Bone Miner Res 2001; 16: 2276-83
44 Duke PM, Litt IF, Gross RT. Adolescents' self-assessment of sexual maturation. Pediatrics 1980; 66: 918-20
45 Duppe H, Gardsell P, Johnell O, Nilsson BE, Ringsberg K. Bone mineral density, muscle strength and physical activity. A population-based study of 332 subjects aged 15-42 years. Acta Orthop Scand 1997; 68: 97-103

46 Duppe H, Gardsell P, Nilsson B, Johnell O. A single bone density measurement can predict fractures over 25 years. Calcif Tissue Int 1997; 60: 171-4
47 Eisman JA. Genetics of osteoporosis. Endocr Rev 1999; 20: 788-804
48 Engstrom L. A people on their feet or on their knees? Symposia 2005.
49 Ericsson I. Motor skills, attention and academic achievements - An intervention study in school year 1-3. Thesis 2003, School of Education, Malmö University.
50 Eriksen EF. Normal and pathological remodeling of human trabecular bone: three dimensional reconstruction of the remodeling sequence in normals and in metabolic bone disease. Endocr Rev 1986; 7: 379-408
51 Faulkner KG. Bone matters: are density increases necessary to reduce fracture risk? J Bone Miner Res 2000; 15: 183-7
52 Faulkner RA, McCulloch RG, Fyke SL, De Coteau WE, McKay HA, Bailey DA, Houston CS, Wilkinson AA. Comparison of areal and estimated volumetric bone mineral density values between older men and women. Osteoporos Int 1995; 5: 271-5
53 Fewtrell MS. Bone densitometry in children assessed by dual x ray absorptiometry: uses and pitfalls. Arch Dis Child 2003; 88: 795-8
54 Forwood MR, Burr DB. Physical activity and bone mass: exercises in futility? Bone Miner 1993; 21: 89-112
55 Forwood MR, Turner CH. Skeletal adaptations to mechanical usage: results from tibial loading studies in rats. Bone 1995; 17: 197S-205S
56 Fournier PE, Rizzoli R, Slosman DO, Theintz G, Bonjour JP. Asynchrony between the rates of standing height gain and bone mass accumulation during puberty. Osteoporos Int 1997; 7: 525-32
57 Friedlander AL, Genant HK, Sadowsky S, Byl NN, Gluer CC. A two-year program of aerobics and weight training enhances bone mineral density of young women. J Bone Miner Res 1995; 10: 574-85
58 Frost HM. A determinant of bone architecture. The minimum effective strain. Clin Orthop Relat Res 1983: 286-92
59 Frost HM. The mechanostat: a proposed pathogenic mechanism of osteoporoses and the bone mass effects of mechanical and nonmechanical agents. Bone Miner 1987; 2: 73-85
60 Frost HM. The role of changes in mechanical usage set points in the pathogenesis of osteoporosis. J Bone Miner Res 1992; 7: 253-61
61 Frost HM. On the estrogen-bone relationship and postmenopausal bone loss: A new model. J Bone Miner Res 1999;14:1473-7.
62 Gardsell P, Johnell O, Nilsson BE. Predicting fractures in women by using forearm bone densitometry. Calcif Tissue Int 1989; 44: 235-42
63 Garn SM. The course of bone game and the phases of bone loss. Orthop Clin North Am 1972;3:503-20.
64 Geusens P, Cantatore F, Nijs J, Proesmans W, Emma F, Dequeker J. Heterogeneity of growth of bone in children at the spine, radius and total skeleton. Growth Dev Aging 1991; 55: 249-56
65 Gilsanz V. Boechat MI, Roe TF, Loro ML, Sayre JW, Goodman WG. Gender differences in vertebral body sizes in children and adolescents. Radiology 1994;190:673-7.
66 Gilsanz V. Bone density in children: a review of the available techniques and indications. Eur J Radiol 1998; 26: 177-82
67 Glastre C, Braillon P, David L, Cochat P, Meunier PJ, Delmas PD. Measurement of bone mineral content of the lumbar spine by dual energy x-ray absorptiometry in normal children: correlations with growth parameters. J Clin Endocrinol Metab 1990; 70: 1330-3
68 Gleeson PB, Protas EJ, LeBlanc AD, Schneider VS, Evans HJ. Effects of weight lifting on bone mineral density in premenopausal women. J Bone Miner Res 1990; 5: 153-8
69 Gustavsson A, Olsson T, Nordstrom P. Rapid loss of bone mineral density of the femoral neck after cessation of ice hockey training: a 6-year longitudinal study in males. J Bone Miner Res 2003; 18: 1964-9
70 Haapasalo H, Kannus P, Sievanen H, Heinonen A, Oja P, Vuori I. Long-term unilateral loading and bone mineral density and content in female squash players. Calcif Tissue Int 1994; 54: 249-55

