# LUND UNIVERSITY

**Effect of Physical Activity on Bone, Muscle and Fracture Risk during Growth**

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# Effects of Physical Activity on Bone, Muscle and Fracture Risk during Growth

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# Abstract

Osteoporosis and related fractures are a global health problem. Physical activity, especially during growth, has been suggested as a strategy to improve musculoskeletal health in the population. Previous prospective pediatric exercise intervention studies, however, are short-term, mostly covering less than one year and use bone traits as surrogate endpoints for fractures. The Pediatric Osteoporosis Prevention (POP) study is a prospective, controlled exercise intervention study, which was designed to annually assess musculoskeletal development and fracture risk in response to increased physical education in school in children aged 7-9 years and onwards, at a population-based level. The data presented in this thesis cover the results for the first two to four years from the POP study.

The intervention included 40 minutes/day of school physical education in one school whereas children from three schools in the same area served as controls achieving the Swedish standard of average 60 minutes of physical education per week. All children with school start between the years 1999 and 2008, assigned to the four participating schools in the POP study, were included in the fracture registration. In a subsample of children with school start during two years, annual measurements of musculoskeletal traits were done. Also, as part of this project, potential skeletal benefits achieved by consistently walking or cycling to school compared to if going by bus or car for two years were investigated.

Fractures were prospectively registered in a cohort of 2625 children for up to two years and in 2395 children for up to four years. Muscle strength was evaluated by isokinetic Peak Torque (PT) of the knee extensors and flexors at 60 and 180 °/second by a computerized dynamometer and neuromuscular performance by Vertical Jump Height (VJH) in 129 children in the intervention and 103 children in the control group, for two years. Bone mineral content (BMC; g) and bone width (cm) were followed by means of dual X-ray absorptiometry (DXA) for four years, in the measured subsample of 121 children in the intervention and 100 children in the control group.

The fracture risk was not higher in children receiving increased physical education in school compared to children in the control group either at the two-, three- or four-year evaluations. The rate ratio (RR) (95% CI) for fractures was 1.11 (0.78, 1.57) at the four year evaluation. The annual gain in knee extensor PT at 180°/second was significantly higher for both genders in the intervention compared to

the control group. Boys in the intervention group also had a greater annual gain in knee flexion PT at 180 º/second and girls a greater gain in VJH. The mean annual gain in lumbar spine BMC and femoral neck width was higher in both genders in the intervention compared to the control group at the four year follow-up. The mode of school transportation for two years did not influence either the annual gain in BMC or bone size in these pre-pubertal children.

This thesis concludes that increased physical education in school for two years enhanced muscle strength and increased physical activity for four years increased bone mass and size in these at study start seven- to nine-year-old children without affecting the fracture risk.

# Abbreviations

| | |
|---|---|
| ANCOVA | Analysis of covariance |
| BMC | Bone mineral content, (g) |
| BMD | Bone mineral density, (g/cm²) |
| BMI | Body mass index, (kg/m²) |
| CI | Confidence Interval |
| CSA | Cross-sectional area, (cm²) |
| CSMI | Cross-sectional moment of inertia, $(\text{cm}^4)$ |
| CV | Coefficient of variation |
| DPA | Dual-photon absorptiometry |
| DXA | Dual-energy X-ray absorptiometry |
| Ex | Extension |
| Fl | Flexion |
| FN | Femoral neck |
| HSA | Hip structural analysis |
| LS | Lumbar spine |
| MVPA | Moderate to vigorous physical activity |
| PA | Physical activity |
| PBM | Peak bone mass |
| POP | Paediatric Osteoporosis Prevention (study) |
| pQCT | Peripheral quantitative computed tomography |
| QUS | Quantitative ultrasound |
| RCT | Randomized controlled trial |
| RR | Rate ratio |

| | |
|---|---|
| SD | Standard deviation |
| SPA | Single-photon absorptiometry |
| TB | Total body |
| VJH | Vertical jump height, cm |
| VPA | Vigorous physical activity |
| WHO | World Health Organization |
| Z | Section modulus, ($cm^3$) |

# Original Papers

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

I.      **An increase in school-based physical education increases muscle strength in children**
        Löfgren B, Daly RM, Nilsson J-Å, Dencker M, Karlsson M.

        Medicine & Science in Sports & Exercise 2012: published online ahead of print November 27, 2012.


II.     **Influence of a 3-Year Exercise Intervention Program on Fracture Risk, Bone Mass, and Bone Size in Prepubertal Children**
        Löfgren B, Detter F, Dencker M, Stenevi-Lundgren S, Nilsson J-Å, Karlsson M.

        Journal of Bone and Mineral Research 2011; 26(8): 1740–7


III.    **A 4-Year Exercise Intervention in Children Increases Bone Mass without Increasing Fracture Risk**
        Löfgren B, Dencker M, Nilsson J-Å, Karlsson M.

        Pediatrics 2012; 129(6):e1468-76


IV.     **The mode of school transportation in pre-pubertal children does not influence the accrual of bone mineral or the gain in bone size - two year prospective data from the paediatric osteoporosis preventive (POP) study.**
        Löfgren B, Stenevi-Lundgren S, Dencker M, Karlsson M.

        BMC Musculoskeletal Disorders 2010; 11:25.

# Glossary

| | |
|---|---|
| Accuracy | in this context means how well a measured value corresponds to the true value |
| Concentric contraction | a contraction during shortening of the muscle |
| Eccentric contraction | a contraction during lengthening of the muscle |
| Exercise | physical activity that is planned, structured with repetitive bodily movement performed to improve or maintain one or more components of physical fitness |
| Muscle endurance | the ability to exert muscle force over time |
| Muscle strength | the amount of force that can be produced by a muscle in a single contraction. |
| Isokinetic | movement at a constant angular velocity around the axis of rotation |
| Isometric contraction | a contraction during which the muscle length remains unchanged |
| Isotonic | loading involves movement of a constant external load |
| Peak torque | maximum force applied around a pivot point |
| Physical activity | any bodily movement produced by the contraction of skeletal muscles that result in energy expenditure |
| Precision | the extent to which repeated measurements under the same conditions give the same results |
| Reliability | refers to the consistency of measurements |
| Validity | the extent to which an instrument or method actually measures what it is intended to |

# Introduction

Bone tissue

Bone is a highly dynamic, vascularized and innervated tissue that undergoes constant remodeling and adaptation to serve the needs of the body.

Hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$, a crystalline form of calcium phosphate, hardens the skeleton and constitutes the building blocks of bone that together with the triple helix protein collagen type 1 makes up the main part of bone. Only 1-2% of the calcium in our body is circulating in the blood; most of the rest is bound in the skeleton.

The main three types of bone cells -osteoblasts, osteoclasts and osteocytes -work in units called basic multicellular units (BMU). The BMU concept was first described in the 1960s by an orthopedic surgeon, Dr. Harold Frost (78). The osteoclasts are multinucleated large cells that are derived from progenitors of the monocyte/machrophage family of hematopoietic stem cells, cells that resorb the bone structure. The osteoblasts are cells derived from mesenchymal stem cells that first appear as bone lining cells, cells that produce new bone (126). Osteocytes are very long-lived differentiated osteoblasts which lie embedded in bone matrix, a cell type that makes up approximately 90 % of all bone cells in adults (124). Osteocytes are irreversibly differentiated osteoblasts within lacunae embedded in the bone matrix which are connected to each other and also to osteoblasts and bone-lining cells on the bone surface. It is believed that osteocytes are mechano-sensors capable of transducing mechanical stimuli in to a biological response and play a key role in the complex autocrine and paracrine mechanisms in bone remodeling (126).

Bone matrix and osteocytes are organized in onion-shaped rings called osteons (fig.1.) or Haversian canals, in which the canals contain blood vessels, lymphatic tissue, and sometimes nerves in the center of the osteon. Lacunae are the small cavities in which the osteocytes lie, connected to each other by small tunnels called canaliculi (57,126).
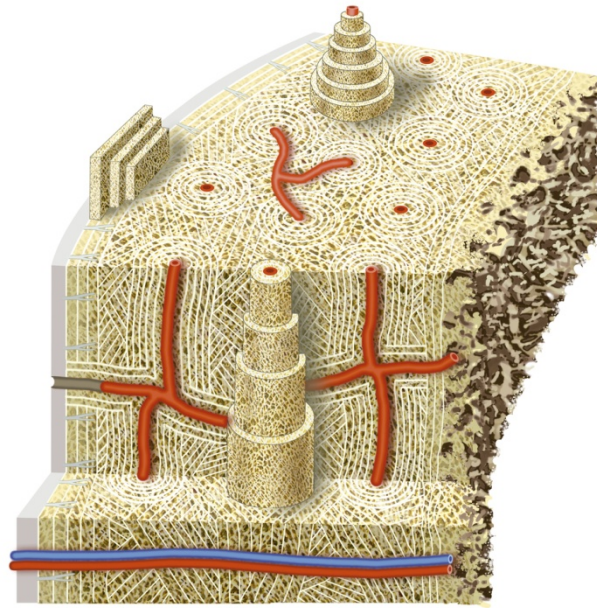
**Fig. 1. Bone tissue organized in osteons**

The bone tissue in a human body serves the following main purposes: giving support and protection for the inner organs; storing minerals, especially calcium; allowing body movement; and being the site where blood cells are produced. The skeletal system has an ingenious three dimensional design, like numerous other examples in nature resulting from evolution, unmatched by anything man-made. Our bones are optimized to allow body movement. Bone tissue has several opposing properties; it is light yet strong and stiff but also flexible (210). Different bones have different functions and hence greatly varying properties and resistance to fracture, as seen both in humans and in other animals (52). Bone in a young adult can be harder than granite in terms of withstanding a compression or tension force (208,211).

Bone modeling is the formation of bone by osteoblasts, that is, changing the size of the skeleton without the prior removal of bone by osteoclasts. This process primarily takes place during growth, but can also be seen in adults in response to mechanical loading or during fracture repair. Remodeling, which is continuous throughout life, is when bone is rebuilt into a new shape by osteoblasts after removal by osteoclasts (131,210). Approximately 10 % of the skeleton in adults is replaced every year (241).

Bone tissue can be classified as either cortical (compact) bone (75-85%) or trabecular (cancellous) bone (15-25%), which consists of the same cell types but with different organization and macroscopic morphology. The outer surface of a bone is called the periosteal and the inner surface the endosteal surface. Trabecular bone is found primarily in the metaphysis regions of long bones and in the vertebrae. Bone turnover is much higher in trabecular bone, which is more metabolically active than cortical bone mainly due to the higher surface versus volume ratio (3,179). Cortical bone is dense and strong and makes up the protective shell (cortex) of bones. Trabecular bone is a complex, much less dense network of plate- or rod-like shapes minimizing the weight yet maximizing the strength of the bone (167).

Hormones and calcium

Parathyroid hormone (PTH) is the main regulator of serum calcium, but vitamin D and to some extent calcitonin also regulate blood calcium levels. PTH increases blood calcium levels by increasing the bone resorption by increasing osteoclastic activity and reabsorption of calcium in the kidneys so that less calcium is lost in the urine. Calcitonin has the opposite effect. PTH also activates vitamin D in the kidneys, which increases calcium absorption from the intestines (10). PTH may also increase calcium uptake in the intestines via a direct mechanism (168). When calcium levels in blood is elevated PTH induced bone remodeling is instantly reduced (240). Most cells and tissue types in our bodies have a vitamin D receptor. Vitamin D is essential for calcium uptake in the intestines and calcium resorption from bone (100). Vitamin D deficiency causes rickets in children and osteomalacia in adults (softening of the bone due to calcium depletion) (100). When our skin is exposed to sunlight (ultraviolet B radiation), 7-dehydrocholesterol is converted to vitamin D. Also, vitamin D can also be ingested. Calcium and vitamin D3 (1, 25 dihydroxy cholecalciferol) both reduce the fracture risk in people over age 50 independently of each other (224).

Besides the effects on bone, vitamin D seems to have a direct effect on skeletal muscle and neuromuscular function and reduces the risk of falling at least in elderly people, especially when given in higher doses ( >700IU/day) or to people with vitamin D deficiency (8,22-24,185).

