PhD Thesis
Bone Health in Danish Children -

The Childhood Health Activity and Motor Performance School Study, Denmark
The CHAMPS study, DK

Malene Søborg Heidemann, MD

Faculty of Health Sciences
University of Southern Denmark
Hans Christian Andersen Children’s Hospital
Odense University Hospital
March 2013
Drawing of a DXA scan by Amalie 8 years old (2nd grade) Sundhøjskolen 2008
# Table of content

Committee and address ............................................................................................................. 4
Preface ........................................................................................................................................ 5
Abbreviations .............................................................................................................................. 7

1. Introduction .............................................................................................................................. 8
   1.1. Why is it important to focus on bone health in childhood? .................................................. 8
   1.2. Bone development ............................................................................................................... 9
      1.2.1. During growth .................................................................................................................. 9
      1.2.3. The impact of puberty on bone mineralisation ............................................................... 10
      1.2.4. Peak bone mass (PBM) ................................................................................................... 12
      1.2.5. Influences of genetics and lifestyle on bone health ....................................................... 12
      1.2.6. The impact of physical activity on bone mass .............................................................. 13
   1.3. Measuring body composition in childhood ........................................................................ 14
   1.4. Measuring bone mass and densitometry in childhood ..................................................... 15
   1.5. Challenges with the interpretation of DXA outcome in childhood .................................. 16
   1.6. Measuring physical activity in childhood ........................................................................... 17
   1.7. School based physical education intervention programmes and bone health ............... 19
   1.8. The need for new information ........................................................................................... 21

2. Aims of the study ....................................................................................................................... 22

3. Materials and methods ........................................................................................................... 23
   3.1. The CHAMPS study, DK .................................................................................................... 23
   3.2. Study design ....................................................................................................................... 23
   3.3 Participants of the sub study ................................................................................................ 23
   3.4 Ethical considerations ......................................................................................................... 24
   3.5 Data collection ..................................................................................................................... 24
      3.5.1. Anthropometrical data .................................................................................................... 24
      3.5.2. Dual Energy X ray absorptiometry ............................................................................... 24
      3.5.3. Pubertal self-assessment ............................................................................................... 25
      3.5.4. Physical activity ............................................................................................................ 25
      3.5.5. Data reduction and analysis .......................................................................................... 26
      3.5.6. Short Messaging Service-Track-Questionnaire (SMS-T-Q) ........................................... 27
   3.6 Statistical analyses .............................................................................................................. 27
      3.6.1. Study I ........................................................................................................................... 28
      3.6.2. Study II .......................................................................................................................... 30
      3.6.3. Study III ......................................................................................................................... 31

4. Results ....................................................................................................................................... 32
   4.1. Participants characteristics ................................................................................................. 32
   4.2. Study I .................................................................................................................................. 36
   4.3. Study II ................................................................................................................................ 39
   4.4. Study III ................................................................................................................................ 43

5. Discussion .................................................................................................................................. 47
   5.1. Study I .................................................................................................................................. 47
   5.2. Study II .................................................................................................................................. 48
Committee and address

Principal Supervisor
Christian Mølgaard, Professor, MD, PhD
Hans Christian Andersen Children’s Hospital Odense University Hospital, Research Unit for Paediatrics and Department of Nutrition, Exercise and Sport, Faculty of Science, University of Copenhagen

Supervisors
Niels Wedderkopp, Professor, MD, PhD
Institute of Regional Services Research, University of Southern Denmark
Anders Jørgen Schou, MD, PhD
Hans Christian Andersen Children’s Hospital Odense University Hospital, Research Unit for Paediatrics
Steffen Husby, Professor, MD, DMedSci
Hans Christian Andersen Children’s Hospital Odense University Hospital, Research Unit for Paediatrics

Evaluating committee:
Magnus Karlsson, Professor, MD, PhD,
Department of Orthopaedics, Skåne University Hospital.
Ole Rintek Madsen, Consultant, DMSc,
Institute of Clinical Medicine, Orthopaedics and Internal Medicine, Gentofte Hospital
Pernille Hermann Clinical Associate Professor, MD, PhD, (Chairman)
Department of Medical Endocrinology, Clinical Institute, University of Southern Denmark

The thesis is based on the following papers

I. The Intensity of Physical activity Influences Bone Mineral Accrual in Childhood

II. The Impact on Children’s Bone Health of a School-based Physical Education Program and Participation in Leisure Time Sports.

III. The Influence of Anthropometry and Body Composition on Children’s Bone Health.
Preface

This PhD thesis presents results obtained from The Childhood Health, Activity and Motor Performance study, Denmark – The CHAMPS study- DK. In 2007 the city council of the municipality of Svendborg, Denmark decided to create sports schools with the intention to improve physical health of children (the Svendborg project). The CHAMPS study was made responsible for the evaluation of this project. The study is on going since August 2008 and has the overall aim to investigate the effect of additional physical education in a school based curriculum on children’s health. The studies of this thesis have focus on bone health in childhood.

I wish to express sincere gratitude to many people who have played an important role in the planning and conductance of this study. I wish to thank my principal supervisor Professor, PhD Christian Mølgaard for invaluable and inspirational guidance and support throughout the process. Thank you for advice and always highly qualified feedback on my works. I wish to thank my supervisors Professor, DMedSci Steffen Husby for believing in the project and being helpful initiating the project, Professor Niels Wedderkopp for including the bone health project in the CHAMPS study. Thank you for support and help in all phases of the study. Many thanks to PhD, MD, Anders Schou for the initiation of the project and for including me in the idea in the first place. Thank you for support and scientific guidance. I owe gratitude to associate professor Lone Agertoft for introducing me to the DXA scan.

I wish to thank associate professor René Holst for supervision, highly qualified statistical assistance and critical review of my manuscripts and for friendship. I owe many thanks to my colleagues in the CHAMPS study: Heidi Klakk, Eva Jespersen, Claudia Franz, Christina Christiansen and Niels Christian for sharing hard work, but most of all laughs and many good moments. Thanks to friends at RICH for companionship to conferences in Cornwall and Madeira. Thanks to all my fellow PhD students in the Paediatric Research Unit: Michael Callesen, Peter Toftedahl, Lone Marie Larsen, Gitte Zachariasen, Mette Tanvig, Helene Rasmussen, Halfdan Skjerning, Kirsten Risby, Mathias Rathe, and a special thank to Lise Aunsholt, Rikke Nees Pedersen and Susanne Pagh Nissen for sharing experiences with the PhD work and friendship.

The study had not been possible without the help of many students participating in the evaluation of the children and helping with practical matters. A special thank to Ida Guldbæk Louw, Camilla Martens and Dorte Johansen. Thanks to Mette Vogn Hviid for helping during the DXA scans and helping with many practical challenges. I owe many thanks to the secretary group, Joan Frandsen, Helle Wehrenberg, Tove Hellelund and
Heidi Lindemann for invaluable help and support. Thanks to Mette Sorang for inspiration and for expressing her interest for the project and the participating children.

A special thank to all the participating children, their parents and teachers without whom this project had not been possible. Thank you to every one in the “Elitesekretariat” Gitte Minor, Martin Andersen, Jannie Kaae, and Mette Skov Hansen.

Many thanks to those who supported the study financially. The study was supported by grants from the Tryg foundation, Gigt foreningen, Egmontfonden, Lipmann fonden, A. J Andersen fonden, Mærsk Mc Møller fonden, Svendborg Kommune

Finally I wish to thank my husband Erik for supporting and encouraging me at all times and many thanks our three wonderful children Alma, Klara and Frederik and our parents for help and support when ever needed.

Malene Søborg Heidemann

March 2013
## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>BA</td>
<td>Bone Area</td>
</tr>
<tr>
<td>BF%</td>
<td>Body Fat per cent</td>
</tr>
<tr>
<td>BMC</td>
<td>Bone Mineral Content</td>
</tr>
<tr>
<td>BMD</td>
<td>Bone Mineral Density</td>
</tr>
<tr>
<td>aBMD</td>
<td>areal Bone Mineral Density</td>
</tr>
<tr>
<td>vBMD</td>
<td>volumetric Bone Mineral Density</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>DXA</td>
<td>Dual Energy X-ray Absorptiometry</td>
</tr>
<tr>
<td>FM</td>
<td>Fat mass</td>
</tr>
<tr>
<td>LL</td>
<td>Lower Limb</td>
</tr>
<tr>
<td>LM</td>
<td>Lean mass</td>
</tr>
<tr>
<td>LTS</td>
<td>Leisure time sport</td>
</tr>
<tr>
<td>PA</td>
<td>Physical Activity</td>
</tr>
<tr>
<td>PBM</td>
<td>Peak Bone Mass</td>
</tr>
<tr>
<td>PE</td>
<td>Physical Education</td>
</tr>
<tr>
<td>SAQ</td>
<td>Self assessment questionnaire</td>
</tr>
<tr>
<td>SMS (T-Q)</td>
<td>Short Message Service-Track-Questionnaire</td>
</tr>
<tr>
<td>TBLH</td>
<td>Total Body Less Head</td>
</tr>
</tbody>
</table>
1. Introduction

1.1. Why is it important to focus on bone health in childhood?
This question was asked in our research group before we entered The CHAMPS study-DK during the fall 2008.

Many factors have an impact on bone health such as gender, genetics, vitamins, lifestyle and various diseases\(^1,2\). Weight-baring activity increases bone mineral content (BMC) whereas inactivity during bed rest caused by disease decreases BMC\(^3\).

Osteoporosis is a systemic skeletal disease characterized by a low bone mineral density (BMD) and deterioration of the inner microarchitecture of the bone tissue\(^4,5\). Osteoporosis has traditionally been considered a disease of the elderly, however, there is an increasing recognition of the importance of bone mineral acquisition during growth as one way to prevent this disease\(^6\). Approximately 200 million people are affected by osteoporosis worldwide and the prevalence is expected to increase due to aging of the population\(^7\). In the year 2000 there were an estimated 9.0 million osteoporotic fractures worldwide, and the greatest number of the fractures occurred in Europe (34.8%)\(^8\). It is estimated that 30-50% of all women and 15-30% of men will suffer from an osteoporosis related fracture during a lifetime\(^9,10\). Osteoporosis is considered to be one of the most common public diseases and a considerable socio-economic burden. A recent estimate for the direct costs of osteoporotic related fractures are €29 billion in the five largest EU countries (France, Germany, Italy, Spain and UK)\(^8,11\). Although osteoporosis is not a paediatric disease that will affect otherwise healthy children, the magnitude of the problem in the old age emphasises the need for preventive strategies in childhood.

Bone accumulation occurs throughout the entire childhood and adolescence and reaches a maximum, which is called peak bone mass (PBM). This maximum of bone mineral acquisition serves as an important protective advantage as bone mineral density declines as a result of aging, illness or diminished sex steroid production\(^9\). Childhood is a critical time for the development of lifestyle habits conductive to maintaining good bone health throughout life.

The Childhood Health Activity and Motor Performance School Study, Denmark (The CHAMPS study, DK) is a large longitudinal natural experiment including ten public schools in the municipality of Svendborg. The study was conducted to evaluate various possible health effects of extra physical education lessons in a school-based curriculum\(^12\) and the evaluation of children’s bone health provides the basis of this present PhD thesis.
1.2. Bone development

1.2.1. During growth
The skeleton changes across the human lifespan. This is characterised predominantly by bone formation and growth throughout childhood. Followed by a gradual loss of bone density that can accelerate in older adults. The skeleton serves three main functions: It provides support for locomotion and protects vital inner organs, it serves as a reservoir of calcium and phosphate and it contains the bone marrow where haematopoietic cells are developed. The bone tissue is a dynamic tissue that undergoes significant changes throughout life. Bone comprises of a collagen matrix into which, hydroxyapatite crystals containing calcium and phosphate are deposited. The bone mass is a composite tissue consisting of an organic collagen protein and inorganic mineral hydroxyapatite and the arrangement of these composites determine two macroscopically different types of bone tissue. The cortical bone is a dense tissue although containing blood vessels in canaliculi and it is primarily found in the shaft of the long bones. The trabecular bone is a porous tissue with a complex three-dimensional structure and is primarily situated near the joint surfaces and in the vertebrae. The bone mass increase throughout childhood and adolescence and undergoes rapid changes due to growth. The skeletal calcium increases form approximately 25 g at birth to 900 to 1200 g in adult females and males respectively. The density of bone is modulated by a group of cells including the osteoclasts that resorb bone and osteoblasts that refill the cavities with new bone. The osteoclasts dissolve the bone mineral content and followed by this process the osteoblasts produce and deposit organic matrix called osteoid predominantly made by collagen, and protein. This cycle of bone resorption and bone formation is called bone remodeling. The process of bone formation by osteoblasts without prior bone resorption by osteoclasts is called bone modeling. This results in an increase in bone mass and bone size. The bone formation and bone modelling is important for bone growth, particular in childhood and promotes bone strength. Remodeling also plays an important role during growth by optimizing bone structure. With advanced age in the elderly there is an advanced loss of bone mass due to a decrease in osteoblast activity relatively to osteoclast activity. During the first two decades of life the modelling process is dominating and result in the growth of the bones.
Introduction

Figure 1: Schematic view of the modeling and remodeling process.
In modelling osteoblasts and osteoclast action are not linked and rapid changes occur in the amount, shape and position of bone. In remodelling osteoblast action is coupled to prior osteoclast action. Net changes in the amount and shape of bone are minimal unless there is a remodelling imbalance. From Rauch 2004

1.2.3. The impact of puberty on bone mineralisation
Bone undergoes rapid changes during puberty. The onset of puberty corresponded earlier to a biological age of 11 and 13 years of age in girls and boys, respectively 18 but the age of onset of puberty has over the past decades declined 19-21. Puberty is a dynamic period of development marked by rapid changes in body size, shape, body composition, and a rise in sex- and growth hormones. In puberty bone mass increases as the pubertal sex hormones secretion increases and the growth spurt takes place with a dramatic increase in height and weight 22. During this period there is an increase in bone width due to deposition of new
Introduction

Bone on the periosteal surface in boys and girls and more than half of the adult bone is accrued during the pubertal years\textsuperscript{14}. Pubertal growth is characterized by sexual differentiation and rapid growth. During puberty BMD increases, but mainly as a consequence of an increase in bone size. The maximal rates of bone mineral accrual lag behind the peak height velocity and hence the growth in bone size (Figure 2), which results in relatively under mineralized bone and increased fracture risk in the pubertal years\textsuperscript{23-25}. The bone mineral is accrued at different rates at different anatomic sites. Gains in the appendicular skeleton predominate before puberty, whereas spinal growth is influenced by sex-steroids and therefore dominate during puberty\textsuperscript{26}.

**Figure 2:** BMC (TBLH) peak bone mineral content velocity curve illustrating the velocity at peak BMC and peak height velocities by chronological age for boys and girls. *Adapted from Bailey et al.,\textsuperscript{27,28}.*
Introduction

1.2.4. Peak bone mass (PBM)
The bone mass reaches a plateau called peak bone mass (PBM), which is the maximum bone mass an individual obtains during growth. Gains in bone mass are most rapid during adolescence, with 25% of the PBM acquired during the two-year period surrounding peak height velocity 28. The PBM is usually reached in the early twenties, however the exact age at which PBM is reached is uncertain. There are gender differences in the level of PBM with boys reaching higher levels of PBM than girls. These differences are largely due to genetic variations but also different effects of sex hormones during puberty, with oestrogen playing a key role in both genders 29. The PBM is relatively stable until the onset of natural loss with aging. At menopause, women experience an accelerated loss for 3-6 years and thereafter a continued loss of bone mineral for both men and women 27. The PBM may be an important predictor for bone mineral density (BMD) in the elderly and thereby fracture risk later in life and hence, maximising the PBM may be essential in the prevention strategy towards osteoporosis 9.

1.2.5. Influences of genetics and lifestyle on bone health
The BMD is as PBM a key predictor of fracture risk in adulthood and it accounts for up to 70% of the variance in the bone strength and it is estimated that up to 60-80% of the variance in BMD is due to genetics 9. Osteoporosis is a polygenic disease and no single gene explains the disease. In twin studies several genes have been identified to be associated with variations in BMD and many of these genes were identified as candidate genes in the pathways of either bone formation or bone resorption 30.

Many factors play an important role in the development and maintenance of healthy bone mass. Early factors are birth weight and infant weight, which have been linked to bone health later in life 31. There are indications of intrauterine conditions with an impact of the development of bone. These are maternal smoking, diet, physical activity (PA) and vitamin D insufficiency 32. In childhood several chronic diseases may lead to osteoporosis due to decreased formation or increased bone resorption. Among these is use of excessive corticosteroid in diseases with increased inflammatory response, eating disorders such as anorexia nervosa and growth hormone deficiency. Otherwise healthy children do not experience conditions that mimic osteoporosis, but they are able to influence and optimize their bone development through lifestyle.
Introduction

1.2.6. The impact of physical activity on bone mass

It is believed that osteocytes are sensitive to workload, also referred to as strain and thereby mediates a response by increasing the activity of the osteoblasts to increase bone density. The opposite response is observed with physical inactivity and an increased activity of osteoclasts is seen. This was described by Harrold Frost as the mechanostat theory (Figure 3)\(^{33}\). Loading on the bones from physical activity is generally accepted to have a positive effect on BMD and also bone structure in both animal studies\(^ {34,35}\) and human studies. Positive effects on the bones are seen in childhood and in some studies it is suggested that the initial benefits of PA in childhood are long lasting regardless of PA levels later in childhood, adolescence or in adulthood\(^ {36-39}\) although these studies are short term follow up and must be interpreted with caution. Other studies, both cross-sectional and longitudinal indicate that the cessation of sports is associated with greater reduction in aBMD whereas changes in bone size may be permanent\(^ {40}\). The decreased fracture rate among adults who were previously active\(^ {41}\) may also be caused by non-skeletal effects such as increased lean mass or improved coordination and balance. The impact of weight baring exercise appears to be positive and well documented as reviewed by Hind et al., 2007\(^ {3}\), especially during pre-pubertal years. Studies have examined relationships between physical activity and bone mass. Several of these studies were longitudinal and the PA measured included weight baring elements\(^ {42-44}\). In cross-sectional studies of tennis players it was demonstrated how the loaded arm had significantly greater bone size and mass than the non-loaded arm with particular differences depending on pubertal stage\(^ {45,46}\). In studies focusing on the impact of habitual PA on bone health different methods are used for collecting information about the type, duration and intensity of the PA and the bone traits. In general, the conclusion is that the effect of PA on bone mass in childhood is mainly related to vigorous intensity level\(^ {47-50}\).
Introduction

Figure 3: A model of regulation of bone strength development during growth based on Frost’s mechanostat theory. *From Kontulanien SA, 2007*  

---

1.3. Measuring body composition in childhood

The body composition describes the percentages of bone mass, fat mass and muscle mass in human bodies. Numerous assessments methods for body composition exist. The most commonly used methods are bioelectrical impedance, air displacement plethysmography, DXA scans and MR. The indications for measuring body composition apart from research are the need to describe deficiencies or excess of one component related to health risk in order to create suitable interventions to reduce morbidity. In childhood the indication is mainly research. The DXA scans provides estimates of fat mass, lean mass and bone mass. The DXA scan has several strengths as a method for measuring body composition, particular in children. The scan duration is short, especially the GE Lunar Prodigy (used in the present study) that uses narrow angle fan beam. The procedure is non-invasive the accuracy and reproducibility is high in normal weight individuals whereas the accuracy of the GE Lunar Prodigy is significantly reduced in obese individuals. The limitations of DXA is radiation, however the effective dose from a total body scan is low (<1.0μSv) and corresponds to less than 5% of a chest X-ray or 5-15% of naturally occurring background radiation.
Introduction

**Table 1**: Radiation doses from DXA* compared to other procedures or activities, from *MS Fewtrell* 55

| Effective dose (μSv) | Lumbar spine | 0.4–4 | Whole body | 0.02–5 | Chest X-ray | 12–20 | Lateral lumbar spine X-ray | 700 | Daily background | 6–20 (depending on location) | Return transatlantic flight | 80 |

*Range for different machines.