71 Haapasalo H, Kontulainen S, Sievanen H, Kannus P, Jarvinen M, Vuori I. Exercise-induced bone gain is due to enlargement in bone size without a change in volumetric bone density: a peripheral quantitative computed tomography study of the upper arms of male tennis players. Bone 2000; 27: 351-7

72 Haapasalo H, Sievanen H, Kannus P, Heinonen A, Oja P, Vuori I. Dimensions and estimated mechanical characteristics of the humerus after long-term tennis loading. J Bone Miner Res 1996; 11: 864-72

73 Hansen MA, Hassager C, Overgaard K, Marslew U, Riis BJ, Christiansen C. Dual-energy x-ray absorptiometry: a precise method of measuring bone mineral density in the lumbar spine. J Nucl Med 1990; 31: 1156-62

74 Harris SS, Dawson-Hughes B. Plasma vitamin D and 25OHD responses of young and old men to supplementation with vitamin D3. J Am Coll Nutr 2002; 21: 357-62

75 Huddleston AL, Rockwell D, Kulund DN, Harrison RB. Bone mass in lifetime tennis athletes. Jama 1980; 244: 1107-9

76 Hui SL, Slemenda CW, Johnston CC, Jr. Baseline measurement of bone mass predicts fracture in white women. Ann Intern Med 1989; 111: 355-61

77 Hui SL, Slemenda CW, Johnston CC, Jr. The contribution of bone loss to postmenopausal osteoporosis. Osteoporos Int 1990; 1: 30-4

78 Huusko TM, Karppi P, Avikainen V, Kautiainen H, Sulkava R. The changing picture of hip fractures: dramatic change in age distribution and no change in age-adjusted incidence within 10 years in Central Finland. Bone 1999; 24: 257-9

79 Ilich JZ, Skugor M, Hangartner T, Baoshe A, Matkovic V. Relation of nutrition, body composition and physical activity to skeletal development: a cross-sectional study in preadolescent females. J Am Coll Nutr 1998; 17: 136-47

80 Ismail AA, O'Neill TW, Cooper C, Finn JD, Bhalla AK, Cannata JB, Delmas P, Falch JA, Felsch B, Hoszowski K, Johnell O, Diaz-Lopez JB, Lopez Vaz A, Marchand F, Raspe H, Reid DM, Todd C, Weber K, Woolf A, Reeve J, Silman AJ. Mortality associated with vertebral deformity in men and women: results from the European Prospective Osteoporosis Study (EPOS). Osteoporos Int 1998; 8: 291-7

81 Iwamoto J, Yeh JK, Aloia JF. Effect of deconditioning on cortical and cancellous bone growth in the exercise trained young rats. J Bone Miner Res 2000; 15: 1842-9

82 Jamsa T, Vainionpaa A, Korpelainen R, Vihriala E, Leppaluoto J. Effect of daily physical activity on proximal femur. Clin Biomech (Bristol, Avon) 2006; 21: 1-7

83 Javaid MK, Cooper C. Prenatal and childhood influences on osteoporosis. Best Pract Res Clin Endocrinol Metab 2002; 16: 349-67