Daily dose recommendations of calcium are 800 mg for adult men and women, 900 mg for children and pregnant women and 1200 mg for breastfeeding women (SBU 2003). Current national and international recommendations regarding vitamin D intake state at least 400 IU/day for children and adolescents (19,74,236). The recommended daily dose of vitamin D during pregnancy and lactation is 600 IU/day according to the Institute of Medicine in the US, assuming

minimal sun exposure (159). Vitamin D deficiency is very rare in Sweden, but children with dark skin may need supplementation also after the age of two due to low sun exposure (19,74). A serum level of less than 50 nmol/l of vitamin D (25-hydroxyvitamin D) is considered by most experts to be defined as vitamin D deficiency (100). In order to provide reduced fracture risk in women with high fracture risk the daily dose of vitamin D should be 700-800 IU and up to 1000-1200 mg of daily calcium (193).

Growth and puberty

Before puberty the gains in bone mineral content are mainly regulated by growth hormone (GH) and Insulin-like Growth Factor-1 by stimulating osteoblast differentiation and proliferation (59). Bone apposition and endocortical remodeling appears to be linear and with small gender differences until puberty (209). During puberty GH and IGF-1 levels increase dramatically due to increased levels of sex steroids (156). Sex steroids, GH and IGF-1 all regulate growth and all have an anabolic effect on bone and muscle tissue (156). The male sex steroid testosterone acts centrally by increasing GH secretion, while the female sex steroid estrogen increases GH indirectly by reduction of feedback inhibition by IGF-1. Testosterone also has an anabolic effect by increasing protein synthesis (160).

Peak velocity of growth occur earlier (mean 1.5 years) in girls (age 11.8) than in boys. Peak height velocity occurs at approximately age 13 (13.4) in boys and peak bone mineral accrual about one year later in both genders (12,143). Approximately a quarter of an individual's bone mineral is achieved during two years around puberty which can be as much as is later lost during 50-60 years after peak bone mass (11,12). In girls peak bone mineral accrual occurs at the age of 11-14 and in boys approximately at the age of 13-17 (28).

During puberty the differences between the two genders in bone mass acquisition become apparent, mainly due to boys having a longer period of bone maturation which results in larger bone size and cortical thickness (28). In contrast to the effects of exercise on bone and muscle development during growth, there is no nutritional dose-response relationship. However malnutrition has a profound effect on bone and muscle (14). Insufficient energy and protein intake can result in massive bone loss, especially when severe and combined with menstrual dysfunction in girls (14,183,213). Whether dietary calcium level (if not very low) has any effect on bone mineralization during childhood and adolescence is debated (129). However, very low levels of calcium consumption (less than 400mg/day) may be harmful for bone mineralization during growth (129).

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## Bone mass measurements

Bone mass is a general and unspecific (unscientific) term, it is actually bone mineral (content or density) that is measured. The methods for estimating the mineralization of the bone use radiation, either ionizing or non-ionizing. Examples of the latter are Magnetic Resonance Imaging (MRI) and ultrasound. The techniques using ionizing radiation can be either gamma radiation (using isotopes) or X-rays (1), and the amount of ionizing radiation that is absorbed by the bone gives a measure of the amount of mineral (mainly calcium) in the bone.

Bone mass is estimated either as bone mineral content (BMC), which is the amount of mineral (g) measured within a scanned skeletal region or as bone mineral density (BMD) which is the measured mineral partially adjusted for bone size through a defined scanned area (g/cm²). The later measurement is also sometimes referred to as areal BMD. In adults and in clinical practice BMD is the preferred and most frequently used variable. In children and adolescents who constantly increase in size, however, BMC and bone size should be reported separately (21,92).

The first attempts to measure bone mass in vivo were made in 1901 (Price), using radio nucleotides, but only major bone loss could be detected by this technique (173).

## Single-photon absorptiometry (SPA)

Single-photon absorptiometry (SPA) was invented in the early 1960s by Cameron et al (33), soon followed by Nilsson et al (108). This revolutionized research on bone since it made it possible to estimate, non-invasively and in vivo, the amount of mineral (calcium) in the bone. A disadvantage is that only appendicular parts (usually the forearm) can be measured since the method is based on a constant thickness of the soft tissues surrounding the bone, which can be obtained by a cuff filled with water which attenuates the radiation approximately to the same extent as the soft tissue around the bone. The radiation source is usually Iodine-125 or Americium-241. A detector measures the radiation that is going through the bone in relation to the radiation going through the soft tissue (and water), then calculating the relation between the bone- and soft tissue gives an estimate of the bone mineral content. The accuracy is about 9% (170), and the precision 1-2% (1). The SPA technique is still in use and has been shown to give reliable estimates of fracture risk at a population level (50,153).

## Dual-photon absorptiometry (DPA)

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

## Dual-energy X-ray absorptiometry (DXA)

Dual-energy X-ray absorptiometry (DXA) has been available since 1987 and has the advantage compared to previous techniques that the investigated skeletal region does not have to be submerged in or surrounded by water; any body part can be measured, and also total body. Instead of gamma radiation the technique uses X-rays. The effective radiation dose received by the patient during a DXA measurement is low (1-8μSv) which corresponds to 1/1000 of the yearly background radiation dose (1). Accuracy is about 10% (measuring a vertebra). The precision of the technique is about 0.5-2% (1).
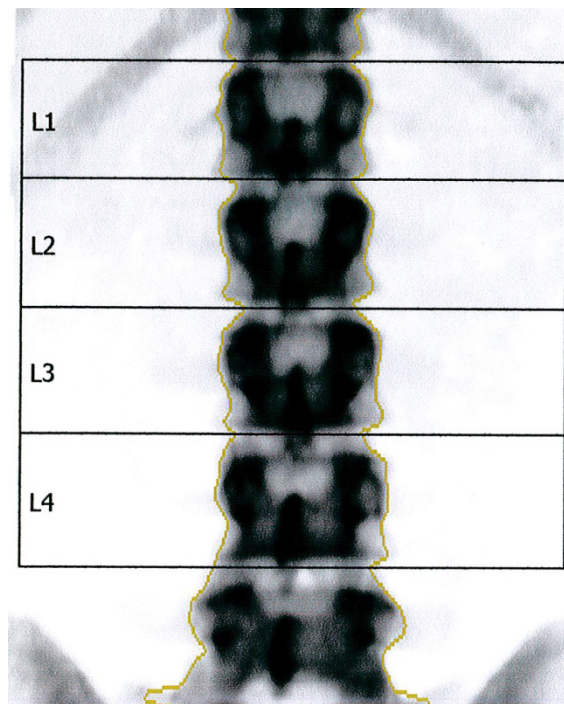


**Fig. 2. A DXA (Lunar DPX-L) scan of the lumbar spine of the author at the beginning of his research career**

**Fig. 3. The author, a skeleton model and a part of a DXA apparatus at the Deptartment of Medicine, Western Hospital, Melbourne**

Quantitative ultrasound (QUS)

Quantitative ultrasound has been proposed to give reliable estimates of skeletal traits. It uses the speed of sound (SOS) to reflect the architecture and elasticity of bone, and broad band attenuation (BUA) to reflect the density of the bone, hence no ionizing radiation is received by the patient using QUS. Although QUS is thought to reflect the elasticity and micro-architecture of the bone, the specific aspects of bone quality that is measured by the technique are not yet known (88). Precision is about 0.3-1.5% and the predictive ability for fractures using QUS is similar to DXA (84,90,125). A disadvantage is that it cannot discriminate between cortical and trabecular bone and bones covered by a thicker layer of soft tissue cannot be examined by this technique, hence only peripheral measurements can be done with QUS. The most commonly used measurement site is the calcaneus bone (84,88,125,216).



**Fig. 4. A Quantitative ultrasound apparatus**

Peripheral quantitative computed tomography (pQCT) and magnetic
resonance imaging (MRI)

Peripheral quantitative computed tomography (pQCT) and magnetic resonance
imaging (MRI) are both techniques with the advantage that they can give a picture
of the true three dimensional properties of bone and the soft tissues surrounding it.
However, both pQCT and MRI are expensive and often inaccessible. pQCT can be
used in the appendicular skeleton and the radiation dose is quite low, less than 10
µSv since radiosensitive organs are distant from the exposed area (56). (QCT can
be used to measure the axial skeleton but the radiation dose is  relatively high,
about 250µSv for a lumbar spine measurement and even better protocols aiming to
reduce effective radiation are needed for the use of this technique (1,56)) MRI has
the advantage that it is a non- ionizing radiation technique. During the last 5-10
years using pQCT for research purposes and in the last few years also MRI has
become increasingly popular, although reference data and the method of choice in
clinical practice examining bone is still DXA.
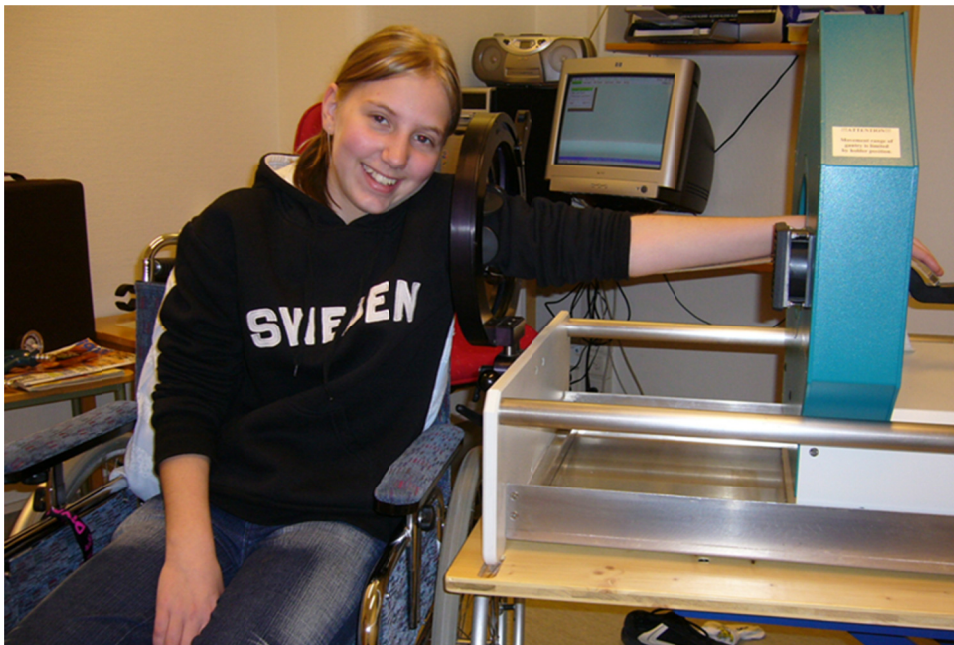


**Fig. 5. A pQCT apparatus** *Photo presented by courtesy of Christian Linden*

Biochemical analysis of bone markers

A variety of different biochemical markers estimating bone turnover in blood or urine are available, but large measurement errors have been associated with analysis of such markers due to technical and biological factors (138,164). Therefore they have not routinely been used either in clinical practice or in research on osteoporosis. However a further enhancement in the precision of bone turnover markers may increase their diagnostic value (36). Bone turnover markers can be classified as markers of either bone formation or resorption. Bone formation markers are derived from osteoblast activity during bone matrix synthesis and can be enzymes released into the blood during bone matrix synthesis (alkaline phosphatase), matrix protein (osteocalcin) or posttranslational processing products of type I collagen (36,48). Examples of bone resorption markers can be products of type I collagen breakdown or less commonly used serum tartrate-resistant acid phosphatase (36,48). Recently it was concluded by the International Osteoporosis Foundation (IOF) that bone turnover markers can be useful in clinical practice when estimating fracture risk and monitoring osteoporosis but international reference standards are needed, currently one bone formation marker (s-PINP) and one marker of bone resorption (s-CTX) are recommended for use in clinical studies (239).