1.4 Measuring bone mass and densitometry in childhood

To assess bone density, size and structure several methods exists. Quantitative ultrasound (QUS) was developed in 1984 for the assessments of calcaneal bone status in adults 56. The QUS measures the speed and attenuation of sound through the appendicular bone. The method is based on ultrasound and has several advantages in childhood. The QUS is portable and suitably for large school studies, there is no radiation exposure and it is technically simple. However, if there are decreased values of the output from the QUS it may not be possible to detect the reason for this since the QUS is dependent on the density and the stiffness as well as micro- and macrostructure 16.

Quantitative computed tomography is another method for assessing bone traits. The method provides a cross-sectional cQCT image slice and measures volumetric BMD (vBMD) in the peripheral skeleton and hence, not size dependent. The method is based on computed tomography and was developed in 1976 57. The advantage of this method is the ability to distinguish between trabecular and cortical bone and it estimates geometric properties. The trabecular bone is more metabolic active than cortical bone and therefore more sensitive to change in BMD. A disadvantage is a tendency to underestimate vBMD when the cortical bone thickness is low 16. The Dual energy x-ray absorptiometry (DXA) was used in this present study. The DXA is the preferred method for assessing BMC and areal BMD 58. The method was developed in the late 1980s. The DXA provides a 2-dimensional measure of a 3-dimensional structure. The method is based on x-ray at two different energies, which are absorbed by the different types of tissue differently. In this
Introduction

way DXA distinguish between hard tissue and soft tissue; bone mass, lean mass and fat mass. The method has several advantages but also limitations as mentioned in section 1.3.

1.5. Challenges with the interpretation of DXA outcome in childhood

When measuring bone density by DXA it is not a true bone density measured since the DXA only measures a cross-sectional area of the bone and not a volume. The estimates of bone by the DXA scan is converted into grams (g) of bone tissue by the projected scan area, measured in cm$^2$ and consequently the density of bone estimated by DXA is defined as areal BMD (g/cm$^2$)=BMC (g)/BA (cm$^2$). In adults the projected BA is relatively stable over time compared to children and does therefore not pose a major problem in a longitudinal perspective. In growing children bone changes in three dimensions over time and thereby the size dependence poses a potential pitfall when evaluating bone changes in longitudinal studies as demonstrated by example in Figure 4.

**Figure 4**: Size dependence of DXA: An example of two “bones” with the same volumetric density, however, different areal BMD, from Carter et al. 61
Introduction

The net result of the example given in Figure 4 is an overestimation of the BMD in large bones and an underestimation of the BMD in small bones. The WHO criteria for diagnosing osteoporosis in adults are based on a T-score defined as the standard deviation of the observed BMD compared with that of a normal young adult. The T-scores are meaningless in childhood. Several methods have been proposed to adjust for size.

One of these methods as described by Ann Prentice in 1994 is the use of multiple regression analysis and simultaneously adjust BMC for BA, weight, height and other relevant factors, i.e. age, gender, pubertal stage. Another method described by Mølgaard et al., 1997 uses a stepwise approach expressing height for age, BA for height and BMC for BA to distinguish three different possible situations in which reduced bone mass may occur: “short” bones, “narrow” bones and “thin” bones. A third approach used for only spine and hip is the use of calculated volumetric bone density (bone mineral apparent density (BMAD)) in which BMC is adjusted for calculated bone volume rather than bone area. Finally a method, which is a modification of the third method where bone volume is additionally adjusted for height to correct for body size was, described by CM Schmidt et al., 2006.

1.6. Measuring physical activity in childhood

Physical activity is defined by the world health organisation (WHO) as any bodily movement by skeletal muscles that require energy expenditure. The energy expenditure or the movement can be measured in different ways and estimate a person’s physical activity (PA) level. The most important dimensions of PA are frequency, duration and intensity. Several methods for measuring or estimating these dimensions of PA are developed and well described. Among these are self-reports based on specifically designed PA questionnaires or recall that rely on the cognitive ability of the participant. Other methods are direct observations, which are time-consuming and unsuitably for large-scale cohort studies and measurements of heart rate or activity monitors (i.e. accelerometers). The accelerometer is frequently used to assess PA in childhood and has several advantages. It is an objective measure; the monitors are easy to use for the participants. The accelerometer captures estimates of duration and intensity and thereby two of the important dimensions of PA. The output from the accelerometer is counts/ minutes and corresponds to metabolic equivalent (MET). The standard metabolic equivalent is a unit used to estimate the amount of oxygen used by the body during physical activity. 1 MET = the energy (oxygen) used by the body at rest, while sitting quietly or reading a book, for example. The harder the body works during the activity, the more oxygen is consumed and
the higher the MET level. Activity that burns 4 to 6 METs is considered moderate-intensity physical activity. Activity that burns > 6 METs is considered vigorous-intensity physical activity. The Actigraph GT3X was used in the present study. The Short Messaging Service-Track-Questionnaire (SMS-T-Q) described in section 4.5.6. was used in study II to assess information about the amount of leisure time PA activities and contain information about type of PA in addition to duration. The Actigraph GT3X has low intra- instrument coefficient of variation as well as inter- instrument CV at lower frequencies (Hz) ranging from CV 0.8-3.7% whereas the CV values increases at higher frequencies. Tracking studies concludes that habits of PA tracks from childhood into adulthood and also that youth PA seem to decrease over time. In a tracking study of PA and sedentary behaviour in childhood from the Iowa Bone Development Study, a moderate tracking correlation was found. The conclusion was that children that are initially labelled as inactive or active may change PA pattern and that prevention efforts should focus on maintaining and increasing PA for all children. Physical activity is an important contributor to health status and prevention of several diseases and it is important with valid tools to estimate PA in order to monitor the development in children and adult’s PA habits.
Introduction

1.7. School based physical education intervention programmes and bone health
A brief summary of selected school-based PA interventions including 6-14 year old children is referred to below. Selected studies from the past 7 years (2006-2012) in which bone health is evaluated are included. Studies with special populations are not included. There have been a number of school-based PE programs with the specific aim to enhance bone health in childhood. The intervention programs vary in length and type, nevertheless many of these have demonstrated positive effects on the bone traits.

The CoSCIS study was a prospective intervention study that recruited n=135 girls and n=108 boys 6-8 years who were included in a school-based curriculum intervention program of 180 minutes per week. The control group comprised of age-matched children (n=62 boys and n=76 girls) who participated in the mandatory 60 minutes of PE per week. BMD was measured by peripheral DXA in the forearm and the calcaneus. The study concluded that the increase in PE for prepubertal children was associated with a higher accrual of bone mineral and bone size after 3 years in girls but not in boys 73.

The POP study is a prospective, controlled exercise intervention study following skeletal traits and fracture incidence in children. Children were recruited from four neighbour elementary schools in a middle-class area in Malmö. Forty-nine girls and 81 boys, 7-9 years of age were included in a school-based curriculum based exercise intervention program of general PA for 40 minutes per school day (200 minutes per week). Fifty healthy age-matched healthy girls and 57 boys assigned to the general Swedish school curriculum of 60 minutes per week and served as controls. Children were examined by DXA scans (total body, femoral neck and lumbar spine) at baseline and at two years follow up. Results showed that there was a significant effect of the intervention on the annual gain in BMC and aBMD of the lumbar spine and the femoral neck in girls 74 and of the lumbar spine in boys 75. At three, four and five year follow up a study of fracture risk was performed and concluded that the school-based intervention did not affect the fracture risk 76-78.
Introduction

The AS! BC is a randomized, controlled school-based intervention study. Children (n=410) aged 9-11 years in the AS! BC study was allocated to intervention (n=281) and control group (n=129). The bone-loading component of the AS! BC consisted of a daily jumping program (Bounce at the Bell) and 15 minutes per day of classroom PA in addition to regular PE. pQCT and DXA were used to evaluate the bone traits. The study concluded that the school-based program with PA enhanced bone strength measured by pQCT at the distal tibia in pre-pubertal boys and beneficial effects on the bone mineral evaluated by DXA were reported.

The Power PE was a prospective 8-month randomized, controlled exercise intervention. Exercise session took place every week of the school year except from holidays. Eighty-one adolescents aged 13.8 ± 0.4 years (n=43 intervention; n=38 control) were examined at baseline and at follow up. The intervention consisted of 10 minutes of jumping in the beginning of every PE lesson (twice per week) so the jumping activities were additional to the mandatory PE lessons. Bone parameters were assessed by QUS-2 Ultrasound Densitometer to evaluate broadband ultrasound attenuation (BUA) of the non-dominant calcaneus. Measures of BMC, BMD and BA of the femoral neck, trochanter, lumbar spine and whole body were made with an XR-36 Quick-scan. The conclusion was that participants of the jumping intervention achieved significantly higher bone mass at femoral neck, trochanter, whole body and calcaneus.

Meyer U and Kriemler 2011, The Kinder Sports Study (KISS), Switzerland.
One-year intervention.
The Kiss is a randomized controlled trial. Children at 6-12 years were recruited to participate in the KISS study, n=243 were randomized to an intervention and n=134 to a control school. The intervention consisted of a multicomponent PA intervention including daily PE lessons containing at least 10 minutes of specific weight-baring exercise. Children were examined at baseline and at one-year follow-up by DXA scans (Total body, femoral neck and lumbar spine). The conclusion was that BMC and BMD were positively affected in pre- and early pubertal boys and girls with higher effects during pre-puberty.
1.8. The need for new information

The relationship between weight-bearing PA is well described. Less is known about the impact of habitual activities on children’s bone health and information is needed to develop recommendations in this field.

In many of the recent school intervention projects the PE programs were designed to enhance bone health by including elements of weight-bearing PA into the intervention program. Information about intervention programs that are easily implemented in public schools is still needed to either confirm or reject the potential beneficial effects of such school intervention programs. Children attend leisure time sport (LTS) regardless of school type and knowledge and valid data in this field is limited regarding the effect of LTS on bone health. Many parameters influence bone development in childhood, adolescence and in adulthood. Due to increased prevalence of obesity many studies have been conducted to establish the impact of fat mass on bone development. Knowledge in the field about the relationship between anthropometric and body composition measures impact on bone development in childhood in a longitudinal perspective is limited.
2. Aims of the study

The overall aim of this PhD thesis was to achieve more knowledge about the longitudinal relationships between physical activity, growth and body composition on bone accrualment in children. The aims of the three studies were:

Study I

- To evaluate the effect of physical activity at different intensity on children’s bone health measured by DXA scans.
- To evaluate the effect of changes in proportion of the time in PA at different intensity levels.

Study II

- To evaluate the effect of attending schools with four additional PE lessons per week during a two-year follow-up period on children’s bone health measured by DXA scans compared to children in public schools attending the mandatory two PE lessons per week.
- To evaluate the effect of attending leisure time sport on children’s bone health, regardless of school type.

Study III

- To investigate the parameters that influenced bone accrualment during a two-year period with particular interest in measurements related to anthropometry (height and BMI) and body composition (lean mass and body fat percentage)

- To investigate possible gender differences in these effects.
3. Materials and methods

3.1. The CHAMPS study, DK

3.2. Study design
Nineteen primary schools in the municipality of Svendborg (population of 27.000), Denmark, were invited to participate in the CHAMPS project as intervention schools. The overall purpose of the CHAMPS project is to examine the possible health related effect caused by attending extra physical education lessons in public schools. The study is an on-going observational cohort study including approximately 1200 children attending preschool to fourth grade. The study can be described as a natural experiment, in which the variations in exposure (the sports-schools versus the traditional schools) and outcomes were analysed with the intent of making causal inferences on the effect of the intervention in other words; the researcher has not manipulated the exposure to the event or intervention.

Ten of the 19 schools agreed to be sports schools, but only the participating six schools were able to finance the extra physical education (PE) lessons. The municipality provided six matched control schools but only four schools agreed to become a control school. The six intervention schools and the four control schools were matched based on school size, urban/rural area and socio-economic position. Parents and children were unaware of the initiation of this project until two months before the following school year avoiding parents making an influenced school choice.

The school leaders and PE teachers of the sports schools were invited to design the set-up for an optimal PE intervention. The six intervention schools chose to implement four additional PE lessons per week to their mandatory PE program. This initiative resulted in a minimum of 4.5 hours of PE per week divided over at least 3 sessions of at least 60 minutes and to educate PE-teachers in specific age-related training principles. The four control schools continued their regular PE curriculum of 2 PE lessons per week resulting in 1.5 hours of PE per week.

3.3 Participants of the sub study
A subsample comprising children attending 2nd to 4th grade (age range 7.7-12 years) at baseline in year 2008 was created for this study. The reason for not including the two youngest classes (preschool to first grade) was logistic as well as ethical considerations of sending children aged 5-7 years to examinations at the hospital followed only by a teacher and without their parents. Children were examined at baseline and at two-year follow up examination. Examinations of the children took place at The Hans Christian Andersen Children’s Hospital, Odense, Denmark and at the Department of Radiology, Odense University Hospital, Denmark. A teacher followed the children every school day; 12 children per
Materials and methods

day for 13 consecutive weeks with the exception of the Christmas and winter holiday. This examination program was repeated at two-year follow up in October 2010- February 2011. Children were examined in the same order as they were at baseline and they were all DXA scanned within a range of 2 years ± 14 days.

3.4 Ethical considerations
All children and parents from the participating schools received information about the study through school meetings during the spring 2008 and written information. Parents signed informed consent forms. Children participating in this particular sub study signed informed consent forms concerning the DXA scan and the hand radiograph (data not presented in this thesis). Participation was at any time voluntary. Permission to conduct The CHAMPS Study–DK was granted by the Regional Scientific Ethical Committee of Southern Denmark (Project number: S-20080047).

3.5 Data collection

3.5.1. Anthropometrical data
Anthropometric measures were measured barefoot in a thin T-shirt and stockings. Body weight was measured to the nearest 0.1 kg on an electronic scale, SECA 861 and height was measured to the nearest 0.5 cm using a portable stadiometer, SECA 214 (both Seca Corporation, Hannover, MD). All data were entered and stored in the DXA machine.

3.5.2. Dual Energy X ray absorptiometry
Dual Energy X ray Absorptiometry (DXA), GE Lunar Prodigy (GE Medical Systems, Madison, WI), equipped with ENCORE software (version 12.3, Prodigy; Lunar Corp, Madison, WI), was used to measure estimates of bone mineral content (BMC), bone mineral density (BMD) and bone area (BA) as well as lean mass (LM) (in this study synonymously with muscle mass) and fat mass (FM). The body fat per cent (BF%) was calculated as

\[ BF\% = \left( \frac{FM}{FM + LM + BMC} \right) \times 100\% \]

The total body less head (TBLH) and lower limb (LL) values were used in the studies of this thesis but values from different regions are available. Machine calibration was done daily and quality assurance tests were performed daily and weekly as recommended by the manufacturer. The scanner computer selected the scanning mode (thin, standard or thick) after the data of the height and weight of the subject was entered to the machine. The typical scan duration was 5 minutes.
Materials and methods

depending on the child’s height and weight. Two technologists (Mette V. Hviid and the author Malene Heidemann (MH)) performed all scans and all data were analysed by one person (MH). The children were positioned on the scanner table by the technologist and were instructed to lie still in a supine position wearing underwear; a thin T-shirt, stockings and a thin blanket for the duration of the DXA scan. The positioning of the child, the quality of the scan and the regions of interest were checked immediately and if these were unsatisfactory the DXA scan procedure was either ended and restarted or performed again. The GE Lunar Prodigy has reproducibility with precision errors (1 SD) of approximately 0.75 % CV (Coefficient of Variation) for bone mass, 2.01% for LM and 1.29% for BF% in children and adolescents with a mean age 11.4 years (5-17 years) in children and adolescents having a mean age 11.4 years (5-17 years) \(^{52,84}\). The reproducibility of the DXA measurements performed in the present studies was not examined due to ethical consideration. However, repeated daily scans of a phantom were performed to assess the coefficient of variation (CV) during the two test periods. The CV values were 0.27-0.33% and corresponded well with the mentioned studies above.

3.5.3. Pubertal self-assessment
Tanner pubertal stages self-assessment questionnaire (SAQ) which consists of drawings of the 5 Tanner stages for pubic hair (boys, girls) and breast development (girls), respectively \(^{85}\) with explanatory text in Danish were used \(^{86}\) to evaluate sexual maturation. Children were presented with standard pictures showing the pubertal Tanner staging and asked to indicate which stage best referred to their own pubertal stage. A validation study of the SAQ used in this study was performed in which n=63/120 invited children participated. Agreement between self-assessment of pubertal maturation and the objective examination performed by an experienced paediatric endocrinologist was calculated. The conclusion was a perfect agreement for girls (weighted kappa (WK) 0.83 CI 0.71-0.93) and a moderate to substantial agreement for boys (WK 0.74 CI 0.56-0.91) (unpublished data).