84 Johnell O, Gullberg B, Allander E, Kanis JA. The apparent incidence of hip fracture in Europe: a study of national register sources. MEDOS Study Group. Osteoporos Int 1992; 2: 298-302

85 Johnell O, Gullberg B, Kanis JA, Allander E, Elffors L, Dequeker J, Dilsen G, Gennari C, Lopes Vaz A, Lyritis G, et al. Risk factors for hip fracture in European women: the MEDOS Study. Mediterranean Osteoporosis Study. J Bone Miner Res 1995; 10: 1802-15

86 Johnell O, Kanis JA, Oden A, Sernbo I, Redlund-Johnell I, Petterson C, De Laet C, Jonsson B. Mortality after osteoporotic fractures. Osteoporos Int 2004; 15: 38-42

87 Johnston CC, Jr., Slemenda CW. Peak bone mass, bone loss and risk of fracture. Osteoporos Int 1994; 4 Suppl 1: 43-5

88 Jones G, Nguyen T, Sambrook PN, Kelly PJ, Gilbert C, Eisman JA. Symptomatic fracture incidence in elderly men and women: the Dubbo Osteoporosis Epidemiology Study (DOES). Osteoporos Int 1994; 4: 277-82

89 Jones HH, Priest JD, Hayes WC, Tichenor CC, Nagel DA. Humeral hypertrophy in response to exercise. J Bone Joint Surg Am 1977; 59: 204-8

90 Jouanny P, Guillemin F, Kuntz C, Jeandel C, Pourel J. Environmental and genetic factors affecting bone mass. Similarity of bone density among members of healthy families. Arthritis Rheum 1995; 38: 61-7