Osteoporosis – An expensive and increasing problem

The World Health Organization (WHO) definition of Osteoporosis from 1994 is "a systemic skeletal disorder characterized by low bone mass and micro-architectural deterioration of bone tissue", resulting in bone fragility and greatly increased fracture risk, which is the major clinical manifestation of the disease (244). The estimated number of hip fractures in 1990 was 1.7 million worldwide and by 2050 this number is estimated to increase to over 6 million mainly due to increasing life expectancy, especially in the developing countries (82). Initially, osteoporosis was considered to be a natural part of the ageing process and a condition primarily affecting women. Today we know that the disease has many other causes apart from estrogen depletion after menopause and that it is neither age- nor gender-specific. The following modifiable main risk factors for the development of osteoporosis have been identified; physical inactivity, low body mass, treatment with cortisone, consumption of alcohol and smoking, low sun exposure and vitamin D deficiency and inadequate calcium consumption (206).

Primary osteoporosis is when the cause of osteoporosis is not the result of a specific disease or disorder but due to ageing, menopause and lifestyle factors while secondary osteoporosis is osteoporosis due to a specified disease or medication (206).

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Risk factors that are not possible to influence are: female gender, genetic/hereditary factors (twin studies have suggested that 60-80 % of bone mass is genetically determined), height, ethnicity, age and age at onset of menopause.

Two specific terms are often used when discussing osteoporosis. T-score is the number of standard deviation (SD) below or above the mean value of young healthy individuals (age 20-40) of the same gender and population while Z-score is the number of SD below or above the age specific mean value of individuals within the same population and gender. The WHO definition and staging of osteoporosis (below) only truly applies to Caucasian women and is only based on BMD T-score measured with DXA. Children and adolescents, men, non-Caucasian women and also very old white women are not covered by the definition which hence has limited value (1).

- Normal bone mineral content - BMD T-score above -1 SD from mean young adult value in the same population

- Reduced bone mass (Osteopenia) - BMD T-score -1 to -2.5 SD from mean young adult value in the same population

- Osteoporosis - BMD T-score < -2.5 SD from mean young adult value in the same population

- Manifest Osteoporosis  - Osteoporosis in addition with at least one fracture related to osteoporosis

In Sweden, where we have among the highest incidences in the world of low-energy-related fractures in the old population, the lifetime risk of having a fracture related to osteoporosis in 50-year-old women is about 50% and for men 25% (114). The most common fracture sites are hip, lumbar spine, distal forearm and proximal humerus. However it is important to remember that it is not osteoporosis per se but the fractures related to osteoporosis that cause pain and impaired mobility for the individual. There are many other risk factors for fracture besides low bone mass and the other risk factors for osteoporosis listed above. These are: susceptibility to falls, low muscle strength and poor neuromuscular function, impaired vision, treatment with long-acting benzodiazepines and other psychotropic drugs (51,206,244).

The "mechanostat" theory- exercise and bone

Twenty-five years ago, Dr. Harold Frost, proposed the idea that bone adapts to the mechanical stress it is exposed to, much as a thermostat regulates temperature, hence the name mechanostat (79). Ever since Professor Bo Nilsson, former head of staff at the Department of Orthopedics in Malmö, invented one of the first devices for evaluating bone mass in the 1960ies (SPA) (108) and published the first paper examining the effects of exercise on bone mass in athletes in 1971 (174), research in this field has blossomed.

Peak bone mass

Peak bone mass (PBM) is the highest bone mass a person reaches during his or her lifetime, which usually occurs in our early twenties. Theoretically, a 10% increase in peak BMD is predicted to delay development of osteoporosis by 13 years (97). Peak BMD has been suggested to be the single most important factor in the development of osteoporosis (93,97). Most of the variance in peak bone density is thought to be due to genetic factors (60-80 %) (71,182,186), but dietary calcium and physical activity also contribute to a large proportion of the variance (122,214). Up to half of the variance in bone mass at about the age of 70 years is, according to estimations, predicted by PBM (106).

Besides bone mass, bone size and geometry independently contributes to bone strength (65,66), which is also illustrated by the fact that individuals with femoral neck (FN) fractures have a smaller FN (but normal vertebral size) compared to controls, and women with spine fractures have smaller vertebrae (but normal FN size) than controls without fracture at these sites (65,212). The reduction in bone mass after menopause in women is partially counteracted by an increased periosteal apposition which then partially preserves bone strength (2).

Do exercise-induced benefits during growth remain into adulthood?

The important question is obviously whether the exercise-induced skeletal benefits achieved during childhood remain into adulthood (13). A recent study of over 1000 young adult men using pQCT indicated that positive effects of physical activity during growth on bone geometry remain for several years when sport activities have been reduced or even discontinued (175). The same authors recently reported the five year results from this study, indicating that physical activity can increase peak bone mass in young adult men (176). Also Baxter-Jones et al. presented evidence that skeletal benefits due to PA during adolescence remain into young adult-hood (16), and lifetime sports participation and leisure

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time PA have been shown to improve bone quality, size and strength in older men (54). Perhaps the most important aim of this type of exercise intervention is to lay the foundation for a life-long physically active lifestyle already in youth, especially since longitudinal studies indicate that levels of physical activity in childhood seem to correlate with later physical activity levels in adulthood (223,225,226). This suggests that it is possible to improve various health-related aspects in the population through a school-based exercise program. Longitudinal exercise studies of former athletes indicate that physical activity during youth and young adulthood protects against fracture in old age (123,232,233).

Effects of exercise on bone are age and maturity-dependent; the late pre- and early pubertal years (Tanner stage 2 and 3) seem to be a "window of opportunity" to influence bone through external stimuli (5,96,127,136,145,147,166,184). RCTs and non-randomized controlled exercise interventions and the effects on bone in children and adolescents are briefly summarized in table 1. The effect on BMC and bone size seen in these exercise interventions are reported in several different ways, most commonly the effects of intervention are reported as % changes per year or during intervention but also as SD change or sometimes only if changes were significantly higher in intervention than controls. This makes a direct comparison of the effectiveness of different interventions difficult, but most interventions report a 1-10% higher gain in BMC per year or during study, in the exercise group compared to control group.

The time-span with good possibilities to enhance bone strength through exercise seems to be longer in boys than in girls (68). The optimal timing also varies for different sites (28,29,141,155). The kind of exercise is most important. The load should be fast, dynamic, high in magnitude, with unusual or abnormal strains and intermittent to produce the most pronounced skeletal response, which was first shown in animal models (130,132,201,202,231). This is reflected in humans by the fact that high-impact sports like weight-lifting, tennis, hockey and soccer give large effects on BMC (15,95,98,115,119,120). Small effects have been observed for long-distance running (162,172,174), while endurance sports without weight bearing, such as swimming or cycling seem to give no or little effect on bone tissue (154,171,172,174,190). It is well documented that the effects of exercise on bone are regional and site specific (116-118,148), which is demonstrated by an elegant model of unilateral loading in racquet-sport players (115,116), where a remarkable increase in BMC was seen in the dominant arm compared to the unloaded non-dominant arm of the players. In this model the influence of exercise can be studied without risk of confounding genetic, endocrine and nutritional factors affecting bone, since both arms belong to the same person. Also the effects of exercise on bone are pronounced before but not after puberty (15,115). In adults exercise effects on bone are small or moderate compared to the exercise induced benefits seen in children and adolescents. Later studies using pQCT and MRI,

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have also confirmed the results on bone size and true volume (67,68,204,205). Daly et al. used MRI and the unilateral loading model, examining the link between muscle size and bone parameters, testing the hypothesis that the increase in bone parameters during growth and in response to exercise is primarily mediated through muscle tissue, since muscles cause the largest loads and strains on bone (207). However, the muscle area could only explain 12 to 16% of the variance in bone mass, size and bending strength in this study (55).

**Table 1. Effects on bone in prospective controlled (randomized and non-randomized) exercise interventions in children during pubertal maturation**

| Reference | Age and Number of Participants | Type of Exercise/PA | Study Duration | Effects on Bone Increase higher in Cases vs. Controls |
|---|---|---|---|---|
| **Tanner stage 1 (Pre-pubertal)** | | | | |
| Bradney et al. (1998)(31) | 38 boys 10.4±0.2 years | Weight bearing 30 min three times a week | 8 months | BMD: TB, LS, legs CT: legs |
| MacKay et al. (2000) (158) | 144 children 6.9-10.2 years | High-moderate impact 10-30 min three times a week | 8 months | BMD: Tr |
| Fuchs et al. (2001) (80) | 99 children 7.6±0.2 years | High impact jumping | 7 months | BMC : FN, LS BMD: LS BW: FN |
| Petit et al. (2002, part I) (184) | 68 girls 10.0±0.6 years | High impact 10-12 min three times a week | 7 months | No effects |
| Van Langendonck et al. (2003) (238) | 42 twin girls 8.7±0.7 years | High impact three times a week | 9 months | BMC: PF, FN |
| Specker et al. (2003) (215) | 178 girls 3.9±0.6 years | High impact 30 min five times a week ±calcium | 12 months | BMC: legs |

| | | | | |
|---|---|---|---|---|
| MacKelvie et al. (2004) (147) | 64 boys 10.2±0.2 years | High impact 10-12 min three times a week | 20 months | BMC: FN HSA: Z |
| Laing et al. (2005) (127) | 143 girls 6.0±1.5 years | Gymnastics 60 min once a week | 24 months | BMC: TB, LS, PF BMD: TB, PF BA: TB, PF |
| Valdimarsson et al. (2006) (237) | 103 girls 7.7±0.6 years | PE classes 40 min five times a week | 12 months | BMC: LS, Tr |
| Linden et al. (2006) (136) | 99 girls 7.6±0.6 years | PE classes 40 min five times a week | 24 months | BMC: LS, legs BMD: TB, LS, legs BW: LS |
| Linden et al. (2007) (137) | 138 boys 7.8±0.6 years | PE classes 40 min five times a week | 12 months | BMC: LS BMD: LS BW: LS |
| Alwis et al. (2008) (5) | 137 boys 7.8±0.6 years | PE classes 40 min five times a week | 24 months | BMC: LS BW: LS |
| Alwis et al. (2008) (6) | 99 girls 7.6±0.6 years | PE classes 40 min five times a week | 24 months | No Effects HSA |
| Hasselstrom et al. (2008) (91) | 379 children 6.8±0.4 years | Extra PE classes 45 min two times a week | 36 months | Girls BMC: Distal Forearm BA: Distal Forearm Boys: No Effects |
| Greene et al. (2009) (87) | 42 girls 6-10 years | 50 high-moderate impact jumps three times a week | 7 months | No Effects HSA |
| Meyer et al. (2011, part 1) (161) | 158 children 8.7±2.1 years | Extra PE classes 45 min including 10 min jumping two times a week | 9 months | BMC: TB, LS, FN |

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**Tanner stage 2-3
(Early Pubertal)**

| | | | | |
|---|---|---|---|---|
| Morris et al. (1997) (166) | 71 girls 9.5±0.9 years | Moderate impact 30 min three times a week | 10 months | BMC:TB, LS, FN, PF BMD: TB, LS, FN |
| Heinonen et al. (2000, part 1) (96) | 58 girls 11.0±0.9 years | High impact 20 min two times a week | 9 months | BMC: LS, FN |
| MacKelvie et al. (2001, part 2) (146) | 107 girls 10.5±0.6 years | High impact 10-12 min three times a week | 7 months | BMC: LS BMD: LS, FN |
| Petit et al. (2002 part 2) (184) | 106 girls 10.5±0.6 years | High impact 10-12 min three times a week | 7 months | BMD: Tr, FN HSA: Z CT: FN |
| Iuliano-Burns et al. (2003) (109) | 64 girls 8.8±0.1 years | Moderate impact 20 min three times a week ±calcium | 9 months | BMC: LS, lower leg |
| MacKelvie et al. (2003) (145) | 75 girls 9.9±0.6 years | High impact 10-12 min three times a week | 20 months | BMC: FN, LS |
| McKay et al. (2005) (157) | 122 children 10.1±0.5 years | Jumping 3 x 3 min five times a week | 8 months | BMC: PF, Tr BA: PF No Effects HAS |
| Courteix et al. (2005) (47) | 113 girls 8-13 years | Exercise mean 7.2 hrs a week vs 1.2 hrs a week | 12 months | BMD: TB, LS, FN |
| Macdonald et al. (2008) (144) | 197 girls 213 boys 10.2±0.6 years | High impact 15 min five times a week | 11 months | Boys BMC: LS, TB Girls BMC: FN HSA: Z |

| | | | | |
|---|---|---|---|---|
| Lofgren et al. (2011) (140) | 92 girls 131 boys 7.8±0.6 years | PE classes 40 min five times a week | 36 months | Boys BMC: LS BW: LS HSA: No effects Girls BMC: LS, FN BW: LS HSA: CSA |
| Meyer et al. (part 2) (2011) (161) | 133 children 11.1±0.6 years | Extra PE classes 45 min including 10 min jumping two times a week | 9 months | BMC: TB, LS, FN |
| Lofgren et al. (2012) (139) | 96 girls 125 boys 7.8±0.6 years | PE classes 40 min five times a week | 48 months | Boys BMC: LS BW: FN No Effects HSA Girls BMC: TB,LS, FN, Tr BW: FN, LS HSA: CSA, Z, CSMI |