3.5.4. Physical activity
Physical activity (PA) was assessed using the Actigraph GT3X accelerometer. The GT3X is a light, solid-state triaxial accelerometer, designed to monitor human activity and provide an estimate of energy expenditure. The accelerometer has the ability to measure the rate of acceleration/movement in three different directions: the z-axis/ medio-lateral axis, x-axis/ anterior-posterior axis and the y-axis/ vertical axis. The data from the vertical axis were used in this study as only these are validated and well described \(^{68}\). Assessments of
Materials and methods

PA were performed during November 2009 to January 2010 (the middle of the two-year test period). The children attended 3rd-5th grade. The signal (counts for each movement) was digitalized and passed through a filter with band limits of 0.25-2.5 Hz in order to help eliminate extraneous accelerations that were not due to human movement (e.g., vibration). The accelerometer was set to record PA data every 2 seconds (2-sec. epoch). Researchers from the project personally delivered the accelerometers to the children at the schools. Both verbal and written information and instructions were given to children along with their parents. The children were instructed to wear the device from the time they woke up in the morning until bedtime in order to capture their entire PA for each day, for 7 full, consecutive days, thus including all weekdays and a full weekend. The only exception was to remove the monitor when showering or swimming in order to prevent damage to the device. After the measurement period the accelerometers were recollected and data downloaded to a computer. The period of seven days of measurement was selected in accordance with the findings of Trost et al., implying that an average of 7 days is required in order to reliably characterize a child’s habitual PA behaviour.

3.5.5. Data reduction and analysis

The data were downloaded to a computer, and the customized program, Propero was used to clean and break down raw accelerometer outcome data, and PA was adjusted for various factors in order to minimize bias. The children were informed to wear the accelerometer during waking hours only, however it is probable that some children did not remove the accelerometer during sleep at night. In order to avoid bias, Propero was set up to include only activity in different time blocks depending on grades (2nd grade: 07.00-20.30 hours, 3rd grade: 07.00-21.00 and hours, 4th grade: 07.00-21.00 hours). This was a decision made from the assumption that these time intervals were considered appropriate for Danish children in 2nd to 4th grade, also during weekends. Furthermore, to distinguish between true intervals of inactivity and “false inactivity” recorded when the monitor had been taken off, all strings of zero for 20 min or more were defined as “accelerometer not worn” and subsequently deleted from the summation of activity. Thus, these periods did not contribute to the required minimum of valid registered activity.

Activity data were included for further analyses, if the child had a minimum of 4 days with 10 hours per day of valid recording after the removal of non-wear time. Recording time did not need to be consecutive time. Cut-off points for activity intensity levels were defined according to Evenson et al. For each individual there could therefore be different numbers of days with valid registration of physical activity. The data (counts/minutes) were differentiated into a percentage of the total wear time spent in sedentary, low, moderate and high activity.
Materials and methods

Table 2: Classification of physical activity intensity based on Evenson accelerometer cut-off points and corresponding MET thresholds as described in chapter 1.6.  

<table>
<thead>
<tr>
<th>Physical activity intensity</th>
<th>Accelerometer cut points</th>
<th>Units of metabolic equivalent (MET)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sedentary activity</td>
<td>≤ 100 counts/min</td>
<td>METs &lt; 1.5</td>
</tr>
<tr>
<td>Light physical activity</td>
<td>&gt; 100 counts/min</td>
<td>1.5 ≤ METs &lt; 4</td>
</tr>
<tr>
<td>Moderate physical activity</td>
<td>≥ 2296 counts/min</td>
<td>4 ≤ METs &lt; 6</td>
</tr>
<tr>
<td>Vigorous physical activity</td>
<td>≥ 4012 counts/min</td>
<td>METs ≥ 6</td>
</tr>
</tbody>
</table>

3.5.6. Short Messaging Service-Track-Questionnaire (SMS-T-Q)
Information about sports participation used in study II was measured weekly by “Short Messaging Service-Track-Questionnaire” (SMS-T-Q) version 2.1 (New Agenda Solutions, SMS-Track ApS, Esbjerg). SMS-Track is a web based IT-system (SMS Survey) developed as a tool for frequent surveillance. The method functions as a “follow up” procedure and was used in study II to investigate leisure time sports (LTS) participation over time. The questionnaire was automatically sent to the parent’s mobile phone once a week including a question about LTS: “How many times did [NAME OF CHILD] engage in sports during the last week”? The parents answered with a relevant number between 0 and 8. The answers 0 to 7 represented the unique number of times engaging in sports, whereas 8 indicated “more than 7 times”. The returned answers were automatically recorded and inserted into a database. To improve compliance rate, a reminder was automatically sent, if participants had not responded 48 hours and 96 hours after receiving the initial message. Parent reports were considered appropriate in this cohort as self-reported questionnaires in young children are considered unreliable. SMS-T-Q was introduced to the first three schools in November 2008 and thereafter one school at a time was randomly included every month with all 10 schools included by August 2009.

3.6. Statistical analyses
In all three studies descriptive statistics were calculated for all dependent and independent variables at baseline and follow up the results were presented as means, standard deviations (SD), range, and medians and lower and upper quartiles.
Materials and methods

(only study I) for continuous variables and frequencies by pubertal stages were stratified by gender. Shapiro-Wilk’s test and q-q plots were used along with residual plots from the regressions, to check assumptions of normality of the data. These gave no reason for concern in either of the studies. Linearity between the transformed responses and the explanatory variables under consideration were assessed graphically and explanatory variables were transformed when needed. Multilevel linear regression model was used (using xtmixed command from STATA 12.1) to assess the relationship between BMC, BMD and BA accretion and variables of interest. Data were sampled in a hierarchical structure (schools, classes, children) and each level in this structure adds to the random variation in the data and therefore the choice of using a random effect model was made. Backward elimination was used for reduction from an initial model, containing all the explanatory variables, at each stage; the variable chosen for exclusion was the one leading to the smallest reduction in the regression sum of squares. Effects with p-values < 0.05 were considered significant. All statistical calculations were performed using the STATA (version 12, Stata Corp LP, College Station, TX).

3.6.1. Study I

Mean number of minutes, in each activity intensity level per day of the child was generated from the accelerometer output. These were further converted into percentages of activity in each of the three intensity intervals, “sedentary”, “low” and “moderate to high”. The proportions are denoted \( \pi_s, \pi_l \) and \( \pi_{mh} \) respectively, and are subject to the constraints

\[
\pi_s + \pi_l + \pi_{mh} = 100\%
\]

and

\[
0 < \pi_s, \pi_l, \pi_{mh} < 100\%.
\]

This form is not suitable when considering linear regression models. Note also that only two of them can vary freely within the given boundaries. In order to handle the above-mentioned boundaries a method not previously described was developed for this presented study. To express the physical activity variables in a way that handles the constraints, we choose one level as a reference. The proportions of the two remaining intensity levels are converted into log odds relative to the proportion of the reference level.
Materials and methods

In this case low activity was chosen as the reference level. This gives us two variables that will represent the physical activity:

\[
\theta_{mh} = \log \left( \frac{\pi_{mh}}{\pi_l} \right)
\]

and

\[
\theta_i = \log \left( \frac{\pi_s}{\pi_l} \right)
\]

The relationship between BMC, BMD and BA accretion and the two categories of physical activity intensity levels as described above were assessed by multilevel regression analyses. BMC, BMD or BA at follow up was adjusted for BA (only BMC), height, log(weight), age, gender, puberty stage at follow up and BMC, BMD or BA at baseline, all representing fixed effects and with school and class as random effects. By adjusting for baseline DXA outcome we captured the bone accrual during the two year follow up period. The study aimed to evaluate the relationship between proportions of physical activity at different intensity levels, sedentary, low and moderate-high activity, and the outcome variables BMC, BMD and BA.

A linear regression model gives the best fitting straight line to describe the association between a variable and an outcome. A multiple linear regression model for the effect of say two exposure variables, \(x_1\) and \(x_2\), on an outcome variable \(y\) is given by:

\[
y = \beta_0 + \beta_1 x_1 + \beta_2 x_2.
\]

In our context the exposure variables are the physical activity variables, \(x_1 = \log \left( \frac{\pi_{mh}}{\pi_l} \right)\) and \(x_2 = \log \left( \frac{\pi_s}{\pi_l} \right)\) that represents the physical activity on logit scale, and we consequently test the impact of on our outcome variables \(y_{BMC}, y_{BMD}\), and \(y_{BA}\). To explain the model we consider what happens when the distribution between the physical activity categories change from \((\pi_s, \pi_l, \pi_{mh})\) to \((\pi'_s, \pi'_l, \pi'_{mh})\)

The change in the mean value is then:

\[
\Delta \mu = \mu' - \mu = \beta_1 \log \left( \frac{\pi'_{mh}}{\pi_l} \right) + \beta_2 \log \left( \frac{\pi'_s}{\pi_l} \right) - \beta_1 \log \left( \frac{\pi_{mh}}{\pi_l} \right) + \beta_2 \log \left( \frac{\pi_s}{\pi_l} \right)
\]
Materials and methods

\[ \Delta \mu = \beta_1 \left\{ \log \left( \frac{\pi'_{mh}}{\pi_{lh}} \right) - \log \left( \frac{\pi_{mh}}{\pi_{lh}} \right) \right\} + \beta_2 \left\{ \log \left( \frac{\pi'_{s}}{\pi_{s}} \right) - \log \left( \frac{\pi_{s}}{\pi_{s}} \right) \right\} \]

\[ = \beta_1 \log \left( \frac{\pi'_{mh}}{\pi_{mh}} \right) + \beta_2 \left( \frac{\pi'_{s}}{\pi_{s}} \right) - (\beta_1 + \beta_2) \log \left( \frac{\pi_{l}}{\pi_{l}} \right) \]

The values of \( \beta_1 \) and \( \beta_2 \) hence determine the size of the change in \( \Delta \mu \) as a function of the log of the relative changes within each intensity level.

Consider in particular the cases (displayed graphically) of an exchange in activity between two groups, keeping the third constant. If for example \( \pi'_{l} = \pi_{l} \) we get:

\[ \Delta \mu = \mu' - \mu \]

\[ = \beta_1 \log \left( \frac{\pi'_{mh}}{\pi_{mh}} \right) + \beta_2 \left( \frac{\pi'_{s}}{\pi_{s}} \right) - (\beta_1 + \beta_2) \log \left( \frac{\pi_{l}}{\pi_{l}} \right) \]

\[ = \beta_1 \log \left( \frac{\pi'_{mh}}{\pi_{mh}} \right) + \beta_2 \left( \frac{\pi'_{s}}{\pi_{s}} \right) - (\beta_1 + \beta_2) \log 1 \]

\[ = \beta_1 \log \left( \frac{\pi'_{mh}}{\pi_{mh}} \right) + \beta_2 \left( \frac{\pi'_{s}}{\pi_{s}} \right) \]

The graphs (Figure 4) provided to interpret the results were conducted to display the prediction of the mean values for different combinations of the three levels of physical activity; sedentary (\( \pi_{s} \)), low (\( \pi_{l} \)), moderate to high (\( \pi_{mh} \)).

3.6.2. Study II

In the initial model we examined the impact on follow up bone traits of baseline bone traits, height, log(weight) gender, puberty, all at follow up, school type, mean leisure time sports participation (LTS) interactions between school type and LTS participation, gender and puberty, gender and school type and gender and mean LTS participation. We adjusted for baseline bone traits to control for the children’s initial size In addition BMC was adjusted for BA at follow up to avoid possible size-related artefacts in the analysis of bone mineral data 63.
3.6.3. Study III
Two different models were designed to describe the relationship between each of the outcome variables BMC and BA and the explanatory variables. The first set of models described how the outcome variables depend on anthropometric measures (height, BMI) in combination with age, gender and puberty. The second set of models was designed to describe the same variables by BC variables (LM, BF%) and age, gender and puberty. In all cases gender specific roles of anthropometric and BC related measures were accommodated by interaction terms in the regressions models. The variance inflation factor was used to assess potential multi collinearity between explanatory variables. Variables causing conflicts of independence were removed from the models. Box-Cox analyses were used to identify transformations that would ensure normality of the residuals. BMC was transformed by \((BMC)^{0.2}\) and BA was transformed by \((BA)^{0.4}\) in model I. BMC was transformed by \((BMC)^{0.5}\) in model II. The Box-Cox analyses indicated no need for transformation of BA in model II. Linearity between the transformed responses and the explanatory variables were assessed graphically. None of the explanatory variables were transformed.

BMD is defined as a BMC/BA and hence assessed by the regression models for these two quantities. Data (BMC and BA) were transformed to meet the requirements of normality. The usual interpretations of the parameters therefore apply on the transformed scale. The fitted models for the impact on bone health of each of the outcome variables are presented on the ordinary scale in Figure 5 for the explanatory variables of particular interest.
4. Results

4.1. Participants characteristics
A total of 1512 children from the preschool year to 4th grade (age range 5.5-11 years) were invited to participate in The CHAMPS study-DK from baseline in September 2008, of which 1210 (80 %) accepted.

A sub group was created for the three studies. This group comprised of children from 2nd to 4th grade (7.2-12 years) at baseline. Of these, 742/800 (93%) accepted the invitation to participate and 682/742 (92%) participated at two-year follow up (49% boys, 51% girls). The characteristics of the participants at baseline and follow up regarding age, gender, anthropometry, densitometry, accelerometer data are reported in Table 3a-3c.

**Figure 5:** Flowchart of the participants
Table 3a: Baseline (2008) and follow up (2010) characteristics of the boys participating in The CHAMPS study-dk stratified by school type.

<table>
<thead>
<tr>
<th>Key variables</th>
<th>Boys baseline (2008) (n=344)</th>
<th>Boys follow-up (2010) (n=335)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sports-schools (n=180)</td>
<td>Traditional schools (n=164)</td>
</tr>
<tr>
<td>Age (yrs.)</td>
<td>9.6(0.9)</td>
<td>9.6(0.9)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>33.3(6.6)</td>
<td>32.7(6.4)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>140.6(7.5)</td>
<td>139.6(8.1)</td>
</tr>
<tr>
<td>BMI</td>
<td>16.7(2.3)</td>
<td>16.7(1.9)</td>
</tr>
<tr>
<td>DXA measures (body composition)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>25.5(3.4)</td>
<td>25.0(3.3)</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>6.2(4.1)</td>
<td>6.2(3.9)</td>
</tr>
<tr>
<td>Body fat percentage(%)</td>
<td>17.5(8.0)</td>
<td>17.9(7.5)</td>
</tr>
<tr>
<td>DXA measures (bone)(TBLH)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMC (g)</td>
<td>896.9(186.0)</td>
<td>879.7(196.5)</td>
</tr>
<tr>
<td>BMD (g/cm²)</td>
<td>0.76(0.05)</td>
<td>0.76(0.05)</td>
</tr>
<tr>
<td>BA (cm²) (Lower limb)</td>
<td>1158.1(174.8)</td>
<td>1139.5(177.1)</td>
</tr>
<tr>
<td>BMC (g)</td>
<td>432.1(100.4)</td>
<td>424.7(108.5)</td>
</tr>
<tr>
<td>BMD (g/cm²)</td>
<td>0.87(0.07)</td>
<td>0.87(0.08)</td>
</tr>
<tr>
<td>BA (cm²)</td>
<td>489.1(78.9)</td>
<td>480.5(80.3)</td>
</tr>
<tr>
<td>Pubertal status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tanner stage (n)</td>
<td>1/2/3/4/5</td>
<td>81/87/9/0/0</td>
</tr>
</tbody>
</table>

Note: Results are reported as mean (SD) sorted by gender and schools. Results for pubertal stage are reported as numbers.
**Table 3b:** Baseline (2008) and follow up (2010) characteristics of the girls participating in The CHAMPS study-dk stratified by school type.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sports-schools (n=222)</td>
<td>Traditional schools (n=151)</td>
</tr>
<tr>
<td>Age (yrs.)</td>
<td>9.5(0.9)</td>
<td>9.6(0.8)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>32.1(6.0)</td>
<td>32.9(7.2)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>138.1(7.2)</td>
<td>138.7(7.3)</td>
</tr>
<tr>
<td>BMI</td>
<td>16.7(2.1)</td>
<td>16.9(2.4)</td>
</tr>
<tr>
<td>DXA measures (body composition)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>22.9(3.1)</td>
<td>22.9(3.4)</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>7.5(3.7)</td>
<td>8.3(4.4)</td>
</tr>
<tr>
<td>Body fat percentage(%)</td>
<td>22.6(7.2)</td>
<td>24.0(7.8)</td>
</tr>
<tr>
<td>DXA measures (bone)(TBLH)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMC (g)</td>
<td>843.6(203.6)</td>
<td>849.5(205.9)</td>
</tr>
<tr>
<td>BMD (g/cm²)</td>
<td>0.75(0.06)</td>
<td>0.75(0.06)</td>
</tr>
<tr>
<td>BA (cm²)</td>
<td>1103.2(179.4)</td>
<td>1109.0(183.9)</td>
</tr>
<tr>
<td>(Lower limb)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMC (g)</td>
<td>410.1(102.1)</td>
<td>413.1(105.7)</td>
</tr>
<tr>
<td>BMD (g/cm²)</td>
<td>0.86(0.08)</td>
<td>0.86(0.08)</td>
</tr>
<tr>
<td>BA (cm²)</td>
<td>467.7(74.6)</td>
<td>470.7(78.7)</td>
</tr>
<tr>
<td>Pubertal status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tanner stage (n)</td>
<td>160/54/4/3/0</td>
<td>121/29/1/1/0</td>
</tr>
</tbody>
</table>

**Note:** Results are reported as mean (SD) sorted by gender and schools. Results for pubertal stage are reported as numbers.
Results