91 Kanis JA. Diagnosis of osteoporosis and assessment of fracture risk. Lancet 2002; 359: 1929-36

92 Kanis JA, Johnell O, Oden A, Sembo I, Redlund-Johnell I, Dawson A, De Laet C, Jonsson B. Long-term risk of osteoporotic fracture in Malmö. Osteoporos Int 2000; 11: 669-74
93 Kanis JA, Johnell O, Oden A, Sembo I, Redlund-Johnell I, Dawson A, De Laet C, Jonsson B. Long-term risk of osteoporotic fracture in Malmo. Osteoporos Int 2000; 11: 669-74
94 Kannus P, Haapasalo H, Sankelo M, Sievanen H, Pasanen M, Heinonen A, Oja P, Vuori I. Effect of starting age of physical activity on bone mass in the dominant arm of tennis and squash players. Ann Intern Med 1995; 123: 27-31
95 Kannus P, Haapasalo H, Sievanen H, Oja P, Vuori I. The site-specific effects of long-term unilateral activity on bone mineral density and content. Bone 1994; 15: 279-84
96 Kannus P, Niemi S, Parkkari J, Palvanen M, Heinonen A, Sievanen H, Jarvinen T, Khan K, Jarvinen M. Why is the age-standardized incidence of low-trauma fractures rising in many elderly populations? J Bone Miner Res 2002; 17: 1363-7
97 Kannus P, Palvanen M, Niemi S, Parkkari J, Jarvinen M. Epidemiology of osteoporotic pelvic fractures in elderly people in Finland: sharp increase in 1970-1997 and alarming projections for the new millennium. Osteoporos Int 2000; 11: 443-8
98 Kannus P, Palvanen M, Niemi S, Parkkari J, Jarvinen M, Vuori I. Increasing number and incidence of osteoporotic fractures of the proximal humerus in elderly people. Bmj 1996; 313: 1051-2
99 Karlsson M, Bass S, Seeman E. The evidence that exercise during growth or adulthood reduces the risk of fragility fractures is weak. Best Pract Res Clin Rheumatol 2001; 15: 429-50
100 Karlsson MK, Hasserius R, Obrant KJ. Bone mineral density in athletes during and after career: A comparison between loaded and unloaded skeletal regions. Calcified Tissue International 1996; 59: 245-248
101 Karlsson MK, Johnell O, Obrant KJ. Bone-Mineral Density in Professional Ballet Dancers. Bone and Mineral 1993; 21: 163-169
102 Karlsson MK, Johnell O, Obrant KJ. Bone-Mineral Density in Weight Lifters. Calcified Tissue International 1993; 52: 212-215
103 Karlsson MK, Johnell O, Obrant KJ. Is bone mineral density advantage maintained long-term in previous weight lifters? Calcif Tissue Int 1995; 57: 325-8
104 Kelley GA. Aerobic exercise and bone density at the hip in postmenopausal women: a meta-analysis. Prev Med 1998; 27: 798-807
105 Kelley GA, Kelley KS, Tran ZV. Resistance training and bone mineral density in women: a meta-analysis of controlled trials. Am J Phys Med Rehabil 2001; 80: 65-77
106 Kelly PJ, Eisman JA, Sambrook PN. Interaction of genetic and environmental influences on peak bone density. Osteoporos Int 1990; 1: 56-60
107 Kelly PJ, Morrison NA, Sambrook PN, Nguyen TV, Eisman JA. Genetic influences on bone turnover, bone density and fracture. Eur J Endocrinol 1995; 133: 265-71
108 Kontulainen S, Heinonen A, Kannus P, Pasanen M, Sievanen H, Vuori I. Former exercisers of an 18-month intervention display residual aBMD benefits compared with control women 3.5 years post-intervention: a follow-up of a randomized controlled high-impact trial. Osteoporos Int 2004; 15: 248-51
109 Kontulainen S, Kannus P, Haapasalo H, Heinonen A, Sievanen H, Oja P, Vuori I. Changes in bone mineral content with decreased training in competitive young adult tennis players and controls: a prospective 4-yr follow-up. Med Sci Sports Exerc 1999; 31: 646-52
110 Kontulainen S, Kannus P, Haapasalo H, Sievanen H, Pasanen M, Heinonen A, Oja P, Vuori I. Good maintenance of exercise-induced bone gain with decreased training of female tennis and squash players: a prospective 5-year follow-up study of young and old starters and controls. J Bone Miner Res 2001; 16: 195-201
111 Kontulainen S, Sievanen H, Kannus P, Pasanen M, Vuori I. Effect of long-term impact-loading on mass, size, and estimated strength of humerus and radius of female racquet-sports players: a peripheral quantitative computed tomography study between young and old starters and controls. J Bone Miner Res 2003; 18: 352-9

112 Kontulainen SA, Kannus PA, Pasanen ME, Sievanen HT, Heinonen AO, Oja P, Vuori I. Does previous participation in high-impact training result in residual bone gain in growing girls? One year follow-up of a 9-month jumping intervention. Int J Sports Med 2002; 23: 575-81

113 Kroger H, Kotaniemi A, Kroger L, Alhava E. Development of bone mass and bone density of the spine and femoral neck--a prospective study of 65 children and adolescents. Bone Miner 1993; 23: 171-82

114 Kroger H, Kotaniemi A, Vainio P, Alhava E. Bone densitometry of the spine and femur in children by dual-energy x-ray absorptiometry. Bone Miner 1992; 17: 75-85

115 Lanyon LE. Control of bone architecture by functional load bearing. J Bone Miner Res 1992; 7 Suppl 2: S369-75

116 Lanyon LE, Rubin CT, Baust G. Modulation of bone loss during calcium insufficiency by controlled dynamic loading. Calcif Tissue Int 1986; 38: 209-16

117 Lindsay R. Clinical utility of biochemical markers. Osteoporos Int 1999; 9 Suppl 2: S29-32

118 Lips P. Vitamin D deficiency and osteoporosis: the role of vitamin D deficiency and treatment with vitamin D and analogues in the prevention of osteoporosis-related fractures. Eur J Clin Invest 1996; 26: 436-42