**Tanner stage 4-5 (Pubertal)**

| | | | | |
|---|---|---|---|---|
| Blimkie et al. (1996) (27) | 36 girls 16.3±0.3 years | Weight training three times a week | 7 months | No Effects |
| Witzke et al. (2000) (247) | 53 girls 14.6±0.5 years | Resistance exercise 30-45 min three times a week | 9 months | No Effects |
| Heinonen et al. (part 2) (2000) (96) | 68 girls 13.3±0.9 years | High impact 20 min two times a week | 9 months | No Effects |
| Nichols et al. (2001) (169) | 67 girls 15.9±0.1 years | Resistance training 30-45 min three times a week | 15 months | BMD: FN |

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| Stear et al. (2003) (217) | 144 girls 17.3±0.3 years | Moderate impact 45 min three times a week ± calcium | 16 months | BMC: TB, LS, PF, Tr |
| Weeks et al. (2008) (243) | 44 girls 37 boys 13.8±0.4 years | High impact jumping 10 min two times a week | 8 months | Girls BMC: LS, FN Boys BMC: TB, LS, Tr QUS: BUA calcaneus |

Significant increase in intervention compared to controls seen in the parameters/ sites: Bone Area (BA), Bone Width (BW), Broadband ultrasound attenuation (BUA), Cross sectional area (CSA), Cross sectional moment of inertia (CSMI) Distal Forearm, Femoral Neck (FN), Hip structure analysis (HSA), Lumbar Spine (LS) Proximal Femur (PF), Total body (TB), Trochanter (Tr), Section modulus (Z).
Table in part adapted from the thesis; Physical Activity, Bone mass and Bone Structure in pre-pubertal Children (2009) by Gayani Alwis, with the kind permission of the author.

## Bone strength

Bone strength can be defined as "the force required to produce a mechanical failure under a specific loading condition" (17). The resistance of bone to such a mechanical failure, i.e. fractures, depends on both the material and the structural properties of bone (17). Loading of the long bones is normally axial or bending compression. Compression is a shortening of the bone as an axial force acts upon it. Tension gives a lengthening of a bone, hence when a bending force acts on bone it will be compressed on one side and tension acts on the other side.

Hip structure analysis is an attempt to estimate structural bone strength through the two-dimensional DXA hip scan (248). The following parameters describing hip structure are calculated assuming the bone to be cylindrical. Cross-sectional area (CSA) is an estimation of a bone's capacity to withstand a compressive force (i.e. from an axial load). The automatic identification of the weakest cross-section level of FN is done by the HSA software. Cross-sectional moment of Inertia (CSMI) is calculated by $(\pi/4)\ (R_o^4 - R_i^4)$, where $R_o$ is the outer radius and $R_i$ is the inner radius and gives an estimate of the structure's resistance to a bending force and is independent of the material properties. Since strength is proportional to the fourth power of the radius, a small increase in periosteal width produces a large increase in strength. Section modulus (Z) also describes the resistance to a bending force of a tubular structure (i.e. bone). Z=CSMI divided by the outer radius. The correlation between DXA measured CSMI and direct measure on cadavers has

been shown to be very high ($r^2$=0.96) (248), but in reality the correlation is probably less since limb positioning and a sometimes moving subject may influence the measurement, especially when measuring children. There are some limitations to the technique since it calculates the parameters from a two-dimensional DXA image (17).
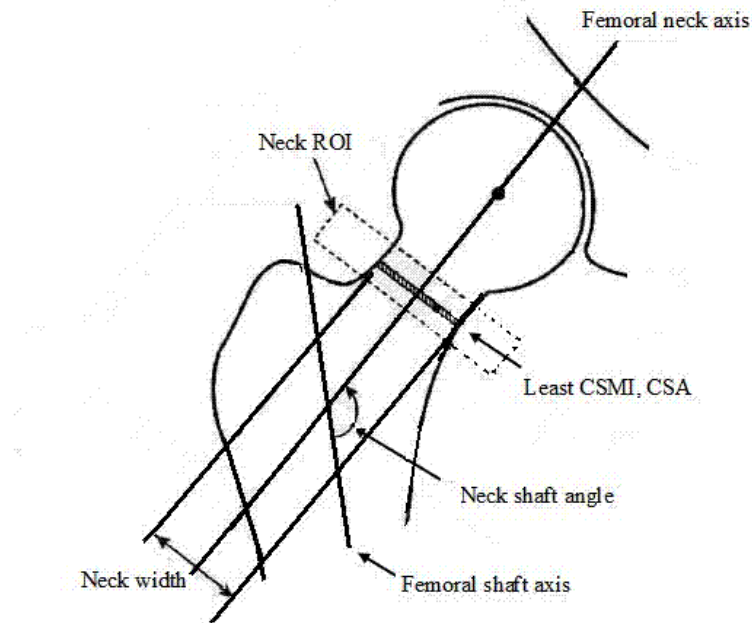


**Fig. 6. Hip structure analysis (HSA)** *Figure presented by courtesy of Gayani Alwis*

Skeletal muscle

A muscle fiber, the cell of muscular tissue, can contain up to thousands of thin strands called myofibrils, which are built up by two overlapping protein filaments, actin and myosin. Each muscle fiber is innervated by a single motor neuron which can innervate thousands of muscle fibers. During normal daily activities such as movement and lifting, three types of contraction occur in muscle: concentric, eccentric and isometric. All three types of contraction usually occur at the same

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time in different muscle groups during a motion (199). The number of muscle fibers remains the same from or soon after birth, but the muscle fiber size increases 5-10-fold during growth, probably depending on the function or intensity of work load to which the muscle is exposed to (188).

## Muscle strength

Muscle strength reflects the tension that is created when actin slide past myosin filaments within the muscle fibrils. It is defined as "the amount of force that can be produced by a muscle in a single contraction" (199). Muscle endurance is the ability to generate muscle force over time (199).

Muscle strength seems to increase in a linear fashion before puberty in both genders without associated muscle hypertrophy (220). In adolescent boys resistance training and gains in muscle strength are associated with muscular hypertrophy (220). In children muscle strength is associated with age, height and or body stature, weight, gender, and sexual maturity (4,60,110,199). A resent meta-analysis evaluating effects of resistance training on muscle strength in children and adolescents concluded that the possibility to gain muscle strength seems to increase with maturation and age (but with no clear boost of this gain during puberty) (20). However, several other studies have reported a marked increase in the effect of training on muscle mass in males compared to females during late puberty, most probably due to androgen effects on protein synthesis (99,188,198).

## Neuromuscular function and physical performance

Vertical jump height (VJH) has been widely used to assess neuromuscular function in both children and adults (53,151,152,218,219,242). The maximum ability to jump straight upwards is measured, which is a complex movement involving ankle, knee and hip joints of both legs. In a vertical jump both muscle power and muscle coordination are needed, both having a strong neural component (188). Muscle power (which is not the same as muscle strength and to which it is only moderately related) is considered to be an important factor in the ability to perform rapid movements at maximum effort (such as jumping) (151). Several different protocols for measuring VJH are used, which makes it difficult to compare results between different studies. The test can also be performed with or without arm swing, which in children has been shown to give up to 23% higher test results (101). Some studies suggest that since neuromuscular function is not

fully developed before puberty, the test might not be suitable for children under age 13 (30,188), but the test has, as mentioned, been widely used in previous pediatric studies including previous reports from this cohort (9,101,107,177,218,219).

## Fracture risk during growth

Some reports infer that vigorous physical activity increases the risk of fractures in children, also suggesting vigorous physical activity to be an independent risk factor for fracture (40). Low bone mass is obviously another risk factor for fracture also in children (39,42). Further, low muscle strength seems to increase the fracture risk in children, especially low forearm strength (41). Adiposity has been suggested as another risk factor for fracture in children (58,86). Fracture risk in children peaks during early puberty, possibly because rates of bone turnover then are high, with a large gain in bone size but bone mineral accrual lags behind gains in height and weight (13,85,115). Socioeconomic factors do not seem to influence fracture risk in children (187) and nor do the educational achievements of the children's mothers (37,38). The most common fracture sites in children and adolescents are the wrist and distal forearm, followed by hand (128). Interestingly, one report infers a possible dose-response relationship between the risks of forearm or wrist fracture and time spent watching television, video or video games in children and adolescents aged 9-16 years (142). In general, age increase fracture risk in children and boys are more prone to fracture than girls (128).

## Fracture risk in elderly people

Falling is one of the most important risk factors for fracture in elderly people where bone mineral density is a less useful predictor of fracture on an individual level (111). Physical activity, especially strength and balance training, can help prevent falls in older people (194). BMD or BMC is however a reliable tool when estimating fracture risk on a group or population level, explain 60-70% of the fracture risk, especially combined with other risk factors (51). Besides falls and low bone mass, known risk factors for fracture in elderly are: impaired vision, medication with long-acting benzodiazepines and other psychotropic drugs, low muscle strength, poor neuromuscular function and poor balance. A recent large meta-analysis on interventions for the prevention of falls in elderly revealed that exercise programs containing strength and balance training and also Tai-Chi training effectively reduce falls and fractures in older people (83).

Physical activity

Humans, like all other animals, are built to hunt or to be hunted i.e. nature and evolution has adapted our species over millions of years to survive in an environment where our survival depended on our ability to move. Not surprisingly lack of PA has been associated with a huge variety of chronic diseases affecting; the cardiac, vascular, respiratory, metabolic, and musculoskeletal systems and has been associated with an increased risk of all causes of early death (7,103-105,133-135,234). In modern everyday life in the western world, we live increasingly sedentary lives. Technical advances, especially during the last century with new means of transportation, large food supplies, computers, etc. makes our immediate needs to be physically active decrease.

Physical activity is hard to estimate and measure with accuracy. The most commonly used method is self-report by various questionnaires, which has the obvious downside that they can only give a subjective measure of PA levels but the advantage of being cheap and easy to administer. The objective measurement techniques include doubly labeled water (DLW) which gives reliable estimates of energy expenditure over time but is very expensive and gives no information about intensity, duration or frequency of the activity(200,227). Using heart rate monitors (HRM) gives the potential confounders of emotional stress, body size and temperature, age and fitness of the measured individual. Pedometers, which cannot give information about the duration or intensity but only the volume of activity (amount of steps taken by the measured individual) (200,227).

During the last three decades the proportion of physical education in school has decreased from 20% to currently 7.5% in Sweden (72,178). In Europe only three countries out of 25 offered at least 2 hrs/week of physical education in primary and secondary school during 2002-2003(72,178). There is compelling evidence that PA behaviors during childhood, at least moderately reflect later PA patterns during adulthood (223,225,226).

This thesis is about how physical activity influences musculoskeletal parameters and fracture risk during growth, studying children during their first four years in school, primarily through an increased physical education program in school, but also evaluating potential effects of walking or cycling to school.

# Aims

The general aims of this project were to prospectively study the effects of physical activity on bone, muscle and fracture risk in school children, aged 7-9 years at baseline.

The specific aims of this thesis were as follows:

- To evaluate the effects of increased physical education in school for two years on muscle strength and neuromuscular function in pre-pubertal children
- To evaluate the effects of increased physical education in school on bone mass, bone size, hip structure and fracture risk for up to four years in the pre-pubertal and early pubertal period
- To investigate whether the mode of school transportation for two years influences bone mass and bone size in pre-pubertal children

# Material and Methods

The Paediatric Osteoporosis Intervention (POP) Study

The Paediatric Osteoporosis Intervention (POP) study was launched in 1999. Four government funded schools in the same socioeconomic area in Malmö in southern Sweden were invited to participate in the study. The inspiration to start the project derived partially from a previous study with increased physical education in school in geographically nearby Sösdala, where Dr. Martin Sundberg found positive effects on bone mass in peri-pubertal boys but no significant effects on bone in girls. The children participating in the Sösdala project were aged 12-16 and received increased physical education from 100 min/week to 160 min /week (221). Sundberg speculated in his thesis whether the intervention would have been more effective if the children had been at a younger age (pre-pubertal) at study start (221).