Table 3c: Descriptive statistics of the participants with complete datasets n=602. October 2010 to March 2011.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Gender</th>
<th>Mean (SD)</th>
<th>25th %</th>
<th>Median</th>
<th>75th %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs.)</td>
<td>Boys</td>
<td>11.5 (0.89)</td>
<td>10.8</td>
<td>11.4</td>
<td>12.2</td>
</tr>
<tr>
<td></td>
<td>Girls</td>
<td>11.5 (0.87)</td>
<td>10.7</td>
<td>11.4</td>
<td>12.2</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>Boys</td>
<td>151.2 (8.88)</td>
<td>145</td>
<td>151.1</td>
<td>156.5</td>
</tr>
<tr>
<td></td>
<td>Girls</td>
<td>150.5 (8.3)</td>
<td>144.4</td>
<td>150.4</td>
<td>156.5</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>Boys</td>
<td>40.7 (8.46)</td>
<td>34.5</td>
<td>39.3</td>
<td>45.2</td>
</tr>
<tr>
<td></td>
<td>Girls</td>
<td>40.9 (8.81)</td>
<td>34.8</td>
<td>40.05</td>
<td>46</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>Boys</td>
<td>8.2 (5.02)</td>
<td>4.6</td>
<td>6.8</td>
<td>10.2</td>
</tr>
<tr>
<td></td>
<td>Girls</td>
<td>10.5 (5.0)</td>
<td>6.8</td>
<td>9.5</td>
<td>13.03</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>Boys</td>
<td>30.7 (4.97)</td>
<td>27.2</td>
<td>30.1</td>
<td>33.2</td>
</tr>
<tr>
<td></td>
<td>Girls</td>
<td>28.4 (4.96)</td>
<td>24.5</td>
<td>27.8</td>
<td>31.8</td>
</tr>
<tr>
<td>BMC (g)</td>
<td>Boys</td>
<td>1189 (267)</td>
<td>1007</td>
<td>1169</td>
<td>1314</td>
</tr>
<tr>
<td></td>
<td>Girls</td>
<td>1183 (313)</td>
<td>945.5</td>
<td>1142</td>
<td>1363</td>
</tr>
<tr>
<td>BMD (g/cm²)</td>
<td>Boys</td>
<td>0.83 (0.06)</td>
<td>0.79</td>
<td>0.83</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td>Girls</td>
<td>0.83 (0.08)</td>
<td>0.78</td>
<td>0.83</td>
<td>0.88</td>
</tr>
<tr>
<td>BA (cm²)</td>
<td>Boys</td>
<td>1405 (222)</td>
<td>1246</td>
<td>1399</td>
<td>1540</td>
</tr>
<tr>
<td></td>
<td>Girls</td>
<td>1390 (249)</td>
<td>1211.2</td>
<td>1372.3</td>
<td>1545.5</td>
</tr>
<tr>
<td>Accelerometer (Days registered)</td>
<td>Boys</td>
<td>6.1 (0.97)</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Girls</td>
<td>6.1 (0.96)</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Sedentary Activity (%)</td>
<td>Boys</td>
<td>62</td>
<td>58</td>
<td>62</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>Girls</td>
<td>64</td>
<td>61</td>
<td>64</td>
<td>68</td>
</tr>
<tr>
<td>Low activity (%)</td>
<td>Boys</td>
<td>29</td>
<td>26</td>
<td>29</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>Girls</td>
<td>29</td>
<td>26</td>
<td>29</td>
<td>31</td>
</tr>
<tr>
<td>Moderate to high activity (%)</td>
<td>Boys</td>
<td>9</td>
<td>6</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Girls</td>
<td>7</td>
<td>6</td>
<td>7</td>
<td>9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pubertal stages</th>
<th>Boys (n)</th>
<th>Girls (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tanner stage 1 (n)</td>
<td>55</td>
<td>97</td>
</tr>
<tr>
<td>Tanner stage 2 (n)</td>
<td>139</td>
<td>124</td>
</tr>
<tr>
<td>Tanner stage 3 (n)</td>
<td>87</td>
<td>102</td>
</tr>
<tr>
<td>Tanner stage 4 (n)</td>
<td>15</td>
<td>23</td>
</tr>
<tr>
<td>Tanner stage 5 (n)</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Fishers exact test</td>
<td></td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

Note: *The Fishers exact refers to the comparison of the pubertal stages between boys and girls at follow-up.
Figures are means (SD) presented along with median, 25th % and 75th%
Results

4.2. Study I

Complete datasets (DXA scans and accelerometer) were obtained in 602/742 (81%) children.

The relationship between BMC and the PA intensity levels represented by $\theta_{mh}$ and $\theta_s$ were assessed (Table 4). There was a positive relationship between $\theta_{mh}$ and BMC and a positive relationship between $\theta_s$ and BMC. There was a gender interaction with $\theta_s$ leading to an additional negative effect for boys on BMC.

**Example 1**: The fitted model predicts that for a girl a change in proportions of activity levels from $(\pi_s, \pi_l$ and $\pi_{mh}) = (60\%, 30\%, 10\%)$ to $(65\%, 27\%, 8\%)$ will lead to a change in BMC of $20.94 \times \log\left(\frac{0.65}{0.27}\right) + 27.77 \times \log\left(\frac{0.1}{0.3}\right) - 20.94 \times \log\left(\frac{0.6}{0.3}\right) = 2.7$ g, whereas it will be -4.0 g for a boy (see section 4.6.1 Study I).

**Example 2**: A change in proportions of activity levels from $(\pi_s, \pi_l$ and $\pi_{mh}) = (70\%, 22\%, 8\%)$ to $(75\%, 15\%, 10\%)$ will lead to a change in BMC of $20.94 \times \log\left(\frac{0.75}{0.15}\right) - 20.94 \times \log\left(\frac{0.08}{0.22}\right) - 27.77 \times \log\left(\frac{0.70}{0.22}\right) = 25$ g, whereas it will be 8.9 g for a boy.

**Table 4**: Effect of the physical activity on BMC at follow up

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient estimate</th>
<th>Standard error</th>
<th>P- value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMC baseline</td>
<td>0.44</td>
<td>0.03</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BA follow up</td>
<td>0.97</td>
<td>0.04</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gender</td>
<td>35.20</td>
<td>9.50</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>$\theta_{mh} = \log\left(\frac{\pi_{mh}}{\pi_l}\right)$</td>
<td>20.94</td>
<td>6.58</td>
<td>0.001</td>
</tr>
<tr>
<td>$\theta_s = \log\left(\frac{\pi_s}{\pi_l}\right)$</td>
<td>27.77</td>
<td>8.17</td>
<td>0.001</td>
</tr>
<tr>
<td>Gender # $\theta_s$</td>
<td>-36.03</td>
<td>13.11</td>
<td>0.006</td>
</tr>
<tr>
<td>Height follow up</td>
<td>-3.96</td>
<td>0.17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Puberty follow up</td>
<td>29.99</td>
<td>3.52</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gender # puberty</td>
<td>-16.95</td>
<td>5.14</td>
<td>0.001</td>
</tr>
</tbody>
</table>

*Note: $\pi_{mh}, \pi_s, \pi_l$ represents the percentage of the total time spent in, moderate-high, sedentary and low intensity activity respectively. #: Interaction. The girls were chosen as the reference level for gender.*
Results

The relationship between BMD and the PA intensity levels represented by $\theta_{mh}$ and $\theta_s$ were assessed (Table 5). There was a positive relationship between $\theta_{mh}$ and BMD. There was no significant relationship between $\theta_s$ and BMD and no gender interaction.

Table 5: Effect of the physical activity on bone mineral density at follow up

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient estimate</th>
<th>Standard error</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMD baseline</td>
<td>0.966</td>
<td>0.02</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>$\theta_{mh} = \log(p_{mh} / p_l)$</td>
<td>0.009</td>
<td>0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Height follow up</td>
<td>0.001</td>
<td>0.0002</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Log(weight follow up)</td>
<td>0.039</td>
<td>0.008</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gender</td>
<td>0.017</td>
<td>0.005</td>
<td>0.001</td>
</tr>
<tr>
<td>Puberty follow up</td>
<td>0.016</td>
<td>0.002</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gender # puberty</td>
<td>-0.014</td>
<td>0.003</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Intercept</td>
<td>-0.150</td>
<td>0.018</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Note: $p_{mh}$, $p_l$ represents the percentage of the total time spent in, moderate-high and low intensity activity respectively. #: Interaction. The girls were chosen as the reference level for gender.

The relationships between BA and the PA levels represented by $\theta_{mh}$ and $\theta_s$ were assessed (Table 6). There was a positive relationship between $\theta_{mh}$ and BA but no significant relationship between $\theta_s$ and BA and furthermore there was no significant gender interaction.

Table 6: Effect of the physical activity on bone area at follow up

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient estimate</th>
<th>Standard error</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BA baseline</td>
<td>0.62</td>
<td>0.49</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>$\theta_{mh} = \log(p_{mh} / p_l)$</td>
<td>22.18</td>
<td>4.20</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Height follow up</td>
<td>6.87</td>
<td>0.69</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Log(weight follow up)</td>
<td>285.20</td>
<td>18.12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Puberty follow up</td>
<td>24.47</td>
<td>2.32</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gender</td>
<td>-26.33</td>
<td>5.52</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Intercept</td>
<td>-1405.85</td>
<td>86.01</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Note: $p_{mh}$, $p_l$ represents the percentage of the total time spent in, moderate-high and low intensity activity respectively. The girls were chosen as the reference level for gender.
Results

**Figure 6:** Graphs presenting the effect of changes in different configurations of the proportion of total time in physical activity and sedentary, low and moderate to high level activity on the bone traits BMC, BMD and BA.

*Note: The different intensity levels were fixed at the mean values. The exchange between the two remaining intensity levels were between the proportions of the total time in activity, at the 10th, 25th, 50th, 75th and 90th percentiles. The solid lines refer to the girls whereas the dotted lines refer to the boys.*
Results

The graphs in Figure 6 illustrate the impact on bone health represented by BMC, BMD and BA when a certain level of physical intensity was kept fixed at the mean value allowing an exchange of time spent in the two remaining intensity levels to occur. This exchange occurred between the time spent in the two remaining intensity levels at their 10th, 25th, 50th, 75th and 90th percentiles. The solid lines refer to the girls whereas the dotted lines refer to the boys.

In the first column sedentary intensity level is fixed at the mean value 64% and 62% of the total time in activity for girls and boys respectively. An exchange between low-level activity and moderate to high level activity can occur and when increasing the proportion of time in low-level activity the BMC, BMD and BA values will decrease for both genders. In the second column low intensity PA is fixed at the mean level 29% of the total time in activity for boys and girls, and an exchange between moderate to high and sedentary can occur. The bone traits increases as the proportion of time spent in moderate to high-level PA increases opposed to sedentary level PA. In the third column moderate to high level PA is fixed at the mean value 7% and 9% of the total time in activity for girls and boys respectively. An exchange between sedentary level activity and low-level activity reveals an increase in bone outcome when the proportion of time in sedentary level activity increases opposed to low-level activity.

4.3. Study II

Complete datasets were obtained in 633/742 (85%) children. Fifty-six per cent of the children attended sport schools and 44% traditional schools at baseline and follow-up (Table 7). Of the children who did not participate in LTS, we found that the majority of this group of children attended sports schools n=131/195 (67%). The response rate of SMS (Q-T) was 95.9 %.

The relationship between school-type and BMC, BMD and BA accretion was assessed separately. All subsequent results refer to the final models for bone traits at follow up.
Results

BMC (TBLH) (Table 8) was adjusted for BMC at baseline, BA, height and puberty, all at follow up. Puberty had a positive effect for girls but was insignificant for boys. The amount of LTS was significant with different effects for boys and girls. The estimated increase of BMC for each extra hour of LTS for boys was 9.48 g and the estimated increase of BMC for girls was $9.48 - 6.63 = 2.85$ g. All effects of school type were insignificant.

BMC (LL) (Table 8) was adjusted for the same variables as for BMC (TBLH). However, height at follow up was insignificant whereas log(weight) was significant with a positive effect. The amount of LTS was significant with different effects for boys and girls. All effects of school type were insignificant.

Table 8: The impact on BMC accruement of different variables, during a two-year period. Multilevel regression analyses with backward elimination of insignificant variables were performed.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient estimate</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TBLH$^4$</td>
<td>LL$^5$</td>
</tr>
<tr>
<td><strong>BMC Baseline</strong></td>
<td>0.42***</td>
<td>0.68***</td>
</tr>
<tr>
<td><strong>BA Follow-up</strong></td>
<td>0.99***</td>
<td>0.55***</td>
</tr>
<tr>
<td><strong>Height Follow up</strong></td>
<td>-4.20***</td>
<td>---</td>
</tr>
<tr>
<td><strong>Log(weight) Follow up</strong></td>
<td>---</td>
<td>105.96***</td>
</tr>
<tr>
<td><strong>Puberty Follow up</strong></td>
<td>13.82***</td>
<td>9.25***</td>
</tr>
<tr>
<td><strong>Gender#Puberty$^1$</strong></td>
<td>16.12***</td>
<td>6.62***</td>
</tr>
<tr>
<td><strong>Mean LTS$^3$</strong></td>
<td>9.48***</td>
<td>6.76***</td>
</tr>
<tr>
<td><strong>Gender#Mean LTS$^2$</strong></td>
<td>-6.63*</td>
<td>-4.15***</td>
</tr>
</tbody>
</table>

*Note:*$^1$ The interactions between gender and puberty and gender and mean sport respectively, with boys representing the reference group.$^2$ Leisure time sport (LTS), $^3$ Total body less head (TBLH), $^5$ Lower limb (LL) $^*p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$
Results

BMD (TBLH) (Table 9) was adjusted for BMD at baseline, height, log(weight) at follow up and gender and a significant positive interaction was observed between gender and puberty for only girls. The amount of LTS was significant. School type and interactions between school type and gender and school type and mean LTS participation were all insignificant. (All p>0.05)

BMD (LL) was adjusted for the same variables as BMD (TBLH). The amount of LTS was significant. School type and interactions between school type and gender and school type and the amount of LTS were all insignificant.

**Table 9:** The impact on BMD accrualment of different variables, during a two-year period. A multilevel regression analyses with backward elimination of insignificant variables were performed.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Coefficient estimate (β)</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TBLH</td>
<td>LL</td>
</tr>
<tr>
<td>BMD Baseline</td>
<td>0.963***</td>
<td>0.975***</td>
</tr>
<tr>
<td>Height Follow up</td>
<td>0.001***</td>
<td>---</td>
</tr>
<tr>
<td>Log(weight) Follow up</td>
<td>0.043***</td>
<td>0.088***</td>
</tr>
<tr>
<td>Gender^1</td>
<td>-0.017***</td>
<td>-0.019***</td>
</tr>
<tr>
<td>Gender#puberty^2</td>
<td>0.016***</td>
<td>0.019***</td>
</tr>
<tr>
<td>Mean LTS^3</td>
<td>0.004***</td>
<td>0.007***</td>
</tr>
</tbody>
</table>

*Note:*^1 Boys are the reference group. *^2 The interactions between gender and puberty (the difference between boys and girls) with boys representing the reference group. *^3Leisure time sport, *^4Total body less head (TBLH), *^5 Lower limb (LL) *p<0.05, **p<0.01, ***p<0.001
Results

BA (TBLH) (Table 10) was adjusted for BA at baseline, height, log(weight), age, puberty at follow up. There was a significant interaction between gender and puberty leading to an additional positive effect on BA for girls. The amount of LTS was significant. School type and interactions between school type and gender and school type and the amount of LTS were all insignificant (All p>0.05).

BA (LL) (Table 10) was adjusted for the same variables as BA (TBLH). The amount of LTS was however insignificant. School type and interactions between school type and gender and school type and the amount of LTS were all insignificant (All p>0.05).

Table 10: The impact on BA accrual of different variables, during a two-year period. A multilevel regression analysis with backward elimination of insignificant variables was performed.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Coefficient estimate (β)</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TBLH&lt;sup&gt;4&lt;/sup&gt;</td>
<td>LL&lt;sup&gt;5&lt;/sup&gt;</td>
</tr>
<tr>
<td>BA Baseline</td>
<td>0.65***</td>
<td>0.65***</td>
</tr>
<tr>
<td>Height Follow up</td>
<td>7.09***</td>
<td>2.64***</td>
</tr>
<tr>
<td>Log(weight) Follow up</td>
<td>273.48***</td>
<td>104.92***</td>
</tr>
<tr>
<td>Age Follow up</td>
<td>-6.55*</td>
<td>-2.27*</td>
</tr>
<tr>
<td>Puberty Follow up</td>
<td>16.96***</td>
<td>3.86***</td>
</tr>
<tr>
<td>Gender#puberty&lt;sup&gt;1&lt;/sup&gt;</td>
<td>10.99***</td>
<td>-1.92***</td>
</tr>
<tr>
<td>Mean LTS&lt;sup&gt;3&lt;/sup&gt;</td>
<td>4.65*</td>
<td>---</td>
</tr>
</tbody>
</table>

Note: <sup>1</sup>The interactions between gender and puberty with boys representing the reference group. 
<sup>3</sup>Leisure time sport, <sup>4</sup>Total body less head (TBLH), <sup>5</sup>Lower limb (LL). *p<0.05, **p<0.01, ***p<0.001
Results

4.4. Study III
The accretions of BMC and BA were assessed by two different sets of predictors using multilevel regression models with schools, classes and children as random effects. Model I used simple anthropometric variables whereas model II used BC variables. Results are listed in Table 11 and Table 12 respectively. Except for BA in model II, the outcome variables were transformed by suitable power transformations to ensure normality. The estimated effects described below are therefore only linear on the scale of the transformed outcomes. The effects do however retain the same directions when back transformed, as the transformations are monotonic.

Model I
The relationship between BMC accretion and anthropometry was assessed. Of the examined explanatory variables; height, BMI, gender and puberty in girls positively predicted the accrual of BMC. The effect on BMC of puberty in boys was insignificant (Table 11). Gender interactions with height and BMI were removed from the model due to multi co-linearity.

The relationship between BA accretion and anthropometry was assessed. From the initial model the explanatory variables height and BMI predicted the BA accrueement positively. The effect on BA of puberty was insignificant (Table 11). Gender interactions with height and BMI were removed from the model due to multi co-linearity.
Results

Table 11: Model I. Coefficient estimates of the significant parameters for the regression of the Box Cox transformed BMC and BA. Backward elimination of insignificant parameters was performed.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Coefficient estimate for the regression of ((BMC)^{0.2}) (\beta) (CI)</th>
<th>P Value</th>
<th>Coefficient estimate for the regression of ((BA)^{0.4}) (\beta) (CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height</td>
<td>0.022 (0.020-0.023)</td>
<td>&lt;0.001</td>
<td>0.113 (0.111-0.115)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body mass index (BMI)</td>
<td>0.0185 (0.0182-0.0187)</td>
<td>&lt;0.001</td>
<td>0.128 (0.119-0.137)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gender</td>
<td>0.029 (0.017-0.041)</td>
<td>&lt;0.001</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Puberty girls</td>
<td>0.012 (0.009-0.016)</td>
<td>&lt;0.001</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>0.89 (0.85-0.92)</td>
<td>&lt;0.001</td>
<td>-1.47 (-1.67- -1.28)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Note: In the initial model one \((BMC)^{0.2}\) and \((BA)^{0.4}\) were adjusted for height, BMI, gender, puberty and the interaction between gender and puberty. The girls were chosen as the reference level for gender.

Model II

The relationship between BMC accretion and BC was assessed in model two (Table 12). From the initial model the examined explanatory variables, LM, BF%, age, puberty predicted the BMC accretion. There was an interaction between gender and BF% with a higher effect for boys than for girls (Table 12).

The relationship between BA accretion and BC was assessed. There were positive associations between LM, BF%, age and an interaction between gender and puberty with a positive impact of puberty in girls.

BMD was described as a function of BMC and BA it was therefore redundant to perform a regression for the outcome variable BMD. The result of the functional description of BMD is displayed in Figure 2.