119 Liu L, Maruno R, Mashimo T, Sanka K, Higuchi T, Hayashi K, Shirasaki Y, Mukai N, Saitoh S, Tokuyama K. Effects of physical training on cortical bone at midtibia assessed by peripheral QCT. J Appl Physiol 2003; 95: 219-24

120 Liu YX, Wikland KA, Karlberg J. New reference for the age at childhood onset of growth and secular trend in the timing of puberty in Swedish. Acta Paediatr 2000; 89: 637-43

121 Lohman T, Going S, Pamenter R, Hall M, Boyden T, Houtkooper L, Ritenbaugh C, Bare L, Hill A, Aickin M. Effects of resistance training on regional and total bone mineral density in premenopausal women: a randomized prospective study. J Bone Miner Res 1995; 10: 1015-24

122 MacKelvie KJ, Khan KM, McKay HA. Is there a critical period for bone response to weight-bearing exercise in children and adolescents? a systematic review. Br J Sports Med 2002; 36: 250-7; discussion 257

123 MacKelvie KJ, Khan KM, Petit MA, Janssen PA, McKay HA. A school-based exercise intervention elicits substantial bone health benefits: a 2-year randomized controlled trial in girls. Pediatrics 2003; 112: e447

124 MacKelvie KJ, Petit MA, Khan KM, Beck TJ, McKay HA. Bone mass and structure are enhanced following a 2-year randomized controlled trial of exercise in prepubertal boys. Bone 2004; 34: 755-64

125 MacLaughlin J, Holick MF. Aging decreases the capacity of human skin to produce vitamin D3. J Clin Invest 1985; 76: 1536-8

126 Marshall D, Johnell O, Wedel H. Meta-analysis of how well measures of bone mineral density predict occurrence of osteoporotic fractures. Bmj 1996; 312: 1254-9

127 Matkovic V, Jelic T, Wardlaw GM, Ilich JZ, Goel PK, Wright JK, Andon MB, Smith KT, Heaney RP. Timing of peak bone mass in Caucasian females and its implication for the prevention of osteoporosis. Inference from a cross-sectional model. J Clin Invest 1994; 93: 799-808

128 Mazess R, Collick B, Trempe J, Barden H, Hanson J. Performance evaluation of a dual-energy x-ray bone densitometer. Calcif Tissue Int 1989; 44: 228-32

129 McKay HA, Petit MA, Schutz RW, Prior JC, Barr SI, Khan KM. Augmented trochanteric bone mineral density after modified physical education classes: a randomized school-based exercise intervention study in prepubescent and early pubescent children. J Pediatr 2000; 136: 156-62

130 Melton LJ, 3rd, Crowson CS, O'Fallon WM. Fracture incidence in Olmsted County, Minnesota: comparison of urban with rural rates and changes in urban rates over time. Osteoporos Int 1999; 9: 29-37

131 Michel BA, Lane NE, Bjorkengren A, Bloch DA, Fries JF. Impact of running on lumbar bone density: a 5-year longitudinal study. J Rheumatol 1992; 19: 1759-63

132 Molgaard C, Thomsen BL, Prentice A, Cole TJ, Michaelsen KF. Whole body bone mineral content in healthy children and adolescents. Arch Dis Child 1997; 76: 9-15

133 Nelson DA NS, and Gilsanz V. Childhood and Adolescence. Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism. The American Society for Bone and Mineral Research, Washington, D.C, 2006: 55-63