The POP study was initiated at the Department of Orthopedics in Malmö, although several other departments and clinics have been involved in the project as well. The Department of Clinical Physiology and Nuclear Medicine has investigated the effect of the intervention on aerobic fitness and body fat (62-64). The Faculty of Education and Society at Malmö University has studied the effects of the intervention on motor skills, attention and school performance (73). The Department of Pediatric Psychiatry examined effects of physical activity on attention deficit hyperactivity disorder (ADHD) (89).

The POP study is a population-based, prospective, controlled exercise intervention study, which was designed to annually assess musculoskeletal development in response to a school-based physical activity program in children aged 7-9 years onwards.  At baseline, the school curriculum in children assigned to the physical activity intervention changed from the Swedish standard of 60 min of physical education per week to 40 minutes per day; the controls continued with an average of 1 to 2 lessons (average 60 min) of physical education per week. Lessons were led by the teachers and included general activities within the standard school curriculum such as ball games, running, jumping and climbing activities. As school physical education is compulsory, all children had to participate. During the vacation periods, no additional exercise training was provided. The study was conducted according to the Helsinki Declaration of 2000 and was approved by the

Ethics Committee of Lund University (LU 453-98; 1998-09-15). Informed written consent was obtained from parents or guardians of all participating children before study start.



**Fig. 7. Participants in the POP study during physical education** *Photo presented by courtesy of Christian Linden*

Measurements

All children in the in the intervention school (the "Ängslätt" school) and the three control schools (the "Fridhem", "Mellanhed" and "Ribersborg" schools) starting in first and second grade at study start in 1999 were invited for annual musculoskeletal and anthropometric measurements, this subgroup with measurements done will be referred to as the "subgroup" or the "sub-cohort" .

Study subjects measurements

**Paper I**: A total of 55 of the 61 girls and 84 of the 89 boys in the intervention schools who were invited to participate in the study agreed (attendance rate: 93%). One girl was excluded as she was 11 months younger than all other girls and two boys were excluded for medical reasons. During the follow-up, two boys and five girls moved or declined further participation. In the control cohort, 64 of the 158 girls and 68 of the 169 boys who were invited to the measurements agreed to participate (attendance rate: 40%). One girl and one boy were excluded for medical reasons. During the follow-up, 13 girls and 10 boys moved or declined further participation. An additional four boys had to be excluded due to technical errors in the follow-up measurements. Therefore, Paper I include 49 girls and 80 boys in the intervention group and 50 girls and 53 boys in the control group with prospective measurements at the two-year follow-up. In **Paper II**, evaluating the three-year follow-up in this cohort, one additional girl and four boys had moved out of the region or declined further participation in the intervention group. This left 48 girls and 76 boys in the intervention group. At the three-year follow-up in the control group, an additional two girls and two boys had moved out of the region or declined further participation, and four girls missed their measurements, leaving 44 girls and 55 boys for inclusion in the control group.

**Paper III**: During the four-year follow-up period, out of the 55 girls and 84 boys included in the intervention group at baseline six girls and eight boys moved out of the region or declined further participation. One girl was excluded as she was almost one year younger than all other girls. Two boys were excluded due to medication known to influence bone metabolism, and one boy was excluded due to technical errors in the measurement, leaving 48 girls and 73 boys to be included in the intervention group. Out of the 64 girls and 68 boys who accepted participation at baseline in the control schools, 15 girls and 13 boys had moved out of the region or declined further participation at follow-up, one girl and two boys were excluded due to medication known to influence bone metabolism, and one boy adopted from Colombia was excluded as being the only non-Caucasian, leaving 48 girls and 52 boys in the control group.

**Paper IV**: All the measured children participating in the POP study were pooled and divided in two groups depending on their self-selected mode of school transportation. Out of the 133 boys included at the two-year follow-up (described under paper I) 57 boys consistently walked or cycled to school, 24 went to school by car or bus and 5 boys did not answer questions about school transportation and 47 boys showed no consistent pattern in their mode of school transportation. Out of the 99 girls included at the two-year follow-up (described under paper I), 48 girls consistently walked or cycled to school while 17 girls went by car or bus, 28

girls altered between active and passive transportation to school and 6 girls did not answer the questions about school transport.

**Paper I**

**Intervention**

**Girls**

Baseline
- 55 accepted
- 1 excluded young age

Dropouts
- 5 declined or moved

Follow-up
- 49 girls included 2-years

**Controls**

Baseline
- 64 accepted

Dropouts
- 13 declined or moved
- 1 excluded medical reason

Follow-up
- 50 girls included 2-years

**Boys**

**Intervention**

Baseline
- 84 accepted

Dropouts
- 2 declined or moved
- 2 excluded medical reason

Follow-up
- 80 boys included 2-years

**Controls**

Baseline
- 68 accepted

Dropouts
- 10 declined or moved
- 4 excluded tech. errors
- 1 excluded medical reason

Follow-up
- 53 boys included 2-years

**Paper II**

**Intervention**

**Girls**

Baseline
- 55 accepted
- 1 excluded young age

Dropouts
- 6 declined or moved

Follow-up
- 48 girls included 3-years

**Controls**

Baseline
- 64 accepted

Dropouts
- 15 declined or moved
- 4 missed measurement
- 1 excluded medical reason

Follow-up
- 44 girls included 3-years

**Boys**

**Intervention**

Baseline
- 84 accepted

Dropouts
- 6 declined or moved
- 2 excluded medical reason

Follow-up
- 76 boys included 3-years

**Controls**

Baseline
- 68 accepted

Dropouts
- 12 declined or moved
- 1 excluded medical reason

Follow-up
- 55 boys included 2-years

**Fig. 8. Flow of study subjects through the study**

## Paper IV

**Girls**

- 99 girls included 2-years POP study out of 119 at baseline **[Subjects]**
- 6 no information about school-transport
- 28 altered or mixed mode of school transport **[excluded]**
- 65 girls included (48 walked or cycled , 17 by bus or car) **[Included]**

**Boys**

- 133 boys included 2-years POP study out of 152 at baseline **[Subjects]**
- 5 no information about school-transport
- 47 altered or mixed mode of school transport **[Excluded]**
- 81 boys included (57 walked or cycled, 24 by bus or car) **[Included]**

## Paper III

**Intervention** **Girls** **Controls**

Intervention:
- 55 accepted
- 1 excluded young age **[Baseline]**
- 6 declined or moved **[Dropouts]**
- 48 girls included 4-years **[Follow-up]**

Controls:
- 64 accepted **[Baseline]**
- 15 declined or moved
- 1 excluded medical reason **[Dropouts]**
- 48 girls included 4-years **[Follow-up]**

**Boys**

Intervention:
- 84 accepted **[Baseline]**
- 8 declined or moved
- 2 excluded medical reason
- 1 excluded tech. errors **[Dropouts]**
- 73 boys included 4-years **[Follow-up]**

Controls:
- 68 accepted **[Baseline]**
- 13 declined or moved
- 2 excluded medical reason
- 1 excluded ethnicity **[Dropouts]**
- 52 boys included 4-years **[Follow-up]**

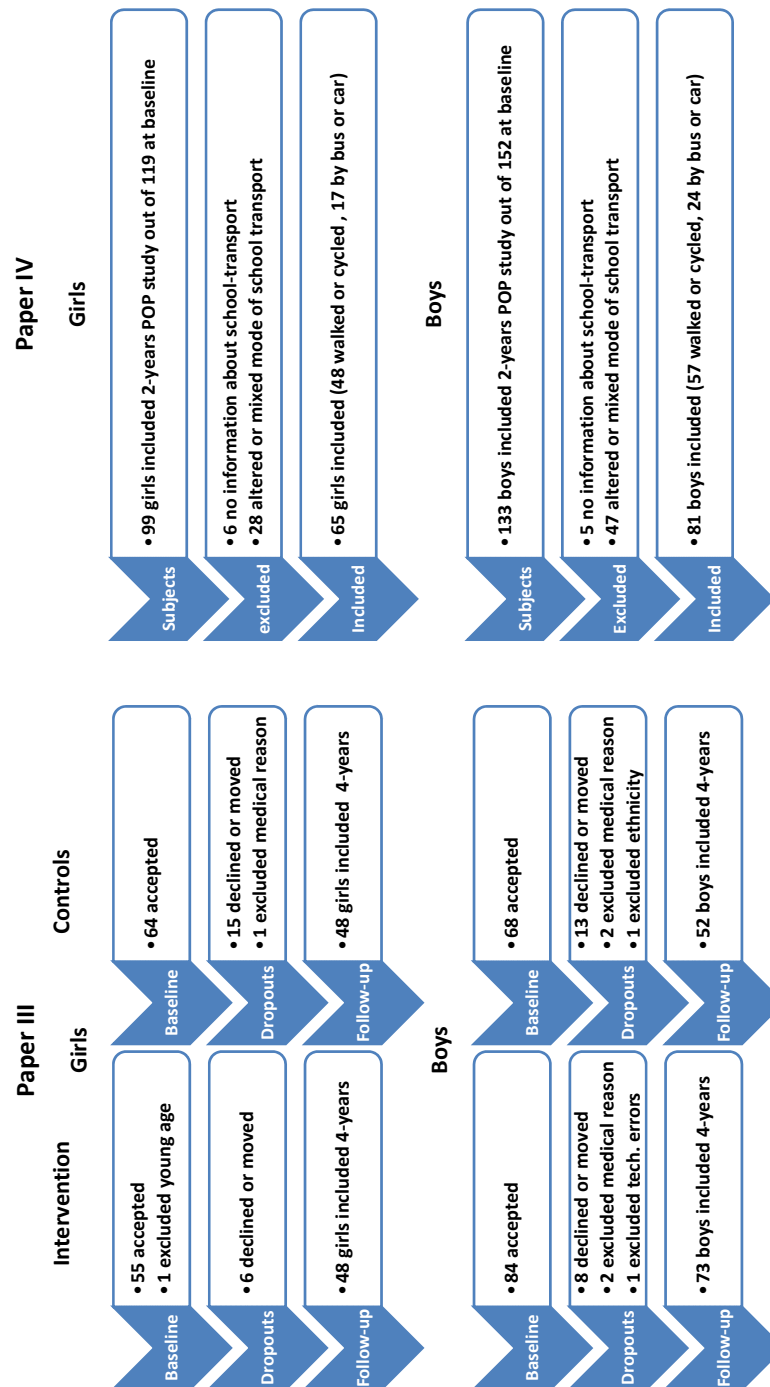**Fig. 8. Flow of study subjects through the study**

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## Drop-out analysis measurements

Drop-out analyses in this sub-cohort revealed that there were no differences in baseline age, height, weight, body mass index (BMI), total body or regional body composition, BMC, muscle strength, or vertical jump height between children that completed the measurements and those that only attended the baseline measurement (data not shown). Furthermore, there were no differences in age, height, weight or BMI when data from the grade one compulsory school health examination were analyzed and compared to the children who participated in the baseline measurements with those who declined (136,137,218,219). This strengthens the view that the data are generalizable.

## Fracture registration

All children with school start between the years 1999 and 2008, assigned to the four participating schools in the POP study, were included in the prospective fracture registration. Since we updated the fracture cohort repeatedly, a total of 2625 children were included in paper I, and 2395 children included in the fracture evaluation in papers II and III. One hundred percent of the studied population was included in the fracture evaluation since the fractures were registered through the hospital archives. Less than 3% of all fractures occurring in the studied population are expected to be treated by private practitioners and might thus be missed in this registration (113). Also, about 14% of the fractures, based on previous studies, are expected to occur and initially be treated at another location (during holidays etc.) (113), but the majority of those fractures will then be registered at follow-up visit in Malmö.