In model I height was the most precise predictor of BMC compared to BMI (lowest AIC value) in both genders. In model II LM was the most precise predictor of BMC compared to BF% in both genders.
Results

Table 12: Model II. Coefficient estimates of the significant parameters for the regression of the Box Cox transformed BMC and BA. Backward elimination of insignificant parameters was performed.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Coefficient estimate for the regression of $\sqrt{BMC}$ $\hat{\beta}$ (CI)</th>
<th>P Value</th>
<th>Coefficient estimate for the regression of $BA$ $\hat{\beta}$ (CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lean mass (LM)</td>
<td>6.03e-04(5.8e-04-6.2e-04)</td>
<td>&lt;0.001</td>
<td>0.035(0.035-0.037)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fat percentage</td>
<td>0.103(0.091-0.114)</td>
<td>&lt;0.001</td>
<td>6.475(5.893-7.056)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age</td>
<td>0.684(0.619-0.749)</td>
<td>&lt;0.001</td>
<td>30.974(27.270-34.678)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fat percentage</td>
<td>0.015(0.004-0.027)</td>
<td>0.008</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>boys</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Puberty girls</td>
<td>0.337(0.246-0.428)</td>
<td>&lt;0.001</td>
<td>13.190(8.122-18.257)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Puberty boys</td>
<td>-0.122(-0.220--0.024)</td>
<td>0.014</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Intercept</td>
<td>5.815(5.386-6.243)</td>
<td>&lt;0.001</td>
<td>-168.253(-193.329-143.178)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Note: In the initial model one $\sqrt{BMC}$ and BA were adjusted for lean mass, fat percentage, the interaction between body fat percentage and gender (the interaction between body lean mass and gender was omitted from the model due to co-linearity), age, puberty and the interaction between gender and puberty. The girls were chosen as the reference level for gender.
Results

**Figure 7:** Graphs representing the effect of changes of height, body mass index, lean mass, body fat percentage on bone outcome (BMC, BA and BMD)

**Note:** The values of age, puberty, height, BMI (model one) and age puberty, lean mass and body fat (model two) were chosen at the mean values. The impact on bone outcome of a change in the parameters of interest (keeping the other explanatory variables constant) is shown above for each parameter of interest in model one (height, BMI) and model two (LM and BF%). The solid lines refer to the girls whereas the dotted lines refer to the boys.
5. Discussion

The findings presented in this PhD thesis are based on the results obtained in three studies of children aged 7-9 years old from the CHAMPS study DK in a two-year follow-up design. All the presented studies are concerning bone health in childhood. The major reason for the interest in bone health is to prevent osteoporosis and fracture risk later in life. The presented studies aimed to describe influences with an impact on bone accrueMENT in childhood in different ways and from different perspectives. In study I the focus was on the impact of the intensity of PA on bone accrueMENT. In study II the focus was to describe the impact of school type and LTS participation on bone accrueMENT and in study III the focus was to describe predictors of bone accrueMENT from two different models of growth. The three studies described a large cohort of healthy children in a longitudinal design and thereby add new perspectives into the research field.

5.1. Study I

The relationship between the proportions of time spent in PA at different intensity levels and bone development represented by BMC, BMD, and BA accrueMENT during two years was examined.

There was a positive relationship of the log odds of moderate to high and low intensity activity and BMC, BMD and BA accrueMENT over a two-year period indicating that changing the proportion of time in PA towards moderate to high intensity would have beneficial effects on bone health. There was a significant relationship between the log odds of sedentary relative to low intensity activity and BMC indicating that changing the proportion of time in PA towards sedentary opposed to low-level intensity also had a positive influence on bone health. This rather surprising but interesting result may reflect that sedentary behaviour is not necessarily negative for the bones compared to a general low PA level.

The positive relationship between weight bearing exercises, and bone health during growth has been well described \(^3,\)\(^{91}\). This study adds information about the intensity of PA rather than the type of PA.

Previous studies have examined the impact of intensity on bone health. Tobias JH et al. (2007) examined a cohort of \(n=4457\) children in the Avon Longitudinal Study of Parents and Children (ALSPAC) study. The study was performed in a cross-sectional design. They found a positive relationship between BA and BMD and moderate PA only \(^92\).
Discussion

Several other studies have presented cross-sectional data on the relationship between habitual PA and bone health, whereas this study provides longitudinal data. Sardina et al. (2008) examined the relationship between intensity and duration of PA and composite indices of femoral neck strength and bone mineral content of the femoral neck, lumbar spine and total body. They concluded that vigorous activity measured by accelerometers emerged as the main PA predictor of femoral neck strength only.

Habits of physical activity can be traced from childhood to adolescence and adulthood, and habits developed in early life may persist into adulthood. It has been suggested that children become less physically active and spend more time in sedentary activity as they age. It therefore seems of great importance to influence these behaviours of PA positively in childhood to prevent future side effects of sedentary behaviour.

Osteoporosis is a growing problem in adults and the elderly. When planning prevention strategies towards osteoporosis, all of the factors that affect bone health including PA patterns should be considered. The results of study I suggest that small changes in PA behaviour in childhood towards more moderate to high level PA opposed to low and sedentary intensity levels are sufficient to achieve beneficial effects on bone traits.

Focus on habitual physical activity during childhood is of major importance to consider when planning preventive strategies towards osteoporosis. Also in relation to bone health it is important to change children’s lifestyle from mainly inactivity to higher activity levels.

5.2. Study II

In study II the effect of a school based PE programme and the effect of participation in LTS on children’s bone health was examined. LTS had a positive impact on bone accrual in children with boys having the greatest effect from LTS participation. However, the analyses revealed no effect of school type on children’s bone mineral accrual during the two-year observation time. The hypothesis of a positive effect on bone traits of the sports schools for children with low amount of LTS participation suggested an examination of the interactions between school type and the amount of LTS participation. Such an effect was not found.

The design of the sports schools in the present study was not specifically intended to influence bone health in childhood and contained no specific elements of
Discussion

repetitive jumping or similar activities, although we would expect a school based PE programme with four extra PE lessons to include weight-bearing elements. The majority of the children who did not participate in LTS, attended sports schools n=131/195 (67%). The reason for this is uncertain but may be due to a behavioural effect of attending a sports school causing parents to reduce their child’s LTS due to practical reasons or that the child become less physically active during the leisure time due to the increased activity during school time even though children in the two school types had equal access to LTS facilities. This effect may represent a part of the reason why extra PE lessons in school do not have the expected effect on bone accrual during the two-year follow up period. However, we found that participation in LTS had a highly significant impact on bone accrualment. We expect that the children participate in LTS due to interest in a specific sport and this group of children may represent a proxy group for the most active children regardless of school type.

The findings in study II differ from several other well-designed studies where participation in different PE programs and the impact on bone health were examined 74, 76, 82, 97. In several of these studies the PE programs were designed to enhance bone health and PE lessons and PE homework contained elements of weight-bearing exercise, and impacts on bone mineralisation were seen 81, 98-101.

Several studies have examined the impact of specific LTS activities of bone accrualment, such as the study where young tennis players were found to have increased BMC on their loaded arm 45 and a study of non-elite gymnastics participation which was associated with musculoskeletal benefits on upper limb bone geometry, strength and muscle function 102. To our knowledge no other study has explored the relationship between general LTS participation and bone health in childhood.

The study presented does not support the creation of sports schools with the presented design if the main purpose is to enhance bone health. The specific weight baring elements included in other of the mentioned studies seems important to induce skeletal benefits.

The study finds positive relationships between LTS participation and bone health, and a hypothesis might be that the intensity achieved during LTS as opposed to PE lessons reaches higher levels and thereby benefits the bone accrualment as demonstrated in study I 103.
Discussion

5.3. Study III
In study III the aim was to describe the influence of anthropometry and BC on bone accrual in a large cohort of healthy Danish children. The main finding of the study was that height; BMI, LM and BF% were all positive predictors of bone accrual in children with height being a more precise predictor than BMI and LM being a more precise predictor than BF% in both genders.

An important reason for focusing on BC and the effect on bone health is that the incidence and prevalence of overweight and adiposity, usually assessed by BMI, has increased in most developed countries over the past decades. Several studies evaluated the effect of overweight and adiposity on bone traits in childhood. In most studies the conclusion is that body mass and the relative changes in the different components of BC are essential in the regulation of bone growth and accrual of bone mineral. In the HELENA study 330 adolescents were examined. The authors aimed to examine the independent association of FM and LM with bone mass. The conclusion was that a high bone mass in adipose adolescents is explained by their higher levels of LM. In study III, model I, both height and BMI predicts BMC, BA and BMD positively as well as LM and BF%.

In study III, the impact of changes in anthropometry (height, BMI) and BC (LM and BF%) on bone health parameters was evaluated and the gender differences of these effects. The two models created in this study reflected two different approaches to explain the development in bone traits in a longitudinal perspective form different sets of parameters. The overall conclusion of model I is that no gender differences in the effect of height and BMI on BMC, BA and BMD are observed. In model II the effect of LM on BMC, BA and BMD is the same in boys and girls and the curves show similar patterns of dependency with height and BMI (Figure 7). Gender differences are observed for the effect of only BF% on BMC, BA and BMD. Girls reach higher values of BMC at lower BF% values than boys with the same BF%. Girls reach higher BA than boys with the same BF% although with parallel curves. The effect of BF% on BMD shows two different curves with girls having higher BMD values at low BF% whereas this relationship is opposite at the higher BF% values.

In a cross-sectional study of 267 students (8-18 years) a correlation between bone mass and BC in both genders was observed with LM and fat mass index being the predictor of bone mass in males and females, respectively. In this longitudinal study all the regression analyses were stratified by gender and height was found to be the most precise predictor of bone traits in both boys and girls in model I and LM was the most precise predictor of bone health model II.
Discussion

Height and BMI have independently effects on BMC, BA and BMD in model I and LM and BF% have independently effects on bone health in model II.

The two models reflected two different approaches to explain the development in bone traits in a longitudinal perspective. The two models explain gender specific differences in the impacts of anthropometry and BC on bone accrueement in a simple easy assessable and cheap model (anthropometry) and a more complex model (BC). Both models predict bone accrueement in similar patterns although only a gender difference is observed with BF% as the predictor of bone accrueement.

5.4. Strengths and limitation

The study had some limitations. The creation of sports schools was a political decision in the municipality of Svendborg therefore the design was not a randomized controlled trial but a natural experiment. The children in the two groups were however, comparable at baseline regarding all measurements presented in this article. Information on the children’s PA level at baseline was not available, and it is not possible to conclude that the patterns of activity were equal at the beginning of the experiment.

Some other methodological aspects need to be mentioned. The collection of accelerometer data was performed at one-year follow-up. We had no knowledge of how this one period of measurements represented the child’s activity level over a longer time period, but we assumed that the measurements reflected the child’s activity level in general. Accelerometers have practical limitations as participant’s willingness to wear them. However, we succeeded in obtaining complete datasets on 81% of the participants. Another positive aspect of our study was that the children included in the analyses were measured with accelerometers at a mean of 6.1 days (4-11 days) and at a mean of 13 hours per day (11-14 hours per day). Trost et al demonstrated that 4-5 days of monitoring were needed to obtain an intra class coefficient of 0.8 in children, which we exceeded. In study I the analyses only addressed the effect of the relative distribution of PA among three levels. Although the total amount of activity may have an impact on the bone health, the data analysed here are not amenable for this question. PA measurements by accelerometers were obtained during the same yearly season for all children. The analyses in any of the studies did not consider potential confounders such as vitamin D status or nutritional habits. Data on the children’s socio-economic status were also not mentioned. However, we presented data obtained from a cohort of healthy children that show homogeneity in relation to anthropometry and BC at baseline.

The strengths of the study include the population size, the high percentage of follow-up measurements and the longitudinal design with the ability to measure
Discussion

accrueement of bone health outcome. Another strength is the data collection, including DXA as the reference standard method, for the measurement of the bone parameters BMC, BA and BMD and body composition (LM and BF%). Valid information on the children’s sports participation was achieved through SMS-T-QS with a large response rate of 95.9 % in a reliable follow up design.

5.5. Closing remarks
The three longitudinal studies presented provide additional and new information on the relationships between physical activity, physical education, body composition and bone accrueement. Physical activity has an impact on bone accrueement with different effects of the different intensity levels of PA. Leisure time sport has a positive impact on bone accrueement regardless of school type (sport schools versus normal schools). Attending a sports school as described in the present study does not have a positive influence on the bone accrueement in the examined children, which in part may be explained by behavioural changes in the children resulting in less LTS participation for children attending sports schools. Measurements of growth and body composition are all positive predictors of bone accrueement. Most important height is shown to be a positive predictor even when adjusted for BMI and vice versa, and lean mass is a positive predictor when adjusted for BF% and vice versa. In other words the predictors are independent of each other within the two models described. Whether the effect of PA and LTS is mediated by changes in body composition remains unanswered in these studies but poses an important and interesting question for further research.
6. Conclusions

6.1. Study I
A positive association was observed between physical activity and bone mineral accretion in childhood. In particular, moderate to high intensity activity had a positive influence on the bone development. The data suggest that changes in the distribution of time spent in different activity intensity levels will influence the bone mineral accrualment during a two year period with moderate to high intensity activity having a positive effect on bone outcome, whereas an increase in low-level and sedentary activity opposed to moderate to high-level activity will have a negative impact on bone health in childhood. This knowledge has public health as well as clinical relevance. Further research should focus on detecting the threshold of the beneficial effects of physical activity on bone health.

6.2. Study II
There was a highly significant positive relationship between participation in LTS and bone health. We did not find any impact on bone development (TBLH and lower limb) for children attending sports schools compared to children in traditional schools.

6.3. Study III
Height and BMI were both independent positive predictors of BMC, BA and BMD in childhood. However, height, BMI and LM did not reveal gender differences in bone mineral accrual as well as BF%. Height is a more precise predictor of BMC, BA and BMD than BMI. LM and BF% are both positive predictors of bone accrualment with LM being a the most precise predictor of bone accrualment in both boys and girls, supporting increased physical activity and training for increased bone accrualment in children. From simple anthropometry measured in every physical examination of a child, valuable predictive information is offered and seems to predict bone accrualment in the same pattern as the more complex measurement of LM.
7. Perspectives

When recognising osteoporosis as an increasing problem and a threat to public health and socioeconomic it is important to develop preventive strategies towards the disease. Childhood is an optimal period of introducing lifestyle changes and providing good habits of PA, which may have beneficial effects on bone health. Reaching a high PBM may possibly delay the development of osteoporosis. It seems important to find recommendations that will enhance bone health in childhood.

In the three studies presented results suggests that the intensity of the PA is important to enhance bone health and small changes in the proportion in PA at different activities are beneficial to bone health. Although no positive effect of attending sports school was revealed the LTS participation has a positive influence on the bone accrue. By supporting LTS and the national recommendations of PA including the recommendations of high intensity PA, children will possibly achieve beneficial effects on their bone health. However, these studies are not suitable for assessing specific general recommendations, as this was not the aim.

Blood samples were collected at baseline (2008) and at two year follow up (2010) and these will hopefully be analysed during 2013 giving excellent opportunities to investigate relationships between biomarkers of bone metabolism and PA, BC and bone health.

In future studies the evaluation of bone health and BC related to fractures and PA will proceed, continuing the analyses collected in this large-scale school study. A special interest in the effects and interactions of puberty, glucose metabolism, and FM on bone outcome will hopefully form the basis of the 5 year follow up measurements on the cohort of The CHAMPS study- DK during the fall 2013 and possibly another PhD study.

Achieving more knowledge about the relationships between PA and BC and the interactions between these parameters effects on bone health is possible by combining the knowledge of the three studies presented.
8. Summary

8.1. Summary in English
This PhD thesis is based upon three studies and the results obtained from The Childhood, Health, Activity and Motor Performance School study, Denmark.

1. In study I we evaluated the effect of physical activity (PA) at different intensity on children's bone health measured by DXA scans. We also evaluated the effect of changes in proportion of the time in PA at different intensity levels.
2. In study II we evaluated the effect of attending schools with four additional physical education (PE) lessons per week on children's bone health measured by DXA scans compared to children in public schools attending the mandatory two PE lessons per week. We also evaluated the effect of attending leisure time sport (LTS) on children's bone health, regardless of school type.
3. In study III we evaluated the parameters that influenced bone accrualment during a two-year period with particular interest in measurements related to body composition (BC) and anthropometry regardless of PA or school type.

All three studies were based on the same cohort of approximately 717 children with a mean age of 9.5 years (range: 7.7-12.0) attending 2nd-4th grade at baseline (fall 2008). The children were attending sports schools (n= 402) defined as public schools with 4 extra PE lessons and traditional schools (n=315) with the two mandatory PE lessons. Two year follow up examination were performed in n=660 children.

In study I we found a positive association between PA and bone mineral accretion in childhood. In particular moderate to high intensity activity had a positive influence on the bone health and a change in the distribution of time spent in different activity intensity levels has an influence on the bone mineral accrualment during a two-year period.

In study II a positive relationship between participation in LTS and bone health was found. There was no impact of school type on bone health (total body less head and lower limb).

In study III we found that different models of growth and BC may describe and predict bone mineral accrual differently. Height and BMI are both independent predictors of bone mineral content, bone area and bone mineral density in childhood. However, BMI and height do not distinguish gender difference in bone mineral accrual as well as lean mass and body fat percentage. Lean mass and body fat percentage are both independent predictors of bone accrualment with lean mass being a better predictor of bone accrualment in boys and in girls.
8.2. Summary in Danish (Resumé på dansk)
Denne Ph.D.-afhandling er baseret på tre undersøgelser der alle er baseret på resultater opnået fra Svendborg studiet.

1. I studie I evaluerede vi effekten af fysisk aktivitet ved forskellig intensitet på børns knogle sundhed målt ved DXA scanninger. Vi evaluerede ligeledes betydningen af ændringer i forhold mellem tid i fysisk aktivitet ved forskellig intensitet og knogle parametre målt ved DXA skanning.

2. I studie II evaluerede vi effekten på børns knogle sundhed målt ved DXA-scanninger af at deltage i skoler med fire ekstra idrætstimer om ugen i forhold til børn i offentlige skoler med to obligatoriske idrætstimer om ugen. Vi vurderede ligeledes effekten på børns knogle sundhed af at deltage fritids sport, uanset skoletype.

3. I studie III undersøgte vi de parametre, som påvirkner knogle opbygningen i løbet af en to årig periode med særlig interesse for målinger relateret til kropssammensætning (muskel masse, fedt masse og fedt procent) samt betydningen af antropometri (højde og vægt) uanset niveau af daglig fysisk aktivitet eller skole type.