134 Nelson ME, Fisher EC, Dilmanian FA, Dallal GE, Evans WJ. A 1-y walking program and increased dietary calcium in postmenopausal women: effects on bone. Am J Clin Nutr 1991; 53: 1304-11
135 Nilas L, Podenphant J, Riis BJ, Gotfredsen A, Christiansen C. Usefulness of regional bone measurements in patients with osteoporotic fractures of the spine and distal forearm. J Nucl Med 1987; 28: 960-5
136 Nilsson B. Medical uses of Ca47. Second panel report, IAEA Technical report series, Vienna. 1964
137 Nilsson BE. Posttraumatic osteopenia. A quantitative study of bone mineral mass in the femur following fracture of the tibia in man using Americium-241 as photon source. Acta Orthop Scand 1966; 37: 1-55
138 Nordstrom A, Karlsson C, Nyquist F, Olsson T, Nordstrom P, Karlsson M. Bone loss and fracture risk after reduced physical activity. J Bone Miner Res 2005; 20: 202-7
139 Nordstrom A, Olsson T, Nordstrom P. Bone gained from physical activity and lost through detraining: a longitudinal study in young males. Osteoporos Int 2005; 16: 835-41
140 Nymark T, Lauritsen JM, Ovesen O, Rock ND, Jeune B. Decreasing incidence of hip fracture in the Funen County, Denmark. Acta Orthop 2006; 77: 109-13
141 O'Connor JA, Lanyon LE, MacFie H. The influence of strain rate on adaptive bone remodelling. J Biomech 1982; 15: 767-81
142 Orwoll E, Klein, RF. Osteoporosis in men. Epidemiology, pathophysiology and clinical characterization. Osteoporosis 1996; 3rd ed. San Diego: Academic Press: 745-784
143 Pacifici R, Rupich R, Vered I, Fischer KC, Griffin M, Susman N, Avioli LV. Dual energy radiography (DER): a preliminary comparative study. Calcif Tissue Int 1988; 43: 189-91
144 Parfitt AM. The cellular basis of bone remodeling: the quantum concept reexamined in light of recent advances in the cell biology of bone. Calcif Tissue Int 1984; 36 Suppl 1: S37-45
145 Parfitt AM. The two faces of growth: benefits and risks to bone integrity. Osteoporos Int 1994; 4: 382-98
146 Parkkari J, Kannus P, Niemi S, Pasanen M, Jarvinen M, Luthje P, Vuori I. Increasing age-adjusted incidence of hip fractures in Finland: the number and incidence of fractures in 1970-1991 and prediction for the future. Calcif Tissue Int 1994; 55: 342-5
147 Petit MA, McKay HA, MacKelvie KJ, Heinonen A, Khan KM, Beck TJ. A randomized school-based jumping intervention confers site and maturity-specific benefits on bone structural properties in girls: a hip structure analysis study. J Bone Miner Res 2002;17:363-72.
148 Prince R, Devine A, Dick I, Criddle A, Kerr D, Kent N, Price R, Randell A. The effects of calcium supplementation (milk powder or tablets) and exercise on bone density in postmenopausal women. J Bone Miner Res 1995; 10: 1068-75
149 Pruitt LA, Taaffe DR, Marcus R. Effects of a one-year high-intensity versus low-intensity resistance training program on bone mineral density in older women. J Bone Miner Res 1995; 10: 1788-95
150 Raab-Cullen DM, Akhter MP, Kimmel DB, Recker RR. Bone response to alternate-day mechanical loading of the rat tibia. J Bone Miner Res 1994; 9: 203-11
151 Riggs BL, Wahner HW, Melton LJ, 3rd, Richelson LS, Judd HL, Offord KP. Rates of bone loss in the appendicular and axial skeletons of women. Evidence of substantial vertebral bone loss before menopause. J Clin Invest 1986; 77: 1487-91
152 Robling AG, Hinant FM, Burr DB, Turner CH. Shorter, more frequent mechanical loading sessions enhance bone mass. Med Sci Sports Exerc 2002; 34: 196-202
153 Rockwell JC, Sorensen AM, Baker S, Leahey D, Stock JL, Michaels J, Baran DT. Weight training decreases vertebral bone density in premenopausal women: a prospective study. J Clin Endocrinol Metab 1990; 71: 988-93
154 Rogmark C, Sernbo I, Johnell O, Nilsson JA. Incidence of hip fractures in Malmo, Sweden, 1992-1995. A trend-break. Acta Orthop Scand 1999; 70: 19-22
155 Ross PD, Genant HK, Davis JW, Miller PD, Wasnich RD. Predicting vertebral fracture incidence from prevalent fractures and bone density among non-black, osteoporotic women. Osteoporos Int 1993; 3: 120-6