All fractures in the studied population were confirmed and classified by the same senior consultant in orthopedic surgery. We used the Landin classification of trauma levels (low-energy trauma, moderate trauma or high-energy trauma) (128), which has been used in several other pediatric fracture studies (39-42).

## School transportation

Physically active transportation to school (i.e. walking and cycling) has been suggested as an important way to increase the level of physical activity during growth (43-45). The mode of school transportation and its effect on level of PA and potential effects on bone traits in pre-pubertal children was evaluated in paper IV. All the children in the subgroup subjected to annual measurements in the POP study were classified according to their chosen mode of transportation to school, according to the questionnaires (described below). The children were divided in to

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those who consistently walked or cycled to school and those who consistently went to school by bus or car. Children who altered their mode of transportation to school were excluded from this comparison.



**Fig. 9. Physically active transportation to school**

Questionnaires

A questionnaire used in several previous pediatric studies, modified for children, but not validated, were used to collect detailed information about: physical activity habits, sports participation during leisure time, nutrition and dietary habits, diseases, medication, allergies, fractures, ethnicity and socioeconomic background factors (5,69,136,137,219,222,237). Minor adjustments of the questionnaire had been made to serve the needs of the POP study. The subgroup of children subjected to measurements filled out the questionnaires annually together with

their parents or guardians, to whom the questionnaire was sent one week before the participant's measurement.


## Assessment of physical activity


### Accelerometers

In order to get an objective estimation of the levels of physical activity, MTI accelerometers (Manufacturing Technology Incorporated, Fort Walton Beach, FL, USA) model 7164, were used for four consecutive days (three weekdays and one day during the weekend), at the two-year follow-up, a cross-sectional single measurement. The accelerometer is about the size of a box of matches ($1.5 \times 4 \times 5$ cm) and weighs approximately 40 grams. It is worn in an elastic band around the waist at all times during the day except when engaged in activities in water since it is not waterproof. This model is a single-plane (vertical) accelerometer, and was calibrated against a standardized vertical movement to minimize inter-instrumental variation. Previously conducted pediatric accelerometer studies have shown that a 4-5-day recording period gives reliable estimates of PA levels (228). Accelerometer data were averaged over a period called an epoch, representing ten seconds. Established age- and weight-specific cut-off points for accelerometer counts representing varying intensities of PA made it possible to estimate time spent (minutes) above different intensity thresholds (77,230). The cut-off points used were 167-583 counts/epoch (>1000 counts per minute (cpm)) for moderate to vigorous physical activity (MVPA) equaling over 3 metabolic equivalents (METs) and >583 counts/epoch (>3500 cpm) for vigorous physical activity (VPA) equaling over 6 METs, for all participants. This is similar to cut-off points used in other pediatric studies (181,192,229). Physical activity above 5000, 6000 and 10,000 cpm was also included in order to capture the most intense activities (known to be osteogenic). All accelerometer data were analyzed using SAS-based software and consecutive sequences of more than 10 minutes of zero counts were automatically deleted based on the assumption that such sequences were caused by the accelerometer not being worn.


### Physical activity assessed by questionnaires

Duration of PA in school and organized PA during leisure time were registered through the questionnaires. Total duration of physical activity was estimated as duration of school physical education and organized leisure time activity per week, and the mean value during the study period was calculated (hours/week).

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## Anthropometrics and maturation

Height in cm was measured by a wall-tapered height meter (Holtain Stadiometer) and weight in kg to the nearest 0.1 kg by an electric scales (Avery Berkel HL 120). Maturation was assessed as Tanner staging by the research nurse.

## Bone and Muscle Measurements

### DXA

Dual X-ray absorptiometry (DXA) (DPX-L version 1.3z, Lunar ®, Madison, WI) measurements were used to evaluate bone mass, lean mass and fat mass (g) in all papers (I-IV) for this thesis. BMC (g) was measured in total body (TB), lumbar spine (LS), femoral neck (FN) and trochanter of the hip. BMC in lumbar spine was evaluated either as BMC total LS (L1-L5) from the TB scan (paper II and III) or as BMC L2-L4 from the LS scan (paper IV). The width of the third lumbar vertebra and the femoral neck were evaluated at the lumbar spine and hip scans. The equipment was calibrated daily with the Lunar® phantom and our technicians also did all measurements and software analyses. The coefficients of variation, evaluated by duplicate measurements in 13 healthy children aged 7-15 (mean age 10 years), were BMC 1.4–3.8%, bone width 1.5–2.2%, total body fat mass 3.7% and total body lean mass 1.5%.

**Fig. 10. The Lunar® DPX-L (DXA) apparatus** *Figure presented by courtesy of Christian Linden*

Hip structure analysis

The hip structural analysis (HSA) software, provided by Lunar Instruments Corporation (Madison, WI), was applied to the hip scan as an evaluation of femoral neck cross-sectional area (CSA, $cm^2$), section modulus (Z, $cm^3$) and cross-sectional moment of inertia (CSMI, $cm^4$). Values three standard deviations (SD) above or below the mean were excluded as being biologically unlikely in accordance with Beck et al. (17,18). The coefficients of variation, evaluated by duplicate measurements in 13 healthy children, were FN CSA 2.2%, FN Z 6.2%, FN CSMI 6.2%.

Muscle strength

Muscle strength was measured as concentric isokinetic peak torque of extensors (quadriceps) and flexors (hamstrings) of the right knee at two velocities (60 and 180 °/seconds), by a computerized dynamometer, Biodex ® (Biodex System III Pro with Biodex advantage software). The child was seated with 85º flexion of the hips, and secured to the chair using standard Biodex procedure with shin, upper crossing torso, pelvic and thigh stabilization straps. The axis of the knee was

aligned with the Biodex axis of rotation. When required, a 10 cm thick pad was used to fill the space between the participants back and the support of the chair. When the lever arm of the Biodex was longer than the lower leg of the participant a small pad was used to adjust for the difference. All participants were instructed to place their arms across their chest during the testing. All subjects received both visual and verbal encouragement during the test, although according to the literature this has not been seen to influence results in children (112). All muscle strength measurements were done by the same two physiotherapists. During the test the knee was positioned at 90º of flexion and went through a 75º range of motion, stopping at 15º of flexion. Concentric isokinetic knee extension and flexion peak torque (PT) was tested at an angular velocity of 60 and 180º/second. After two sub-maximal repetitions (to familiarize the participants with the testing), five maximal repetitions (flexion and extension) at 60º/seconds were performed. After 30 seconds' rest, 10 maximal repetitions at 180º/second for both flexion and extension were done, with the highest peak torque (Nm) recorded for all measurements. All peak torque (Nm) values, were normalized to body weight (kg) and expressed as Nm/kg. The intra-individual test variability, evaluated as coefficient of variability (CV) for repeated measurements in 21 children, was 6.6% for $PT_{Ex60}$, 12.3% for $PT_{Ex180}$, 12.1% for $PT_{Fl60}$ and 9.1% for $PT_{Fl180}$.



**Fig. 11. The Biodex® System III Pro apparatus** *Figure presented by courtesy of Susanna Stenevie Lundgren*

Neuromuscular function- Vertical Jump Height

Vertical jump height (VJH) was measured to estimate neuromuscular function. Performing a vertical jump requires both muscle power and motor coordination, both considered to have a strong neural component. Three maximal vertical jumps were recorded for each subject using an electronic mat (product name "Time it" Eleiko Sport ®, Halmstad, Sweden). The mat is connected to a timer recording the subjects' time in the air, and thereby calculating the height of the jump in centimeters. For each individual the best jump out of three tries was used. The coefficient of variation was 5.9%, as calculated for repeated measurements in 21 children.



**Fig. 12. VJH performed on the "Time it" Eleiko Sport ® electronic mat**
*Figure presented by courtesy of Susanna Stenevi Lundgren*

## Statistical analysis

The statistical analyses regarding all measurements were performed with Statistica® version 7. For the fracture data evaluations in papers I, II and III the statistical calculations were performed with the Statistical Package for the Social Sciences (SPSS) version 14.0-17.0. Data are presented as means with 95% confidence interval or with standard deviations (SD). Fracture risk with 95% CI was estimated by Poisson distribution. $P<0.05$ was regarded as a statistically significant difference.

Gender-specific baseline group differences were tested by Student's t-test and Fisher's exact test. The annual changes of all parameters in paper I, II and IV were calculated by delta values, and for paper III the annual changes were calculated using linear regression slopes for each individual.

Group differences in annual changes were investigated by means of analyses of covariance (ANCOVA) with adjustment for Tanner stage at follow-up.

# Summary of Papers

Paper I

A Two-Year Exercise Intervention in Pre-Pubertal Children Increases Muscle Strength

Introduction

This two-year prospective controlled intervention in pre-pubertal girls and boys evaluated if a moderately intense exercise program improves the growth-related gains in muscular function and muscle strength without affecting fracture risk.

Subjects and methods

Fractures were registered in 417 girls and 500 boys aged 7–9 years who were exposed to a general school-based exercise program of 40 minutes per school day (200 min/week) for 24 months and in 836 age-matched girls and 872 boys who participated in the general Swedish curriculum comprising a mean 60 min/week of physical activity. In a subsample consisting of 49 girls and 80 boys in the intervention group and 50 girls and 53 boys in the control group, body composition was measured by dual-energy X-ray absorptiometry (DXA), muscle strength by isokinetic Peak Torque (PT) of the knee extensors and flexors at 60 and 180 °/second by a computerized dynamometer and neuromuscular performance by Vertical Jump Height (VJH). When comparing the annual gains in the measured parameters, adjustments were made for age at baseline, change in height and the baseline value for each measured parameter.

Results

The rate ratio (RR) for children in the intervention group to sustain a fracture was 1.07 (0.66, 1.68) [mean (95% CI)] compared to controls. The annual gain in knee extensor PT at 180º/second was significantly higher for both girls (p<0.001) and

boys (p<0.01) in the intervention than in the control group. Boys in the intervention group also had a higher annual gain in knee flexion PT at 180 °/seconds (p<0.001) and girls in VJH (p<0.05).
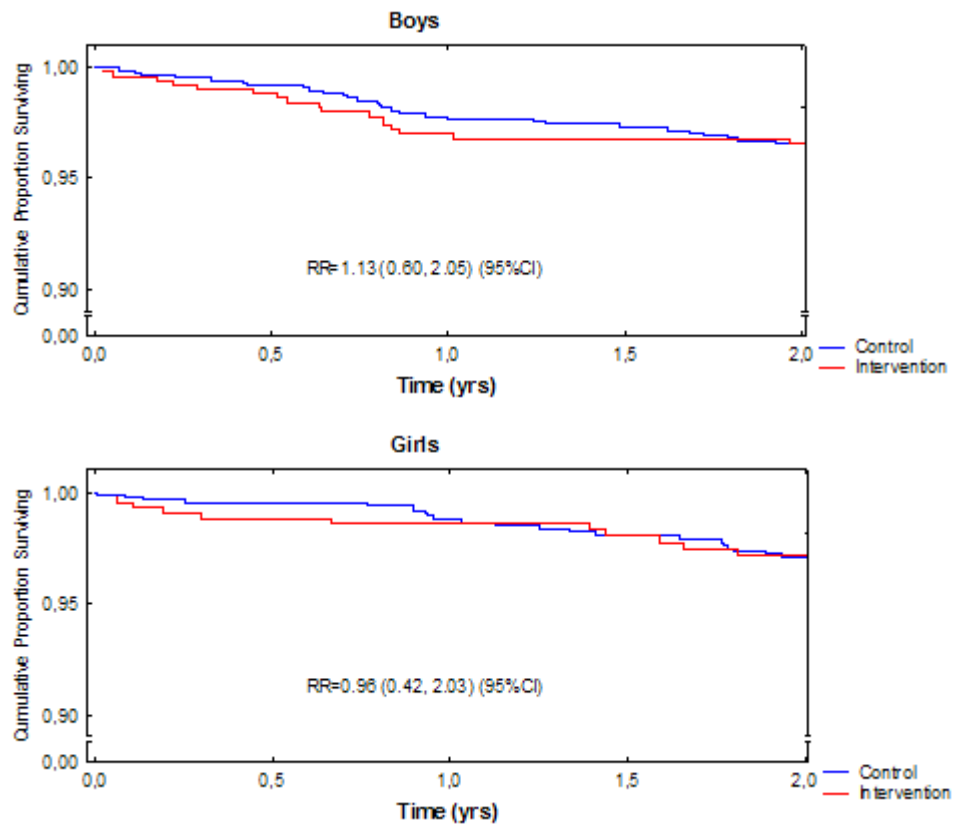


**Fig. 13. Fracture incidens for boys and girls during the study period expressed as cumulative survival functions**

Conclusion

Extending the school-based physical education from 60 to 200 min/week enhanced muscle strength in pre pubertal boys and girls without influencing fracture risk.