Alle tre undersøgelser er baseret på den samme kohorte af 717 børn med en gennemsnitsalder på 9,5 år (7,7 - 12,0) fra 2.-4. klasse ved studiets begyndelse (efteråret 2008). Børnene deltog i idrætsskoler (n = 402) defineret som folkeskoler med 4 ekstra idrætstimer/uge sammenlignet med folkeskoleskoler (n = 315) med to obligatoriske idrætstimer. Ved to års opfølgning blev 660 børn undersøgt.

I studie I var der en positiv sammenhæng mellem fysisk aktivitet og knoglemineralindhold tilvækst i barndommen. Især moderat til høj intensiv aktivitet har en positiv indflydelse på knogle sundhed. Vi fandt, at en ændring i fordelingen af tid brugt på fysisk aktivitet ved forskellig intensitet har indflydelse på knogle mineral tilvæksten i løbet af en to årig observationsperiode.

I studie II var der en positiv sammenhæng mellem deltagelse i fritidssport og knoglesundhed. Der var ingen indvirkning af skoletype på knogle sundhed i den observerede periode.

I studie III kunne forskellige modeller for vækst og kropssammensætning beskrive og prædiktere udviklingen i knoglemineralindhold. BMI og højde er vigtige uafhængige prædiktorer for udviklingen i knogle mineralisering i barndommen men effekten synes ikke at være forskellig for de to køn til. Muskel masse og fedtprocenten er begge prædiktorer for knogle tilvæksten. Højde og muskelmassen konkluderes at være den bedste prædiktor for knogle udviklingen hos piger og drenge.
9. References


89. Johansen B, Wedderkopp N. Comparison between data obtained through real-time data capture by SMS and a retrospective telephone interview. Chiropr Osteopat 2010;18:10.


10. Appendix I

Pubertal self-assessment questionnaire

Girls

Skema vedr. pubertetsudvikling

Piger

Navn: ____________________________  Dato: ____________
ID: ____________________________
Alder: ____________________________

Kønsbøjning

Brystudvikling

- Der er ingen kønsbøjning

- Der er næsten ikke eller ganske lidt kønsbøjning

- Kønsbøjningen er blevet lidt markere, tyder mere

- Kønsbøjningen er næsten som hos en ung, voksen kvinde

- Kønsbøjningen er som hos en ung, voksen kvinde

- Brystvorten er ikke begyndt at vokse

- Brystvorten er lige begyndt at vokse

- Selve bryster er ved at vokse

- Brystet er næsten som hos en ung, voksen kvinde

- Brystet er vokset som hos en ung, voksen kvinde

Menstruation: Ja [ ]  Nej [ ]
Boys

Pubertal self-assessment questionnaire

Skema vedr. pubertetsudvikling

_Drenge_

Navn: ___________________________ Dato: _________
ID: ______________________________
Alder: __________________________

Kønshår

- Der er icke kropsbehåring
- Der er næsten icke eller ganske lidt kornsbehåring
- Kornsbehåringen er blevet lidt mørkere, fylder mere
- Kornsbehåringen er næsten som hos en ung, voksen mand
- Kornsbehåringen er som hos en ung, voksen mand
11. Appendix II Manuscripts

Manuscript I


Manuscript II


Manuscript III

The intensity of physical activity influences bone mineral accrual in childhood: the childhood health, activity and motor performance school (the CHAMPS) study, Denmark

Malene Heidemann1*, Christian Mølgaard1,5, Steffen Husby1, Anders J Schou1, Heidi Klakk3, Niels Chr Møller3, René Holst4 and Niels Wedderkopp2

Abstract

Background: Studies indicate genetic and lifestyle factors can contribute to optimal bone development. In particular, the intensity level of physical activity may have an impact on bone health. This study aims to assess the relationship between physical activity at different intensities and Bone Mineral Content (BMC), Bone Mineral Density (BMD) and Bone Area (BA) accretion.

Methods: This longitudinal study is a part of The CHAMPS study-DK. Whole-body DXA scans were performed at baseline and after two years follow up. BMC, BMD, and BA were measured. The total body less head (TBLH) values were used. Physical activity (PA) was recorded by accelerometers (ActiGraph, model GT3X). Percentages of different PA intensity levels were calculated and log odds of two intensity levels of activity relative to the third level were calculated. Multilevel regression analyses were used to assess the relationship between the categories of physical activity and bone traits.

Results: Of 800 invited children, 742 (93%) accepted to participate. Of these, 682/742 (92%) participated at follow up. Complete datasets were obtained in 602/742 (81%) children. Mean (range) of age was 11.5 years (9.7-13.9). PA at different intensity levels was for boys and girls respectively, sedentary 62% and 64%, low 29% for both genders and moderate to high 9% and 7% of the total time. Mean (range) BMC, BMD, and BA was 1179 g (563–2326), 0.84 g/cm2 (0.64-1.15) and 1393 cm2 (851–2164), respectively. Valid accelerometer data were obtained for a mean of 6.1 days, 13 hours per day.

Conclusions: There was a positive relationship between the log odds of moderate to high-level PA versus low level activity and BMC, BMD and BA. Children with an increased proportion of time in moderate to high-level activity as opposed to sedentary and low-level activity achieved positive effects on BMC, BMD and BA.

Keywords: Dual energy X-ray absorptiometry, Bone health, Physical activity, Accelerometers

Background

Osteoporosis is a highly prevalent disease [1,2], which is costly for society [3]. The disease is characterized by systemic impairment of bone mass, bone strength and alterations in the bone micro architecture resulting in an increased risk of fractures [4]. Research has focused on the treatment of osteoporosis and the consequences of the disease. However, it is equally important to focus on disease prevention during childhood and adolescence. Peak bone mass (PBM) is the highest bone mass an individual obtains during a lifetime [5]. Factors that determine PBM are not fully understood. Studies indicate that both genetic factors and lifestyle, such as diet and physical activity during childhood can contribute to optimal bone development [6]. Low PBM is an important determinant of later osteoporosis and risk of fractures [7].
Previous research has shown positive effects of weight-bearing exercise on bone mineral accrual [8] and PA undertaken in childhood have sustained lasting positive influence on the adult skeleton [9].

Dual Energy X-ray Absorptiometry (DXA) can evaluate bone health by providing estimates of bone mineral density (BMD), bone mineral content (BMC), and bone area (BA). Workload during physical activity can be measured by oxygen consumption (VO$_2$) and heart rate (HR) [10]. These measures correspond well to an individual’s speed or power output [10]. However, neither of these methods were well suited for large-scale population based studies. The recently developed ActiGraph GT3X monitors use a triaxial accelerometer and provide activity counts for each vector as well as a composite vector magnitude of the three axes [11]. This method gives the opportunity to capture the complexity of habitual activity and to stratify the activity intensity into sedentary, low, moderate, and high activity by using the data from the vertical axis. The results from the vertical axis were used in this research study.

It still remains uncertain to what degree physical activity (PA) as measured by accelerometers has an impact on children’s bones, and there is no knowledge about which level, intensity or volume of PA is the most beneficial regarding bone mineral accrual in childhood.

Previous studies have reported cross-sectional data on the relationship between PA and bone health in childhood [12,13], but the longitudinal data on this relationship has not been published. This study provides such longitudinal data. The aim was to evaluate how habitual PA, defined as any PA involving muscle force including walking, running, cycling as well as more passive activities, influences bone health. The specific objective was to assess the relationship between physical activity at different intensities, measured by accelerometers, and BMC, BMD and BA accrual measured by DXA scans during a two-year period.

Methods

Study design

The study is a sub study of the CHAMPS study, DK, a natural experiment [14]. The study embraces ten public schools in the municipality of Svendborg, Denmark, with children from the pre-school year to 4th grade. Six schools chose to implement four additional lessons of physical education (PE) to their usual PE program and to educate PE-teachers in special age-related training principles (“sports schools”), and were matched to four schools continued with two PE lessons as usual (“normal schools”) based on school size and location. The study has been described in details elsewhere [14].

A subsample comprised of children attending 2nd to 4th grade (7.2–12 years) at baseline in year 2008 was formed for this study. These children were invited to participate in DXA scans. The children were examined by DXA at baseline and the follow-up examination was performed after two years. Accelerometer measurements were performed in the middle of the two-year test period. Examinations of the children took place at The Hans Christian Andersen Children’s Hospital, Odense, Denmark.

Ethics

Participation was voluntary. Children and parents received information about the study through school meetings and written information. The parents signed informed consent forms. Permission to conduct The CHAMPS Study–DK was granted by the Regional Scientific Ethics committee (Project number: S-20080047).

Data collection

Anthropometrical data

Body weight was measured to the nearest 0.1 kg on an electronic scale, SECA 861. Height was measured to the nearest 0.5 cm using a portable stadiometer, SECA 214 (both Seca Corporation, Hanover, MD). Body weight was measured in a thin T-shirt and stockings and both anthropometric measures were conducted barefoot.

Dual energy X-ray absorptiometry

Dual Energy X-ray Absorptiometry (DXA), GE Lunar Prodigy (GE Medical Systems, Madison, WI), ENCORE software (version 12.3, Prodigy; Lunar Corp, Madison, WI), measured BMC, BMD and BA. The total body less head (TBLH) values was used. The children were instructed to lie still in a supine position wearing underwear; a thin T-shirt, stockings and a blanket for the duration of the DXA scan. The typical scan duration was 5 min, depending on subjects’ height and weight. The instrument automatically altered scan depth depending on the size of the subject, as estimated from age, height, and weight. Two operators performed the DXA scans. The data form the DXA scans were analyzed by one person (MH). The DXA scanner was reset every day following standardized procedures. The GE Lunar Prodigy has reproducibility with precision errors (1 SD) of approximately 0.75% CV (Coefficient of Variation) for bone mass in children and adolescents with a mean age 11.4 years (5–17 years) [15].

Pubertal self-assessment

The children were presented with standard pictures showing the pubertal Tanner staging [16] and asked to indicate which stage best referred to their own pubertal stage. The Tanner pubertal stages self-assessment questionnaire (SAQ) used in this study consists of drawings of the 5
Tanner stages for pubic hair and breast development, respectively [17]. Explanatory text in Danish supported the self-assessment. Boys were presented with pictures and text of Tanner staging for pubic hair development, whereas girls were presented with pictures and text representing breast development and pubic hair. The procedure took place in a private space with sufficient time to self-assess the pubertal stage.

Physical activity
Assessments were performed in the middle of the two-year test period (November 2009 to January 2010), when the children attended 3rd-5th grade. Physical activity was assessed using the Actigraph GT3X accelerometer. The GT3X is a light, solid-state triaxial accelerometer, designed to monitor human activity and provide an estimate of energy expenditure. It measures the rate of acceleration in the (Cartesian coordinate system) z-axis/medio-lateral axis, x-axis/anterior-posterior axis and the y-axis/vertical axis. In the Actigraph GT3X, the signal is digitalized and passed through a filter with band limits of 0.25-2.5 Hz in order to help eliminate extraneous accelerations not due to human movement (e.g., vibration). The measurements of the vertical axis were used in this study [18]. The accelerometer was set to accumulate PA data every 2 seconds (2-sec. epoch) and subsequently collapsed to 10 seconds epoch [19].

Verbal and written information and instructions were given to the children along with their parents. The children were instructed to wear the device from the time they woke up in the morning until bedtime in order to capture their entire physical activity (PA) for each day, for 7 full consecutive days, thus theoretically including all weekdays and a full weekend. The children should remove the monitor when showering or swimming in order to prevent damage to the device. After the measurement period, the accelerometers were recollected and data downloaded to a computer.

Data reduction and analysis
The customized computer program, Propero [20], was used to process accelerometer data files. Propero was set up to include only activity in different time blocks depending on grades (2nd grade: 07.00-20.30 hours, 3rd grade: 07.00-21.00 hours and 4th grade: 07.00-21.00 hours) to avoid measurements during sleeping hours as some participants forgot to take off the accelerometer during the night. Furthermore, in order to distinguish between true intervals of inactivity and “false intervals” of inactivity recorded when the monitor had been taken off, all strings of consecutive zero for 20 min or more were defined as “accelerometer not worn” and subsequently deleted from the summation of activity. Thus, these periods did not contribute to the required minimum of valid registered activity.

Activity data were included for further analyses if the child had a minimum of 4 separate days with 10 hours per day of valid recording after the removal of non-wear time.

Cut-off points for activity intensity levels were defined according to Evenson et al. [18] (see Table 1).

Statistical analysis
Descriptive statistics were presented as means and SD, and medians and lower and upper quartiles. Explorative plots assessed linearity between the outcome variables (BMC, BMD and BA) and the covariates. Non-linear covariates were transformed to achieve linearity. Shapiro-Wilk’s test and q-q plots were used to check assumptions of normality. Residuals plots were inspected to check for variance of homogeneity. These tests did not indicate any violations of the model assumptions.

A multilevel linear regression model (using the xtmixed option from STATA 12.1), taking into account the hierarchical structure of the data was used to assess the relationship between BMC, BMD and BA accretion and the categories of physical activity intensity levels. Models were checked by residual plots. Effects with p-values < 0.05 were considered significant.

Backward elimination was used for reduction from an initial model, containing all the explanatory variables that included height, log(weight), age and puberty at follow up, bone outcome at baseline and interactions between gender and the activity levels as well as interactions between gender and puberty. BMC was in the final model adjusted for BMC at baseline, BA and height at follow up to avoid the possibility of size-related artifacts in the analysis of bone mineral data [21], and puberty at follow up and gender. BMD was in the final model adjusted for BMD at baseline log(weight); height and puberty at follow up, BA was adjusted for BA at baseline, log(weight), height, age and puberty at follow up and gender, all representing fixed effects. School and class were chosen as random effects. The information in the regression analyses was weighed by the total days of accelerometer measurements accepted (using the pweight option from STATA 12.1). By adjusting for baseline DXA outcomes, we captured the bone accrual during the two-year follow up period.

The accelerometer output generated the child’s number of minutes, in each activity intensity level per day. The data were further converted into proportions π_π, π_γ and π_γh of activity in “sedentary”, “low” and “moderate to high” intensity intervals, respectively.

The inherent ties in the proportions π_π, π_γ and π_γh (adding up to 100%) were handled by choosing low activity level as a reference level and calculating the log
odds of the proportions of the two remaining intensity levels relative to the proportion of the reference level. This way the physical activity was represented by the two parameters \( \theta_{mh} = \log \left( \frac{\pi_{mh}}{\pi_l} \right) \) and \( \theta_s = \log \left( \frac{\pi_s}{\pi_l} \right) \). These parameters cannot be interpreted separately but must be understood as an entity. This difficulty is addressed by visualizing how the bone health measures depend on PA (Figure 1). The effect upon the outcome variables of a change in activity intensity levels depends on the relative change among the proportions and is therefore suitably assessed for changes in particular configurations. This is demonstrated by an example in the result section.

### Results

A total of 1512 children from the preschool year to 4th grade (age range 5.5-11 years) were invited to participate in The CHAMPS study-DK from baseline in September 2008, of which 1210 (80%) accepted.

A sub group was created for the present study. This group comprised of children from 2nd to 4th grade (7.2-12 years) at baseline. Of these, 742/800 (93%) accepted the invitation to participate and 682/742 (92%) participated at two-year follow up (49% boys, 51% girls). Complete datasets were obtained in 602/742 (81%) children. The characteristics of the participants at follow up regarding

---

**Table 1 Classification of physical activity intensity based on Evenson accelerometer cut-off points and MET thresholds [18]**

<table>
<thead>
<tr>
<th>Physical activity intensity</th>
<th>Accelerometer cut points</th>
<th>Units of metabolic equivalent (MET)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sedentary activity</td>
<td>( \leq 100 ) counts/min</td>
<td>METs ( &lt; 1.5 )</td>
</tr>
<tr>
<td>Light physical activity</td>
<td>( &gt; 100 ) counts/min</td>
<td>( 1.5 \leq ) METs ( &lt; 4 )</td>
</tr>
<tr>
<td>Moderate physical activity</td>
<td>( \geq 2296 ) counts/min</td>
<td>( 4 \leq ) METs ( &lt; 6 )</td>
</tr>
<tr>
<td>Vigorous physical activity</td>
<td>( \geq 4012 ) counts/min</td>
<td>METs ( \geq 6 )</td>
</tr>
</tbody>
</table>

---

**Figure 1** Graphs presenting the effect of changes in different configurations of the proportion of total time in physical activity and sedentary, low and moderate to high level activity on the bone traits BMC, BMD and BA.
age, gender, anthropometry, densitometry, accelerometer data are reported in Table 2. Children, n = 152 (97 girls, 55 boys) reported Tanner stage 1, n = 263 (124 girls, 139 boys) Tanner stage 2, n = 189 (103 girls, 87 boys) Tanner stage 3, n = 38 (23 girls, 15 boys) Tanner stage 4 and n = 5 (2 girls, 3 boys) Tanner stage 5.

The relationship between BMC and the PA intensity levels represented by $\theta_{\text{mh}}$ and $\theta_s$ were assessed (Table 3). There was a positive relationship between $\theta_{\text{mh}}$ and BMC ($\hat{\beta}_{\text{mh}} = 20.94$, $p = 0.001$) and a positive relationship between $\theta_s$ and BMC ($\hat{\beta}_{\text{s}} = 27.77$, $p = 0.001$). There was a gender interaction with $\theta_s$ leading to an additional negative effect for boys on BMC ($\hat{\beta}_{\text{bh}, \text{ boys}} = -36.03$, $p = 0.006$).

By way of example, the fitted model predicts that for a girl a change in proportions of activity levels from $(\pi_l, \pi_m$ and $\pi_{mh}) = (60\%, 30\%, 10\%)$ to $(65\%, 27\%, 8\%)$ will lead to a change in BMC of $20.94 \times \log(\frac{0.6}{0.3}) + 27.77 \times \log(\frac{0.3}{0.1}) - 27.77 \times \log(\frac{0.1}{0.001}) = 2.7g$, whereas it will be $-4.0$ g for a boy. In a different example we observe a change in proportions of activity levels from $(\pi_l, \pi_m$ and $\pi_{mh}) = (70\%, 22\%, 8\%)$ to $(75\%, 15\%, 10\%)$ will lead to a change in BMC of $20.94 \times \log(\frac{0.6}{0.3}) - 20.94 \times \log(\frac{0.3}{0.1}) + 27.77 \times \log(\frac{0.1}{0.001}) - 27.77 \times \log(\frac{0.001}{0.00001}) = 25g$, whereas it will be $8.9$ g for a boy.

The relationship between BMI and the PA intensity levels represented by $\theta_{\text{mh}}$ and $\theta_s$ were assessed (Table 4). There was a positive relationship between $\theta_{\text{mh}}$ and BMI ($\hat{\beta}_{\text{mh}} = 0.009$, $p < 0.001$). There was no significant relationship between $\theta_s$ and BMI and no gender interaction.