156 Rubin CT, Lanyon LE. Regulation of bone formation by applied dynamic loads. J Bone Joint Surg Am 1984; 66: 397-402

157 Rubin CT, Lanyon LE. Regulation of bone mass by mechanical strain magnitude. Calcif Tissue Int 1985; 37: 411-7

158 SBU95. The Swedish Council on Technology Assessment in Health Care: Measurement of bone density. SBU report nr 127, Stockholm. 1995

159 Schapira D, Schapira C. Osteoporosis: the evolution of a scientific term. Osteoporos Int 1992; 2: 164-7

160 Schoenau E, Saggese G, Peter F, Baroncelli GI, Shaw NJ, Crabtree NJ, Zadik Z, Neu CM, Noordam C, Radetti G, Hochberg Z. From bone biology to bone analysis. Horm Res 2004; 61: 257-69

161 Seeman E. An exercise in geometry. Journal of Bone and Mineral Research 2002; 17: 373-380

162 Seeman E. From density to structure: growing up and growing old on the surfaces of bone. J Bone Miner Res 1997; 12: 509-21

163 Seeman E. Reduced bone density in women with fractures: contribution of low peak bone density and rapid bone loss. Osteoporos Int 1994; 4 Suppl 1: 15-25

164 Seeman E, Duan Y, Fong C, Edmonds J. Fracture site-specific deficits in bone size and volumetric density in men with spine or hip fractures. J Bone Miner Res 2001; 16: 120-7

165 Seeman E, Hopper JL, Bach LA, Cooper ME, Parkinson E, McKay J, Jerums G. Reduced bone mass in daughters of women with osteoporosis. N Engl J Med 1989; 320: 554-8

166 Shea B, Wells G, Cranney A, Zytaruk N, Robinson V, Griffith L, Ortiz Z, Peterson J, Adachi J, Tugwell P, Guyatt G. Meta-analyses of therapies for postmenopausal osteoporosis. VII. Meta-analysis of calcium supplementation for the prevention of postmenopausal osteoporosis. Endocr Rev 2002; 23: 552-9

167 Skerry TM, Bitensky L, Chayen J, Lanyon LE. Early strain-related changes in enzyme activity in osteocytes following bone loading in vivo. J Bone Miner Res 1989; 4: 783-8

168 Slemenda CW, Christian JC, Williams CJ, Norton JA, Johnston CC, Jr. Genetic determinants of bone mass in adult women: a reevaluation of the twin model and the potential importance of gene interaction on heritability estimates. J Bone Miner Res 1991; 6: 561-7

169 Slemenda CW, Miller JZ, Hui SL, Reister TK, Johnston CC, Jr. Role of physical activity in the development of skeletal mass in children. J Bone Miner Res 1991; 6: 1227-33

170 Smith EL, Gilligan C. Physical activity effects on bone metabolism. Calcif Tissue Int 1991; 49 Suppl: S50-4

171 Snow-Harter C, Bouxsein ML, Lewis BT, Carter DR, Marcus R. Effects of resistance and endurance exercise on bone mineral status of young women: a randomized exercise intervention trial. J Bone Miner Res 1992; 7: 761-9

172 Strandell A, Bergendahl L, Kallings L. Sweden on the move - the school years. Report, Swedish National Institute of Public Health 2001.