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Paper II

Influence of a 3-Year Exercise Intervention Program on Fracture Risk,
Bone Mass, and Bone Size in Pre-pubertal Children

## Introduction

High levels of physical activity during growth, although beneficial for bone mineral accrual and bone size, have been suggested to be associated with an increased exposure to trauma and hence fracture risk. This prospective controlled exercise intervention evaluated the effects of increased physical education in school and followed skeletal traits as well as fracture risk for 3 years.

## Subjects and methods

Fractures were registered prospectively in 2395 children (446 boys and 362 girls in the intervention group and 807 boys and 780 girls serving as controls) aged 7-9 years at baseline for up to 3 years. DXA measurements were performed annually in a subgroup of 76 boys and 48 girls in the intervention and 44 girls and 55 boys in the control group.

## Results

The mean annual gain in the intervention group in lumbar spine BMC was 0.9 SD higher in girls and 0.8 SD higher in boys (both $p<0.001$) and in third lumbar vertebra width 0.4 SD higher in girls and 0.3 SD higher in boys (both $p<0.05$), than in the control group. The rate ratio (RR) for fracture was 1.08 (0.71, 1.62) [mean (95%CI)].

## Conclusion

Increased physical education in school for 36 months increases bone mass and possibly also bone size without affecting fracture risk in 7-9-year-old children.

Paper III

A 4-Year Exercise Intervention in Children Increases Bone Mass without Increasing Fracture Risk

## Introduction

The majority of the previous exercise interventions examining musculoskeletal health in children span less than one year and use bone traits as surrogate endpoints for fracture. This prospective controlled exercise intervention followed not only skeletal development but also fracture incidence for 4 years.

## Subjects and methods

In 446 boys and 362 girls in the intervention and 807 boys and 780 girls in the control group fractures were prospectively registered for up to 4 years from school start. Measurements were performed in a subsample of 73 boys and 48 girls in the intervention and 48 girls and 52 boys in the control group, bone mineral content (g) and bone size (cm) was assessed annually by DXA. The children in the intervention received physical education (PE) in school 200 min per week and the controls followed the standard curriculum of 60-90 min PE per week.

## Results

The rate ratio for fractures was 1.11 (0.78, 1.57) [mean (95% CI)]. The mean annual gain in BMC was 7.0% higher in girls and 3.3% higher in boys in lumbar spine compared to the controls. The annual gain in femoral neck width was 1.7% higher in girls and 0.6% higher in boys in the intervention compared to the control group. In girls in the intervention, significant effects were seen in all the measured bone traits including hip structure parameters compared to girls in the control group (all $p<0.05$). In boys in the intervention significant gains were only seen in the above mentioned lumbar spine BMC and femoral neck width compared to controls.

## Conclusion

Increased physical education in school, for 4 years, increases bone mass and bone size in at study start pre-pubertal children, without affecting the fracture risk.

Paper IV

The mode of school transportation in pre-pubertal children does not influence the accrual of bone mineral or the gain in bone size - two year prospective data from the paediatric osteoporosis preventive (POP) study.

## Introduction

The aim of this 24 months cross-sectional observational study was to evaluate whether the self-selected mode of school transportation in pre-pubertal children influences bone mineral accrual and/or bone size.

## Subjects and methods

Children included in the Paediatric Osteoporosis Prevention study (POP) study were pooled independently of physical education classes in school and divided into two groups depending on mode of school transportation. Thus 57 boys and 48 girls who consistently walked or cycled to school were compared with 24 boys and 17 girls who consistently went by to school by car or bus. BMC (g) and bone width (cm) were evaluated by DXA measurements of total body (TB), lumbar spine (LS) and the hip at baseline and two-year follow-up. Levels of habitual physical activity were assessed by questionnaires and objectively measured by accelerometers. Analysis of Covariance (ANCOVA) was used to adjust for age at baseline, duration of organized PA, annual change in height and BMC or bone width if there were differences in these traits at baseline. All children remained in Tanner stage 1 during the study.

## Results

Children who cycled or walked to school for 24 months did not have a higher annual gain in BMC or bone width compared to children who went to school by bus or car. The same result was found both before and after adjustment for possible confounders. All children independent of mode of transportation to school reached above the recommended level of 60 minutes of moderate to vigorous activity per day.

Conclusion

Physical active transportation to school for 2 years does not influence bone mineral accrual or bone size in pre-pubertal children, possibly because the habitual level of physical activity in these children was high and the average distance to school relatively short.

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# General Discussion

This thesis evaluates the musculoskeletal effects of physical activity in children, hence a definition of physical activity is in place. The most commonly used definition is "any bodily movement produced by the contraction of skeletal muscles that results in energy expenditure" (34), which was coined by Caspersen et al 1985.

It is important to remember that compared to genetic factors and growth, the effects of PA on bone traits are relatively small. About 20-40% of the variance in bone mass can be explained by environmental factors (28). Athletes of both genders have previously been reported to have 10-25% higher BMC compared to non-exercising controls (13,70,118-120,174). Genetic factors have been shown in studies of monozygotic and dizygotic twins to determine 60-80% of bone traits depending on the studied site (71,182,186). Hip and distal forearm seemed to be sites where BMD to a lesser extent were genetically determined than lumbar spine (186).

We do not know whether musculoskeletal gains through PA during growth persist in a longer perspective giving protection against fractures when we are old, even if the evidence supporting this hypothesis is growing (54,175,176,232,233). Actually the opposite hypothesis that bone traits returns to a genetically determined set point in adulthood or old age has been put forward based on observations in an animal model (81). However, the authors of the latter hypothesis also conclude that the issue is open and may depend on the timing, duration and magnitude of exercise during childhood (81). Probably the issue will to some extent continue to be open until the unlikely event of the perfectly designed and conducted exercise intervention spanning from childhood till the death of the participants. It is also likely that musculoskeletal traits are also determined by altered genetic expression, i.e. on a gene expression level rather than just genetic level (which is in my opinion, not completely taken into account in these studies), hence giving us an opportunity to influence musculoskeletal health through PA and other lifestyle factors. Also, in the above-mentioned twin studies by Eisman et al., only adult twin pairs were evaluated, whereas children have a completely different, higher ability to adapt to environmental factors than adults.

In this intervention the increased physical activity that the children in the intervention group received was designed so that practically all children could

participate, hence no specifically designed exercise program known to influence musculoskeletal parameters was used. This design makes it possible to implement the program on a population level at a very low cost; in fact many elementary schools in Sweden and Norway have already started to implement the increased physical education used in the model from the POP study, based on our findings and concept. Also, collaboration with research groups in Denmark, Norway, and Australia has sprung from this project.

Bone measurements

BMC (g) is generally considered to be a more reliable measure than BMD (g/cm²) during growth, since BMD is affected by the body size of the measured individual (191). TB, LS and hip should be used in pediatric studies (21), and some suggest that total body measurements should preferably not include the head since the skull is a non-loaded region, then reported as total body less head (TBLH) (21). In clinical practice the total body, lumbar spine, hip and distal radius region are the most used and relevant sites to measure.

Peripheral quantified computed tomography (pQCT) can give information about the true density and three-dimensional properties of bone, but in most previous studies and most reference data DXA is used, which is still considered the golden standard. DXA also have other advantages: it is relatively accessible and the radiation dose is very low (one DXA measurement approximately equals the radiation dose received during a transatlantic flight from Copenhagen to New York i.e. 1-8 µSv (1), although the effective dose can be slightly higher when measuring children (56)).

An important limitation when estimating bone mineral accrual in children with a two-dimensional technique like DXA is due to the individuals increasing size and the inability of DXA to measure true volume. As recommended by the International Society of Clinical Densitometry (ISCD), we report bone mineral as BMC values rather than BMD and report bone size separately. Further, DXA was designed to measure bone mineral but not size which limits the HSA method because the poor spatial resolution (compared to new techniques such as pQCT and MRI) makes the detection of bone dimensions difficult. Also, a small measurement error results in a large difference in CSMI. Factors such as limb positioning, in particular anteversion of the hip, and inaccuracies during the manual placement of the ROI may give significant measurement errors, which is why the method suggested by Beck et al., excluding biologically unlikely values, was used when analyzing the HSA data (18).

One study implies the level of physical activity to be constant in children, which is referred to as "the activitystat" theory (245) proposed by Wilkin et al. Also, reactivity, which refers to the children reacting to being measured, might be an issue previously suggested to influence accelerometer measurements in children (61).

As previously mentioned, there are difficulties in assessing levels of physical activity, especially in children. It has been suggested that questionnaires do not give fully reliable estimations in children under the age of 10-12 (203). In order to minimize the risk of errors, the children filled in the questionnaires together with their parents.

Accelerometer data implies that children in the intervention had higher proportion of the most intense activities that were recorded, but these data must be interpreted with caution since the highest intensity levels (>10000 cpm) were only recorded a couple of minutes each day. All children in this study had high levels of PA, well above the current international recommendations of over 60 min MVPA (235). Others suggest, however, that over 90 minutes of at least moderate activity might be needed in children to prevent insulin resistance which has a key role in preventing future cardiovascular disease (7). Different types and durations of PA have different physiological effects. Activities that best influence bone and muscle tissue (high impact in short bursts) are quite different from those that enhance cardiorespiratory, cardiovascular and metabolic traits (longer duration of low-moderate intense training) (121), which is part of the reason general guidelines regarding PA in children are difficult to establish (220).

Our findings regarding school transportation suggest that the mode of commuting to school in these children was primarily reflected by the distance to school, with quite small mean distances (600-700 meters) in children who consistently chose active commuting. This pattern was also reported in American children in a survey by the American Heart Association concluding that compared to the previous generation active transportation to and from school had declined, and less than 3% of transport over 2 miles (3 km) and only one third of all transport over 1 mile (1.6 km) was by walking or cycling (180). This also suggests that increased PE in school might be a more feasible strategy to enhance PA levels in children and adolescents (180).

Gender differences and pubertal maturation

The effects of the intervention on bone mineral accrual, bone size and hip structure seem to be more pronounced in girls than in boys, this may be due to girls being closer their peak bone mineral accrual than the boys. The exercise-induced effects on bone are considered to be especially pronounced in Tanner stage 2 and 3 corresponding to the late pre- early peri-pubertal period (Table 1). Girls have a slightly lower participation in organized sports activities outside school than boys, hence the contribution of PA in the intervention was proportionally larger in girls. Also boys in the control group had a higher Tanner stage at follow-up in both the three-year and the four-year follow-up, which may also affect the results (underestimating the effect of intervention in boys); however, this confounder was adjusted for. Also, the gender difference in fracture incidence observed in this cohort; with boys having more fractures than girls, is consistent with and similar to findings in previous studies (46,128).

Regarding muscle strength and VJH, the intervention seem to increase the growth-related gains in muscle strength primarily through neural adaptations since results remained the same after adjustment for annual gain in leg lean mass, (paper I). This theory is also supported in the literature (26,75,189), suggesting that neural adaptation is the primary way PA increases muscle strength in children before puberty, which was until quite recently regarded as not possible in the absence of sex-steroids (26,75,188,189,199). The group-by-gender analysis in paper I suggest that there were no gender differences in the muscle parameter response to the intervention. The findings of small but significant effects of the intervention in VJH for girls but not boys might reflect the fact that the ability to perform a correct VJH might not be completely developed until the early teens (188). The fact that girls are known to be maturationally more advanced than boys in this age might explain effects in VJH in girls but not boys although not yet reflected by tanner stage (all Tanner 1).