The relationships between BA and the PA levels represented by $\theta_{\text{mh}}$ and $\theta_s$ were assessed (Table 5). There was a positive relationship between $\theta_{\text{mh}}$ and BA ($\hat{\beta}_{\text{mh}} = 22.18$, $p < 0.001$) but no significant relationship between $\theta_s$ and BA and further there was no significant gender interaction.

The graphs in Figure 1 illustrate the impact on bone health represented by BMC, BMD and BA when a certain level of physical intensity was kept fixed at the mean value allowing an exchange of time spent in the two remaining intensity levels to occur. This exchange occurred between the time spent in the two remaining intensity levels at their 10th, 25th, 50th, 75th and 90th percentiles. The solid lines refer to the girls whereas the dotted lines refer to the boys.

In the first column sedentary intensity level is fixed at the mean value 64% and 62% of the total time in activity for girls and boys respectively. An exchange between low-level activity and moderate to high level activity can occur and when increasing the proportion of time in low-level activity the BMC, BMD and BA values will decrease for both genders. In the second column low intensity PA is fixed at the mean level 29% of the total time in activity for boys and girls, and an exchange between moderate to high and sedentary can occur. The bone traits increases as the proportion of time spent in moderate to high-level PA increases opposed to sedentary level PA. In the third column moderate to high level PA is fixed at the mean value 7% and 9% of the total time in activity for girls and boys respectively. An exchange between sedentary level activity and low-level activity reveals an increase in bone outcome when the proportion of time in sedentary level activity increases opposed to low-level activity.

**Discussion**

A major reason for the interest in increasing bone health during growth is to prevent fractures due to osteoporosis later in life. This longitudinal study examined the relationship between the proportion of time spent at PA in different intensity levels and bone health represented by BMC, BMD, and BA accrual during two years, and showed a positive relationship of the log odds of moderate to high and low intensity activity and BMC, BMD and BA accrual over a two-year period.

We also found a significant relationship between the log odds of sedentary relative to low intensity activity and BMC as well as a significant gender interaction with an additional negative effect on BMC for boys. The changes in particular configurations between the three categories of PA revealed positive effects on bone traits when increasing the proportion of time in moderate to high-level activity opposed to sedentary and low-level activity behavior, but also a positive effect on bone traits when increasing the proportion of time in sedentary activity on behalf of low level activity. This rather surprising but interesting result may reflect that sedentary behavior is not necessarily negative for the bones compared to a general low activity level. However, this result was only found for BMC and not BMD and BA and should be confirmed in other studies.

The positive relationship between weight-bearing exercises, and bone health during growth has been well described [22]. However, this study provides additional information about the association between children’s habitual PA reported as the relationships between the proportion of time spent in different intensity levels of PA and the outcome variables BMC, BMD and BA accrual.

The strengths of the study included the population size, the large numbers of participants at follow up and the longitudinal design. There was an equal distribution between genders, and children attending sports schools and normal schools. The data collection included the
gold standard method of measuring bone health parameters, DXA, and objective information on the children’s physical activity (PA) level. Assessment of PA by accelerometers provided valid information on the frequency, intensity and duration of PA. The accelerometer output correlated well with ground reaction force (GRF) which is pertinent to bone health [23]. The objective measures of accelerometers had the advantage that the children did not need to recall behaviors of physical activity, and therefore output did not rely on cognitive ability and recall bias.

Accelerometers have limitations as to the technical specifications and the participant’s willingness to wear the accelerometers. However, we succeeded in obtaining complete datasets on 81% of the participants. Another positive aspect of our study was that the children included in the analyses were measured with accelerometers at a mean of 6.1 days (4–11 days) and at a mean of 13 hours per day (11–14 hours per day). Trost et al. demonstrated that 4–5 days of monitoring were needed to obtain an intra class coefficient of 0.8 in children [24], which we exceeded. The analyses only addressed the effect of the relative distribution of PA among three levels. Although the total amount of activity may have an impact on the bone health, the data analyzed here are not amenable for this question.

Table 2 Descriptive statistics of the participants with complete datasets n = 602 at follow up

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sex</th>
<th>Mean (SD)</th>
<th>25th%</th>
<th>Median</th>
<th>75th%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs.)</td>
<td>Boys</td>
<td>11.5 (0.89)</td>
<td>10.8</td>
<td>11.4</td>
<td>12.2</td>
</tr>
<tr>
<td></td>
<td>Girls</td>
<td>11.5 (0.87)</td>
<td>10.7</td>
<td>11.4</td>
<td>12.2</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>Boys</td>
<td>151.2 (8.88)</td>
<td>145</td>
<td>151.1</td>
<td>156.5</td>
</tr>
<tr>
<td></td>
<td>Girls</td>
<td>150.5 (8.3)</td>
<td>144.4</td>
<td>150.4</td>
<td>156.5</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>Boys</td>
<td>40.7 (8.46)</td>
<td>34.5</td>
<td>39.3</td>
<td>45.2</td>
</tr>
<tr>
<td></td>
<td>Girls</td>
<td>40.9 (8.81)</td>
<td>34.8</td>
<td>40.05</td>
<td>46</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>Boys</td>
<td>8.2 (5.02)</td>
<td>4.6</td>
<td>6.8</td>
<td>10.2</td>
</tr>
<tr>
<td></td>
<td>Girls</td>
<td>10.5 (5.0)</td>
<td>6.8</td>
<td>9.5</td>
<td>13.03</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>Boys</td>
<td>30.7 (4.97)</td>
<td>27.2</td>
<td>30.1</td>
<td>33.2</td>
</tr>
<tr>
<td></td>
<td>Girls</td>
<td>28.4 (4.96)</td>
<td>24.5</td>
<td>27.8</td>
<td>31.8</td>
</tr>
<tr>
<td>BMC (g)</td>
<td>Boys</td>
<td>1189 (267)</td>
<td>1007</td>
<td>1169</td>
<td>1314</td>
</tr>
<tr>
<td></td>
<td>Girls</td>
<td>1183 (313)</td>
<td>945.5</td>
<td>1142</td>
<td>1363</td>
</tr>
<tr>
<td>BMD (g/cm²)</td>
<td>Boys</td>
<td>0.83 (0.06)</td>
<td>0.79</td>
<td>0.83</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td>Girls</td>
<td>0.83 (0.08)</td>
<td>0.78</td>
<td>0.83</td>
<td>0.88</td>
</tr>
<tr>
<td>BA (cm³)</td>
<td>Boys</td>
<td>1405 (222)</td>
<td>1246</td>
<td>1399</td>
<td>1540</td>
</tr>
<tr>
<td></td>
<td>Girls</td>
<td>1390 (249)</td>
<td>1211.2</td>
<td>1372.3</td>
<td>1545.5</td>
</tr>
<tr>
<td>Accelerometer (Days registered)</td>
<td>Boys</td>
<td>6.1 (0.97)</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Girls</td>
<td>6.1 (0.96)</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Sedentary Activity (%)</td>
<td>Boys</td>
<td>62</td>
<td>58</td>
<td>62</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>Girls</td>
<td>64</td>
<td>61</td>
<td>64</td>
<td>68</td>
</tr>
<tr>
<td>Low activity (%)</td>
<td>Boys</td>
<td>29</td>
<td>26</td>
<td>29</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>Girls</td>
<td>29</td>
<td>26</td>
<td>29</td>
<td>31</td>
</tr>
<tr>
<td>Moderate to high activity (%)</td>
<td>Boys</td>
<td>9</td>
<td>6</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Girls</td>
<td>7</td>
<td>6</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>Pubertal stages</td>
<td>Boys (n)</td>
<td>55</td>
<td>97</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Girls (n)</td>
<td>139</td>
<td>124</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tanner stage 1 (n)</td>
<td></td>
<td>87</td>
<td>102</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tanner stage 3 (n)</td>
<td></td>
<td>15</td>
<td>23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tanner stage 5 (n)</td>
<td></td>
<td>3</td>
<td>2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

October 2010 to March 2011. Figures are means (SD) presented along with median, 25th% and 75th%.

Note: *The Fishers exact refers to the comparison of the pubertal stages between boys and girls at follow-up.
The girls were chosen as the reference level for gender.

Some other methodological aspects need to be mentioned. The collection of accelerometer data was performed at one-year follow-up. We had no knowledge of how this one period of measurements represented the child's activity level over a longer time period, but we assumed that the measurements reflected the child's activity level in general. This assumption was based on the knowledge that although PA and activity patterns vary from day to day as well as by season [25], children exhibit less day-to-day variability than adults [24]. PA measurements by accelerometers were obtained during the same yearly season for all children. Diet was not considered in this study. However, the children's diet habits and variation in these may serve as a possible confounding factor when monitoring bone health.

Our findings of an association between the intensity levels of PA and bone traits corresponded well with the findings in a study by Tobias JH et al. (2007) in which the results presented were derived from a cross-sectional design [13]. In a previous study, Sardina et al. (2008) examined the relationship between intensity and duration of physical activity and composite indices of femoral neck strength and bone mineral content of the femoral neck, lumbar spine and total body. They concluded that vigorous activity measured by accelerometers emerged as the main PA predictor of femoral neck strength [26]. This corresponded well with our results, although we measured TBLH BMC, BMD, and BA, and not indices of bone strength. We found that greater discrepancies in activity level are necessary to disclose the effects of physical activity on bone accretion.

Several other studies have presented cross-sectional data on the positive relationship between habitual levels of physical activity at different intensity and bone mass, in particular moderate to high intensity PA [12,13,27]. Our study provides longitudinal data from a large two-year follow-up study.

Habits of physical activity can be traced from childhood to adolescence and adulthood [28,29], and habits developed in early life may persist into adulthood. It has been suggested that children become less physically active and spend more time in sedentary activity as they age [30]. However, this suggestion has been questioned in other studies [31,32]. Our graphic presentation of the data emphasizes the importance of spending a higher proportion of the total time in higher activity levels to maintain a beneficial bone mineral accrual during a two-year observational time. Our results suggest that small changes in PA behavior towards more moderate to high level activity opposed to low and sedentary intensity levels are sufficient to achieve these beneficial effects on bone traits.

In recent years, osteoporosis has been recognized as a growing problem in adults and the elderly [1,2]. When planning prevention strategies towards osteoporosis, all of the factors that affect bone health including the size of PBM should be considered. Bone growth during childhood and adolescence is important for reaching optimal PBM [7]. Thus it is crucial to optimize these modifiable factors with regard to bone development in childhood. Although up to 60-80% of the variance in PBM is accounted for by genetic factors, the remaining

### Table 3 Effect of the physical activity on BMC at follow up

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient estimate</th>
<th>Standard error</th>
<th>P- value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMC baseline</td>
<td>0.44</td>
<td>0.03</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BA follow up</td>
<td>0.97</td>
<td>0.04</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Gender</td>
<td>35.20</td>
<td>9.50</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>$\theta_{mh}$ =  $\log\left(\frac{n_{mh}}{n_{l}}\right)$</td>
<td>20.94</td>
<td>6.58</td>
<td>0.001</td>
</tr>
<tr>
<td>$\theta_{l}$ =  $\log\left(\frac{n_{l}}{n_{mh}}\right)$</td>
<td>27.77</td>
<td>8.17</td>
<td>0.001</td>
</tr>
<tr>
<td>Gender $\times$ $\theta_{l}$</td>
<td>-36.03</td>
<td>13.11</td>
<td>0.006</td>
</tr>
<tr>
<td>Height follow up</td>
<td>-3.96</td>
<td>0.17</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Puberty follow up</td>
<td>29.99</td>
<td>3.52</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Gender $\times$ Puberty</td>
<td>-16.95</td>
<td>5.14</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Note: $n_{mh,l}$ represents the percentage of the total time spent in, moderate-high, sedentary and low intensity activity respectively. $\times$ Interaction. The girls were chosen as the reference level for gender.

### Table 4 Effect of the physical activity on BMD at follow up

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient estimate</th>
<th>Standard error</th>
<th>P- value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMD baseline</td>
<td>0.966</td>
<td>0.02</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>$\theta_{mh}$ =  $\log\left(\frac{n_{mh}}{n_{l}}\right)$</td>
<td>0.009</td>
<td>0.001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Height follow up</td>
<td>0.001</td>
<td>0.0002</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Log(weight follow up)</td>
<td>0.039</td>
<td>0.008</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Gender</td>
<td>0.017</td>
<td>0.005</td>
<td>0.001</td>
</tr>
<tr>
<td>Puberty follow up</td>
<td>0.016</td>
<td>0.002</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Gender $\times$ Puberty</td>
<td>-0.014</td>
<td>0.003</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Intercept</td>
<td>-0.150</td>
<td>0.018</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Note: $n_{mh,l}$ represents the percentage of the total time spent in, moderate-high and low intensity activity respectively. $\times$ Interaction. The girls were chosen as the reference level for gender.

### Table 5 Effect of the physical activity on BA at follow up

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient estimate</th>
<th>Standard error</th>
<th>P- value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BA baseline</td>
<td>0.62</td>
<td>0.49</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>$\theta_{mh}$ =  $\log\left(\frac{n_{mh}}{n_{l}}\right)$</td>
<td>22.18</td>
<td>4.20</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Height follow up</td>
<td>6.87</td>
<td>0.69</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Log(weight follow up)</td>
<td>285.20</td>
<td>18.12</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Puberty follow up</td>
<td>24.47</td>
<td>2.32</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Gender</td>
<td>-26.33</td>
<td>5.52</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Intercept</td>
<td>-1405.85</td>
<td>86.01</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Note: $n_{mh,l}$ represents the percentage of the total time spent in, moderate-high and low intensity activity respectively. The girls were chosen as the reference level for gender.
20-40% are influenced by lifestyle factors such as physical activity [7,33].

Focus on habitual physical activity during childhood is of major importance to consider when planning preventive strategies towards osteoporosis. Also in relation to bone health it is important to change children’s lifestyle from mainly inactivity to higher activity levels.

Conclusion
A positive association was observed between physical activity and bone mineral accretion in childhood. In particular moderate to high intensity activity had a positive influence on the bone health. The data suggest that changes in the distribution of time spent in different activity intensity levels will influence the bone mineral accretion during a two year period with moderate to high intensity activity having a positive effect on bone outcome, whereas an increase in low-level and sedentary activity opposed to moderate to high-level activity will have a negative impact on bone health in childhood. This knowledge has public health as well as clinical relevance. Further research should focus on detecting the threshold of the beneficial effects of physical activity on bone health.

Competing interests
All authors state that they have no competing interest.

Authors’ contributions
Study design: MH, CM, NW, SH, HK, NC, AJS. Study conduct: MH, HK, NC. Data collection: MH, HK, NC. Data interpretation: MH, CM, NW, RH and NC. Drafting manuscript: MH, CM, and NW. Revising content: MH, CM, NW, SH, HK, NC, AJS, RH. Approving the final version of the manuscript: MH, CM, NW. SH, HK, NC, AJS, RH. Approving the final version of the manuscript: MH, CM, NW, SH, HK, NC, AJS, RH. MH takes responsibility for the integrity of the data analyses.

Acknowledgements
The authors gratefully acknowledge the work of numerous students and PhD students who have participated in the data collection for the CHAMPS study-DK, and professor Charlotte Leboeuf-Yde for her aid in the process of writing the manuscript. We thank children, parents and teachers in the schools involved in the project, and we are grateful for the cooperation with “Elitesekretariatet” in the municipality of Svendborg. The study had not been possible without financial support from The Nordea foundation, The TRYG foundation, The IMK Foundation, The Region of Southern Denmark, The Egmont Foundation, The A. J. Andersen Foundation, The Danish Rheumatism Association, and TEAM Denmark.

Author details
1Hans Christian Andersen Children’s Hospital, Odense University Hospital, Sdr. Boulevard 29, Odense C DK-5000, Denmark. 2Spine Centre of Southern Denmark, Hospital Lillebaelt, Middelfart, Denmark. 3RIC, Centre of Research in Childhood Health, University of Southern Denmark, Odense, Denmark. 4Institute of Regional Health Research, Department of Biostatistics, University of Southern Denmark, Odense, Denmark. 5Department of Nutrition, Exercise and sport, Faculty of Science, University of Copenhagen, Copenhagen, Denmark.

Received: 28 May 2012 Accepted: 25 February 2013 Published: 2 March 2013

References


Cite this article as: Heidemann et al.: The intensity of physical activity influences bone mineral accrual in childhood: the childhood health, activity and motor performance school (the CHAMPS) study, Denmark. *BMC Pediatrics* 2013 13:32.
The impact on children’s bone health of a school-based physical education program and participation in leisure time sports
The Childhood Health, Activity and Motor Performance School (the CHAMPS) study, Denmark
Malene Heidemann a,⁎, Eva Jespersen b, René Holst c, Anders J. Schou a, Steffen Husby a, Christian Mølgaard a,d, Niels Wedderkopp e
a Hans Christian Andersen Children’s Hospital at Odense University Hospital, Denmark
b RICH, Centre of Research in Childhood Health, University of Southern Denmark, Denmark
c Institute of Regional Health Services Research, Department of Biostatistics, University of Southern Denmark, Denmark
d Department of Nutrition, Exercise and Sports, Faculty of Science, University of Copenhagen, Denmark
e Spine Centre of Southern Denmark, Hospital Lillebaelt, Middelfart, Denmark

Abstract
Available online 30 April 2013

Keywords:
 Dual energy X-ray absorptiometry
 Bone health
 Physical activity
 Short Messaging Service-Track-Questionnaire

Objective. To evaluate the effect of a school based physical education (PE) program and the amount of leisure time sport (LTS) on children's bone health and to examine if LTS influences the impact of school type on children's bone health.

Methods. Children attending “sports” schools (6 × 45 min PE lessons per week) were compared to children at “traditional” schools (2 × 45 min of PE lessons per week) in Svendborg, Denmark. Whole-body DXA scans were performed at baseline (2008) and at a two-year follow-up (2010). Bone mineral content (BMC), bone mineral density (BMD), and bone area (BA) were measured. Multilevel regression analyses examined the impact of school type and LTS participation on bone.

Results. 742/800 (93%) invited children accepted to participate. 682/742 (92%) participated at two-year follow-up. Mean (SD) age was 9.5 years (0.9) at baseline. A positive association between LTS and BMC, BMD (p<0.001) and for BA (p<0.05) (total body less head (TBLH) and lower limb (LL)) was found. All effects regarding school type were insignificant.

Conclusion. A positive impact of attending LTS on bone traits was found. There was no effect on BMC, BMD and BA (TBLH, and LL) for children attending sports schools compared to traditional schools.

© 2013 Elsevier Inc. All rights reserved.