173 Sundberg M. Skeletal growth and effects of physical activity during adolescence. Thesis, University of Lund 2001

174 Sundberg M, Gardsell P, Johnell O, Karlsson MK, Ornstein E, Sandstedt B, Sernbo I. Peripubertal moderate exercise increases bone mass in boys but not in girls: a population-based intervention study. Osteoporos Int 2001; 12: 230-8

175 Sundberg M, Gardsell P, Johnell O, Karlsson MK, Ornstein E, Sandstedt B, Sernbo I. Physical activity increases bone size in prepubertal boys and bone mass in prepubertal girls: a combined cross-sectional and 3-year longitudinal study. Calcif Tissue Int 2002; 71: 406-15

176 Swedish National Institute of Public Health. Report, 2006

177 Tanner JM, Whitehouse RH, Hughes PC, Carter BS. Relative importance of growth hormone and sex steroids for the growth at puberty of trunk length, limb length, and muscle width in growth hormone-deficient children. J Pediatr 1976; 89: 1000-8

178 Theintz G, Buchs B, Rizzoli R, Slosman D, Clavien H, Sizonenko PC, Bonjour JP. Longitudinal monitoring of bone mass accumulation in healthy adolescents: evidence for a marked reduction after 16 years of age at the levels of lumbar spine and femoral neck in female subjects. J Clin Endocrinol Metab 1992; 75: 1060-5

179 Trotter M, Hixon BB. Sequential changes in weight, density, and percentage ash weight of human skeletons from an early fetal period through old age. Anat Rec 1974; 179: 1-18

180 Turner CH. Biomechanics of bone: Determinants of skeletal fragility and bone quality. Osteoporosis International 2002; 13: 97-104

181 Turner CH. Homeostatic control of bone structure: an application of feedback theory. Bone 1991; 12: 203-17

182 Turner CH, Pavalko FM. Mechanotransduction and functional response of the skeleton to physical stress: the mechanisms and mechanics of bone adaptation. J Orthop Sci 1998; 3: 346-55

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

184 Turner CH, Robling AG. Exercises for improving bone strength. Br J Sports Med 2005; 39: 188-9

185 Vainionpaa A, Korpelainen R, Leppaluoto J, Jamsa T. Effects of high-impact exercise on bone mineral density: a randomized controlled trial in premenopausal women. Osteoporos Int 2005; 16: 191-7

186 Valdimarsson O. Physical activity and the female skeleton. Thesis 2005

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

188 van Rijn RR, van der Sluis IM, Link TM, Grampp S, Guglielmi G, Imhof H, Gluer C, Adams JE, van Kuijk C. Bone densitometry in children: a critical appraisal. Eur Radiol 2003; 13: 700-10

189 van Rijn RR, van Kuijk C. Bone densitometry in children. Semin Musculoskelet Radiol 2002; 6: 233-40

190 Wastney ME, Martin BR, Peacock M, Smith D, Jiang XY, Jackman LA, Weaver CM. Changes in calcium kinetics in adolescent girls induced by high calcium intake. J Clin Endocrinol Metab 2000; 85: 4470-5

191 Welch JM, Weaver CM, Turner CH. Adaptations to free-fall impact are different in the shafts and bone ends of rat forelimbs. J Appl Physiol 2004; 97: 1859-65

192 WHO. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis. WHO Technical Support Series 843, Geneva 1994

193 Winters KM, Snow CM. Detraining reverses positive effects of exercise on the musculoskeletal system in premenopausal women. J Bone Miner Res 2000; 15: 2495-503

194 Wolff J. Das Gesetz der Transformation der Knochen. A Hirschwald, Berlin. 1892

195 Vuori I, Heinonen A, Sievanen H, Kannus P, Pasanen M, Oja P. Effects of unilateral strength training and detraining on bone mineral density and content in young women: a study of mechanical loading and deloading on human bones. Calcif Tissue Int 1994; 55: 59-67

196 Yoshikawa T, Turner CH, Peacock M, Slemenda CW, Weaver CM, Teegarden D, Markwardt P, Burr DB. Geometric structure of the femoral neck measured using dual-energy x-ray absorptiometry. J Bone Miner Res 1994; 9: 1053-64

197 Zebase RM WF, Juliano-Burns S, Evans A, Seemasn E. The femoral neck is ellipsoid: the assumption of circularity or parallelepedal shape introduces errors in volumetric bone mineral density. J Bone Miner Res 2004; 19: 366