Fat mass seemed to increase in both genders in the intervention, more pronounced in girls than in boys. The reasons for this finding are not clear, however no dose response relation was found between hours of PE in school and the annual gain in fat mass (data not shown). DXA measurements are highly reliable for bone and lean mass, but not equally reliable for measuring fat mass (CV 3.7%). One explanation might be an increased need of energy due to the extra PA in school, which was then slightly over-compensated.

Fracture epidemiology

The trauma mechanism and whether the fracture occurred during physical education in school or elsewhere could in most cases not be determined even if the referrals and reports were scrutinized. In paper III, a mean fracture incidence of just below 20 events/1000 person years was observed, (in both the control and the intervention group), which is similar to previous reports investigating fractures in children (46,94,128). The most common fracture site was the distal forearm followed by hand fractures, which is also consistent with previous reports regarding childhood fractures (46,94,128). As in most studies with prospective fracture risk evaluation there is obviously a power problem.

Fractures during childhood have been associated with low bone mass gain during puberty at various sites, (76) and also associated with low bone mass and size in young adult men (32), hence suggested to be a marker of future bone fragility.

# Study strengths

Previous exercise interventions examining musculoskeletal effects in children have a relatively short duration (all but 3 less than 20 months, Table 1), and use training programs specifically designed to be osteogenic which many children might find unnatural and boring in the long run. The population-based design of this study is also an advantage since most previous studies include only volunteers, thereby increasing the risk of selection bias. This intervention was conducted in such a way that virtually all children could be targeted and participate. To our knowledge no previous study of this kind has used fractures as endpoint. Fracture risk in the short- and long-term perspective, rather than bone mass and size, is the most relevant outcome for patients as well as physicians. It is also important to investigate whether this kind of intervention confers any adverse effects in terms of increased fracture incidence, since some reports indicate a possible association between vigorous physical activity and fracture risk in children (40). Another advantage compared to some previous attempts to evaluate fracture risk in children was that the fractures were objectively registered and ascertained by X-ray verification and medical reports instead of self-reports of fractures used by others (76).

This intervention focused not only on the skeletal effects of physical activity but also on muscle strength and neuromuscular performance which probably play an underestimated role in fracture prevention (111). It is also interesting that low muscle strength seems to be a risk factor for fracture both in children (41) and in elderly people (83).

The fact that the annual changes in the measured parameters for paper III with a four-year follow-up were calculated as regression slopes for each individual rather than merely a delta-value reduces the risk of measurement errors driving the results, since in the regression slope all the annual measurements are taken into account.

# Study limitations

Individual randomization of the study participants would have been preferable, but impossible to conduct due to resistance by the parents, pupils and teachers. Regarding children invited for measurements, the participation rate in the control schools (40%) was lower than in the intervention school (93%). This may have increased the risk of self-selection bias at baseline. However, as mentioned, this risk seems minimal since the drop-out analysis comparing weight, height and BMI from the first compulsory health examination in school revealed no group differences between children who participated and non-participants (136,137).

Physical activity was primarily assessed using annual questionnaires. Unfortunately we only have one cross-sectional accelerometer measurement 2 years after study start; obviously having baseline accelerometer data as well would have been preferable.

The kind of leisure time sports participation was not taken into account but only time spent in organized physical activities. However, it would not have been feasible to divide the children's leisure time sports participation into subcategories according to different levels of PA intensity, nor would it have served the purpose of this project, which was to evaluate the musculoskeletal effects of increased physical education in school on a population level. Although reactivity in the form of increased PA levels during spare time activities of the children in the control group cannot be ruled out, this would then mask the effects of the intervention. Compliance with the program was not specifically evaluated, but low compliance in the school physical education would lead to a missing grade in the subject at the end of primary school for that pupil, and no child had a missing grade (73).

There was no attempt to make an exact estimate of dietary habits, which in large groups of children in the studied age group (8-12 years) is known to be very difficult to obtain (165,246). However at baseline and the three-year follow-up none of the children excluded dairy products from their diet, which is important since prolonged exclusion of milk products has been shown to influence bone mass and/or fracture risk in children (25,35,197). Also, no analysis of serum vitamin D status was done. However in Sweden vitamin D deficiency is rare among children (19,74).

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Regarding the fracture data it would have been advantageous if the fracture incidents could have been divided into fractures occurring during physical education classes in school and fractures occurring during other activities. However, from the referrals and the reports we could in most cases only find out that there had been a fall, in virtually none of the cases whether this had happened during school classes or during spare time.

Regarding the cross-sectional observation study of the mode of school transportation (paper IV) the study design and the evidence value was not as strong as for papers I-III evaluating the effects of the extra physical education in school. However, the design of study IV was regarded as acceptable since the mean time spent in organized PA (or having extra PA in school) did not differ between groups, hence comparisons of active versus passive mode of school transportation could be made.

Is there a dose-response relationship between physical activity and enhanced skeletal gain during growth?

In this study, there seemed to be a dose response relationship between time spent in organized physical activity and gain in BMC and bone size. When we divided all the children into tertiles based on time spent in organized physical activity, we observed that the largest difference with regards to gain in BMC was observed between tertiles 1 and 2 for both boys and girls. In other words, it seems that it is the least active children who benefit most from the extra physical education. However this is merely speculation since the study was not specifically designed to examine a dose-response of this kind.

What is known from previous studies regarding exercise-induced gain in bone mass is that it is probably better to have several shorter periods of physical activity than few longer periods, which is primarily shown in animal models (49,102,195,196). Bone cells exposed to mechanical stimuli rapidly become less sensitive to mechanical load, and once mechanosensorily saturated further load does not produce any anabolic response in the bone tissue (195,201).  Most likely, it is best to be a little active every day. The bone seems to need a recovery period after loading which is demonstrated in an in vivo rat model where a period of 8 hours' rest fully restored the mechanosensitivity of bone cells (195). Current international guidelines recommend that children participate in at least 60 cumulative minutes of moderate to vigorous physical activity per day, through a variety of activities. In addition to this, it is recommended that children engage in muscle-strengthening and bone-strengthening activity, at least 3 days per week (235).

Potential adverse effects of exercise on bone

Intense training, primarily in females, (both before and after puberty), in combination with insufficient energy intake, can have deleterious effects on BMD in girls and women (14,28,163,183). Similar effects have also been observed in men after strenuous training such as marathon running (150). Also, decreased regional bone density in athletes with medial tibial stress syndrome has been reported (149).

None of the measured participants in this project had been diagnosed with an eating disorder such as a possible anorexia or undernourishment. None of the registered fractures occurring during the study was classified as a stress fracture.

# Conclusion

This thesis suggests that increasing the physical education in school, beginning from first or second grade in school, from the Swedish standard of 60 minutes per week to 40 minutes each school day, increases the gains in bone mass and probably also bone size in a four-year perspective in both genders. Also, hip structure seems to be positively affected in girls (but not boys) having extra physical education in school. This confirms that the previously reported short-term skeletal benefits of this intervention remain in a longer perspective and also into early puberty.

Increased physical education to 200 minutes per week does not confer adverse effects in terms of increased fracture risk.

Increased school-based physical education also enhanced growth-related gains in muscle strength in both genders and in lean mass in girls and possibly also neuromuscular performance in girls in a two-year perspective.

An active mode of transportation to school for two years did not confer additional skeletal benefits in this population of children with relatively high levels of physical activity.

The findings suggest that increased physical education in school is a feasible strategy to improve the musculoskeletal health in a young population.

# Future Perspectives

Further studies are required to follow the musculoskeletal effects of increased school-based physical education, preferably to the end of growth. Not until the studied population in this intervention is followed to peak bone mass, it would be possible to give a reliable estimate of whether this kind of intervention can be used as prevention against future fractures at a population based level.

Future studies should include pQCT measurements to give a more reliable and accurate assessment of the structural and geometrical effects of exercise on bone during growth.

Further, future studies are needed to investigate fracture epidemiology in larger cohorts with a longer follow-up, better powered to answer the question of potential effects on fracture risk by increased physical education in school.

# Summary in Swedish - Populärvetenskaplig sammanfattning

Ger en ökad fysisk aktivitet bland växande barn bättre skelett- och muskelutveckling och påverkar det i så fall frakturrisken?

Benskörhet och låg benmassa medför en ökad risk för frakturer. Låg fysisk aktivitet är också en riskfaktor. En ökad frakturförekomst är i dag ett växande samhällsproblem, och med dessutom en åldrande population samt en generellt mindre fysiskt aktiv livsstil riskerar frakturförekomsten därför att öka. Men det finns hopp. Från tidigare studier bland vuxna vet vi att fysisk aktivitet ökar benmassan (BMD) och förbättrar muskelfunktionen. En hög BMD och bra muskelfunktion minskar risken för fraktur. Andra studier har visat att åren precis före puberteten samt under tidig pubertet förefaller vara den period i livet då skelettet har störst möjlighet att svara på fysisk belastning.

Därför lanserades 1999 Bunkefloprojektet, en studie där vi årligen följer dels barn i en skola som dagligen har en lektionstimme idrott och hälsa i skolan, dels barn i tre närliggande skolor som fortsätter med svensk standard på 1-2 lektionstimmar per vecka. Studieupplägget ger oss möjligheter att utvärdera om ökad fysisk aktivitet kan användas för att förbättra skeletthälsan i befolkningen och i en förlängning minska antalet frakturer. Då studien löper över flera år ger den oss möjligheter att utvärdera om träning ger upphov till långtidseffekter, detta då studien har en betydligt längre uppföljningstid än tidigare publicerade undersökningar. Studien innefattade även utvärdering om daglig skolgymnastik påverkar muskelstyrka och muskelfunktion samt om sättet man transporterar sig till skolan påverkar skelett- och muskelutvecklingen. Alla barn i studien följdes från skolstarten med avseende på förekomst av frakturer och hos 121 barn i skolan med daglig skolgymnastik och 100 i kontrollskolorna mätte vi också årligen skelett och muskelfunktion från skolstarten och framåt. Studien planeras att fortsätta tills ungdomarna är vuxna.

Forskargruppen har tidigare publicerat skelettdata från Bunkefloprojektets första 2 år. Denna avhandling utvärderar vad som händer med skelettet efter 3 till 4 års ökad skolgymnastik, samt hur muskelfunktionen påverkas av ökad fysisk aktivitet i ett tvåårsperspektiv. Dessutom utvärderas om de barn som går och cylar till

skolan får en bättre skelettutveckling än de barn som åker buss eller bil. De resultat vi presenterar kan ses som extra intressanta då barnen under denna period går in i  puberteten och då tidig pubertet är en period där tidigare studier har visat att vi kan förvänta oss extra tydliga effekter. Puberteten är också en period där många ungdomar, främst flickor, väljer att byta till en livsstil med minskad fysisk aktivitet. Annan forskning som tidigare publicerats med liknande studieupplägg har i jämförelse med Bunkefloprojektet kortare uppföljningstid och ingen har utvärderat om en ökning av fysisk aktivitet påverkar frakturförekomsten. Tidigare repporter från vårt projekt (ett och tvåårsdata) indikerar att ökad skolgymnastik har en positiv effekt på benmassan och benstorleken, två storheter som båda påverkar frakturrisken. Delarbete II och III i denna avhandling (3-4 års data från projektet), visar att de tidigare beskrivna positiva korttidseffekterna kvarstår även i ett längre perspektiv och då även när barnen börjar påverkas av pubertetsutvecklingen. Samtliga barn i skolan med ökad skolgymnastik och de tre kontrollskolorna med skolstart 1999-2008 följdes även med avseende på frakturer. Totalt följdes 2395 barn under upp till 4 års tid där resultaten visar att ökad idrott och hälsa i skolan inte påverkar frakturförekomsten. Studien visar också att ökad idrott och hälsa under de två första skolåren ger positiva effekter på utvecklingen av muskelstyrka och muskelfunktion, också fynd av stort intresse då då många forskare tidigare menar att muskelstyrka inte går att påverka hos barn genom träning före puberteten och då förbättrad muskelstyrka i regel är kopplad till minskad frakturrisk. Därmot gav våra fynd inte hållpunkter för att de barn som gick och cyklade till skolan fick en bättre skelettutveckling. Sammanfattningsvis kan man därför konkludera att med en ökning av den fysiska aktiviteten kan skelett och muskelutvecklingen förbättras hos barn som börjar skolan.

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