Introduction
The peak bone mass (PBM) is the maximum attained bone mass in a lifetime. A low PBM is a predisposing factor to later development of osteoporosis (Bachrach, 2001). Several factors affect the magnitude of the PBM such as genetics, and lifestyle including nutrition and physical activity (PA) (Berger et al., 2010; Fewtrell et al., 2009). It is important to optimize the modifiable factors during childhood and adolescence when bone growth occurs in order to achieve the highest possible PBM according to our genetic potential, since low bone mass in elderly people is associated with a high fracture risk (Johnston and Slemenda, 1994). The optimal time for bone mineral accrual is prepuberty and early puberty (Bass et al., 2002).

Adiposity and inactivity in childhood is an increasing problem in high-income countries (Brisbois et al., 2012). The impact of adiposity on bone health in childhood and adulthood is contradictory (Dimitri et al., 2012). However, as commonly known inactivity is highly correlated to adiposity and has a negative impact on bone health and may cause a lower PBM and a later increased risk of osteoporosis. The behavior of inactivity tracks from early childhood into adulthood (Janz et al., 2005) and it is important to influence children’s behavior of physical activity at an early stage in life to ensure future health and good habits of PA.

The objective of the study was to evaluate the effect of a generalized school based PE program on children’s bone health, and to examine if participation in leisure time sport (LTS) influences the impact of

Abbreviations: BA, bone area; BMC, bone mineral content; BMD, bone mineral density; BMI, body mass index; DXA, dual energy X-ray absorptiometry; TBLH, total body less head; LTS, leisure time sport; LL, lower limb; PBM, peak bone mass; SMT-TQ, Short Messaging Service-Track-Questionnaire.

⁎ Corresponding author at: Hans Christian Andersen Children’s Hospital at Odense University Hospital, Sdr. Boulevard 29, 5000 Odense C, Denmark.
E-mail address: mheidemann@dadlnet.dk (M. Heidemann).

0091-7435/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ypmed.2013.04.015
school type on children’s bone health or has an independent impact on bone health in childhood.

Methods
Study design

The study was a part of the CHAMPS study, DK an on-going prospective observational cohort study (Wedderkopp et al., 2012). The CHAMPS-study, is a natural experiment, in which the variations in exposure (i.e. the sports schools versus the traditional schools) and outcomes are analyzed with the intent of making causal inferences (Craig et al., 2012) on the effect of the intervention.

Nineteen primary schools in the municipality of Svendborg, Denmark, were invited to participate in the project, six schools agreed to participate. The final concept for the six sports schools included a minimum of 4.5 h of PE, divided into at least 3 sessions per week, at 60 min each. Parents and children were unaware of the initiation of this project until two months before the following school year. Four traditional schools were matched on size and geographic location (rural or urban) (Wedderkopp et al., 2012).

Children attending 2nd to 4th grade (7.7–12 years) were invited at baseline in year 2008. No child was excluded since none of the children received medication with an influence on bone metabolism. Children were examined at baseline (2008) and at two-year follow-up (2010). Examinations of the children took place at the Hans Christian Andersen Children’s Hospital, Odense, Denmark (Fig. 1).

Ethical considerations

All children and parents from the participating schools received information about the study through school meetings and written information. Parents signed informed consent forms. Permission to conduct the CHAMPS study — DK was granted by the Regional Scientific Ethical Committee of Southern Denmark (project number: S-20080047).

Data collection

Anthropometrical data

Anthropometric measures were measured with children required to go barefoot, wearing only a thin T-shirt, underwear and stockings. Body weight was measured to the nearest 0.1 kg on an electronic scale, SECA 861, and height was measured to the nearest 0.5 cm using a portable stadiometer, SECA 214 (both from Seca Corporation, Hannover, MD).

Dual energy X-ray absorptiometry

Dual energy X-ray absorptiometry (DXA), GE Lunar Prodigy (GE Medical Systems, Madison, WI), equipped with ENCORE software (version 12.3, Prodigy; Lunar Corp, Madison, WI), was used to measure BMC, BMD and BA. The total body less head (TBLH) and lower limb (LL) values were used (Lewiecki et al., 2008). The children were scanned in a supine position wearing underwear, a thin T-shirt, stockings and a blanket for the duration of the DXA scan. The GE Lunar Prodigy has reproducibility with precision errors (1 SD) of approximately 0.75% CV (coefficient of variation) for BMC in children and adolescents with mean age 11.4 years (5–17 years) (Margulies et al., 2005).

Pubertal self-assessment

Tanner pubertal stages self-assessment questionnaire (SAQ) which consists of drawings of the 5 Tanner stages for pubic hair (boys) and breast development (girls), respectively (Tanner, 1962) with explanatory text in Danish was used to evaluate sexual maturation. Children were presented with standard pictures showing the pubertal Tanner stages and asked to indicate which stage best referred to their own pubertal stage (Duke et al., 1980).

Short Messaging Service-Track-Questionnaire

Sports participation was measured weekly by “Short Messaging Service-Track-Questionnaire” (SMS-T-Q) version 2.1 (New Agenda Solutions, SMS-Track ApS, Esbjerg). SMS-Track is a web based IT-system (SMS survey) developed as a tool for frequent surveillance (Shiffman et al., 2008). The method is a “follow-up” procedure and was used in this study to investigate LTS participation over time (Johansen and Wedderkopp, 2010). The questionnaire was automatically sent to the parent’s mobile phone once a week including a question about LTS: “How many times did [NAME OF CHILD] engage in sports during the last week?” The parents were instructed to answer with a relevant number between 0 and 8. The answers 0 to 7 represent the unique number of times engaging in sports, whereas 8 indicated “more than 7 times”. The returned answers were automatically recorded and inserted into a database.

Statistical analyses

An initial analysis found mean (±SD) of key physical characteristics at baseline and at two-year follow-up. Explorative plots assessed linearity between the outcome variables (BMC, BMD and BA) and non-linear covariates were transformed to achieve linearity. Model assumptions of normality of the residuals were checked by Shapiro–Wilk’s test and q–q plots. There was no indication of deviation from normality.

The effect of variables of interest upon the accrual of BMC, BMD and BA at baseline and follow-up was analyzed in a multilevel linear regression model (using xtmixed command from STATA 12.1). Backward elimination was used to reduce the initial model, containing all the explanatory variables including baseline bone traits and height, log(weight), gender, puberty, all at follow-up, school type, the amount

**Fig. 1. Flowchart of the participants.**
of LTS, and interactions between school type and the amount of LTS, gender and puberty, gender and school type and gender and LTS. Adjusting for baseline bone traits controlled for the children’s initial size and BMC was adjusted for BA at follow-up to avoid possible size-related artifacts in the analysis of bone mineral data (Prentice et al., 1994). The significance level was set at p < 0.05.

Results

The children attended 2nd to 4th grade (7.7–12 years) at baseline (2008). Of these, 742/800 (93%) accepted to participate and 682/742 (92%) participated at follow-up (47% boys, and 53% girls). The characteristics of the participants at baseline and at follow are reported in Tables 1 and 2. There were no differences in the participant's height, weight, or DXA values, or in the distribution of pubertal stages between the two school types.

The relationship between school-type and BMC, and BMD and BA accretion was assessed separately using a multilevel regression model using school and class as random effects. All subsequent results refer to the final models for bone traits at follow-up.

BMC (TBLH) (Table 3): Puberty had a positive effect for girls ($\hat{\beta}\text{puberty, girls} = 16.12, p = 0.001$) but was insignificant for boys. The amount of LTS was significant with different effects for boys and girls ($\hat{\beta}_{\text{LTS, boys}} = 9.48, p < 0.001$) and the difference between boys and girls was $\hat{\beta}_{\text{LTS, gender}} = -6.63$ (p < 0.001) this implies an estimated increase of 9.48 g BMC for each extra LTS participation for boys and an increase of 9.48–6.63 = 2.85 g BMC for girls. All effects of school type were insignificant.

BMD (TBLH) (Table 4): The amount of LTS was significant ($\hat{\beta}_{\text{LTS}} = 4.65, p = 0.019$). School type and interactions between school type and gender and school type and the amount of LTS were all insignificant (p > 0.05).

BMD (LL): The amount of LTS was significant ($\hat{\beta}_{\text{LTS}} = 0.007, p < 0.001$). School type and interactions between school type and

table 2

<table>
<thead>
<tr>
<th>Participants</th>
<th>Sports schools</th>
<th>Traditional schools</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline (n%)</td>
<td>402 (56%)</td>
<td>315 (44%)</td>
</tr>
<tr>
<td>Follow-up (n%)</td>
<td>381 (56%)</td>
<td>302 (44%)</td>
</tr>
<tr>
<td>LTS participation, mean times/week (SD)</td>
<td>1.6 (0.9)</td>
<td>1.9 (1.2)</td>
</tr>
<tr>
<td>PE lessons in school (times/week)</td>
<td>4.5</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Note: October 2010 to March 2011 and mean (SD) leisure time sport (LTS) participation during the two-year follow-up period and amount of physical education (PE) lessons in school (times/week).

gender and school type and the amount of LTS were all insignificant (p > 0.05).

BA (TBLH) (Table 5): There was a significant interaction between gender and puberty leading to an additional positive effect on BA for girls ($\hat{\beta}_{\text{puberty, girls}} = 10.99 p < 0.001$). The amount of LTS was significant ($\hat{\beta}_{\text{LTS}} = 4.65, p = 0.019$). School type and interactions between school type and gender and school type and the amount of LTS were all insignificant (p > 0.05).

BA (LL) (Table 5): The amount of LTS was however insignificant. School type and interactions between school type and gender and school type and the amount of LTS were all insignificant (all p > 0.05).

Discussion

In this longitudinal study we examined the effect of a school based PE program and the effect of participation in LTS on children's bone health. LTS had a positive impact on bone accrual in children with boys having the greatest effect from LTS regardless of school type. The analyses revealed no effect of school type on children's bone mineral accrual during the two-year observation time. The hypothesis of a positive effect on bone traits of the sports schools for children with low amount of LTS participation suggested an examination of the interactions between school type and the amount of LTS participation, which were statistically insignificant.

Several studies have shown that weight-bearing exercise stimulates bone mineral accrual in children and adolescents as well as in adults (Hind and Burrows, 2007). We would expect a school

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Baseline (2008) and follow-up (2010) characteristics of the participants of the CHAMPS study — DK.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 344)</td>
</tr>
<tr>
<td>Age (yrs.)</td>
<td></td>
</tr>
<tr>
<td>Boys</td>
<td>Girls</td>
</tr>
<tr>
<td>Sports schools</td>
<td>Traditional schools</td>
</tr>
<tr>
<td>Age (yrs.)</td>
<td>9.6 (0.9)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>33.3 (6.6)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>140.6 (7.5)</td>
</tr>
<tr>
<td>BMI</td>
<td>25.5 (3.4)</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>6.2 (4.1)</td>
</tr>
<tr>
<td>Fat mass (%)</td>
<td>17.5 (8.0)</td>
</tr>
<tr>
<td>Body fat percent (%)</td>
<td></td>
</tr>
<tr>
<td>DXA measures (TBLH)</td>
<td></td>
</tr>
<tr>
<td>BMC (g)</td>
<td>896.9 (186.0)</td>
</tr>
<tr>
<td>BMD (g/cm²)</td>
<td>0.76 (0.05)</td>
</tr>
<tr>
<td>BA (cm²)</td>
<td>1158.1 (174.8)</td>
</tr>
<tr>
<td>DXA measures (lower limb)</td>
<td></td>
</tr>
<tr>
<td>BMC (g)</td>
<td>432.1 (100.4)</td>
</tr>
<tr>
<td>BMD (g/cm²)</td>
<td>0.87 (0.07)</td>
</tr>
<tr>
<td>BA (cm²)</td>
<td>489.1 (78.9)</td>
</tr>
<tr>
<td>Pubertal status</td>
<td>Tanner stage (n)</td>
</tr>
<tr>
<td>1/2/3/4/5</td>
<td>81.8/7/9/0/0</td>
</tr>
</tbody>
</table>

Note: Results are reported as mean (SD) sorted by gender and schools. Results for pubertal stage are reported as numbers.
based PE program with four extra PE lessons to include weightbearing elements. The main part of the PE lessons was conducted either outside or inside sports arenas, only 30 min every week in fifth grade was set aside for swimming lessons. The design of the sports schools in the present study was not specifically intended to influence bone health in childhood and contained no specific elements of repetitive jumping or similar activities.

The intensity of PA is important to bone health in childhood (Sardinha et al., 2008; Tobias et al., 2007) and a potential difference in intensity levels reached during PE lessons and LTS participation may in part explain the positive impact of LTS compared to the extra PE lessons.

The majority of the children who did not participate in LTS, attended sports schools (67%) and the children in traditional schools attended more LTS than children in sports schools. The schools were matched on size and geographic location and the children had equal access to LTS facilities. Part of the reason for our findings may be due to a behavioral effect of attending a sports school causing parents to reduce their child’s LTS due to practical reasons or that the child becomes less physically active during the leisure time due to the increased activity during school time. However, participation in LTS had an impact on bone accrual. We expect that the children participate in LTS due to interest in a specific sport and this group of children may represent a proxy group for the most active children regardless of school type.

Our findings differ from several other well-designed studies where participation in different PE programs and the impact on bone health were examined (Linden et al., 2006; Lofgren et al., 2011; Meyer et al., 2011; Valdimarsson et al., 2006). In several of these studies the PE programs were designed to enhance bone health and PE lessons and PE homework contained elements of weight-bearing exercise, and impacts on bone mineralization were seen (Fuchs et al., 2001; Heinonen et al., 2000; MacKelvie et al., 2002; MacKelvie et al., 2004; Weeks et al., 2008).

Several studies have examined the impact of specific LTS activities of bone accrual, such as the study wherein young tennis players were found to have increased BMC on their loaded arm (Bass et al., 2002) and a study of non-elite gymnastics participation which was associated with musculoskeletal benefits on upper limb bone geometry, strength and muscle function (Burt et al., 2012). To our knowledge no other study has explored the relationship between general LTS participation and bone health in childhood.

This present study does not support the establishment of sports schools to achieve a general impact on bone accrual. The present literature indicates that in order to achieve an impact on bone health PE intervention programs must contain elements of specific weight-bearing exercise to be successful. Our study strongly suggests that attending LTS is beneficial to bone health in childhood.

### Limitations and strengths

Our study had some limitations. The creation of sports schools was a political decision in the municipality of Svendborg therefore the design was not a randomized controlled trial but a natural experiment (Craig et al., 2012). Children in the two groups were however, comparable at baseline regarding all measurements presented in this article. Information on the children’s PA level at baseline was not available. It is not possible to conclude that the patterns of activity were equal at the beginning of the experiment. From previous longitudinal studies it is known that loaded sites are increasing in density. The lower limbs extracted from the total body scan, may not be sensitive to changes predominantly found in the femoral neck. A scan result of the hip could be a better representative of a loaded region since measurements of the hip are more sensitive to the detection of an effect on the hip, however we decided to use TBLH measurements since in addition in the CHAMPS study wanted to analyze the effect of
extra PE lessons on the body composition. Furthermore, in children some authorities recommend to use whole body and spine scans and not hip scan because of difficulties in the interpretation of the results when following a child over time due to change in shape (Lewiecki et al., 2008).

The strengths of the study include the population size, high percentage of follow-up measurements and the longitudinal design. The data collection includes DXA as the reference of measuring bone health parameters. Valid information on the children’s sports participation was achieved through SMS-T-QS with a response rate of 95.5% in a reliable follow-up design. This longitudinal study finds no evidence of an impact of attending a sports school on children’s bone health. However, the study has confirmed the positive effect of increased LTS participation. The lack of a significant effect of sports schools suggests that the effect of increased school based PE is balanced out by a reduced amount of LTS participation relative to the traditional schools.

Conclusion

In conclusion there was a highly significant positive relationship between participation in LTS and bone health. There was no impact on bone health (TBH and lower limb) for children attending sports schools compared to children in traditional schools.

Conflict of interest statement
The authors declare that there are no conflicts of interests.

Acknowledgments

The authors gratefully acknowledge the work of numerous students and PhD students who have participated in the data collection for the CHAMPS study – DK. We thank children, parents and teachers in the schools involved in the project, and we are grateful for the cooperation of “Elitesekretariatet” in the municipality of Svendborg. The study would have not been possible without financial support from the Nordea Foundation, the TRYG Foundation, the IMK Foundation, the Region of Southern Denmark, the Egmont Foundation, the A.J. Andersen Foundation, the Danish Rheumatism Association, and TEAM Denmark.

Author contributions: study design: MH, NW, SH, EJ, and AJS; study conduct: MH, and EJ; data collection: MH, and EJ; data interpretation: MH, RH, CM and NW; drafting manuscript: MH, CM, and NW; revising content: MH, CM, NW, SH, EJ, AJS, and RH; and approving the final version of the manuscript: MH, CM, NW, SH, EJ, AJS, and RH. MH takes responsibility for the integrity of the data analyses.

References


Frewer, M.S., Williams, J.E., Singhal, A., Murgatroyd, P.R., Fuller, N., Lucas, A., 2009. Early Diet and Peak Bone Mass: 20 Year Follow-Up of a Randomized Trial of Early Diet in Infants Born Preterm.


The Influence of Anthropometry and Body Composition on Children’s Bone Health
The Childhood Health, Activity and Motor Performance School
(The CHAMPS) study, Denmark

<table>
<thead>
<tr>
<th>Journal:</th>
<th>Osteoporosis International</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manuscript ID:</td>
<td>OI-2013-03-0182</td>
</tr>
<tr>
<td>Manuscript Type:</td>
<td>Original Article</td>
</tr>
<tr>
<td>Date Submitted by the Author:</td>
<td>13-Mar-2013</td>
</tr>
<tr>
<td>Complete List of Authors:</td>
<td>Heidemann, Malene; Odense University Hospital, Hans Christian Andersen Children Hospital Holst, René; Institute of Regional Health Research, Department of Biostatistics Schou, Anders; Hans Christian Andersen Children’s Hospital, Odense University Hospital, Denmark Klakk, Heidi; RICH, Centre of Research in Childhood Health, University of Southern Denmark Husby, Steffen; Hans Christian Andersen Children’s Hospital, Odense University Hospital, Denmark Wedderkopp, Niels; Spine Centre of Southern Denmark, Hospital Lillebaelt, Middelfart Mølgaard, Christian; University of Copenhagen, Department of Nutrition, Exercise and Sport, Faculty of Science</td>
</tr>
<tr>
<td>Keyword:</td>
<td>Dual energy x-ray absorptiometry, bone mineral accrual, anthropometry, body composition, lean mass, body fat percentage</td>
</tr>
</tbody>
</table>
The Influence of Anthropometry and Body Composition on Children’s Bone Health

The Childhood Health, Activity and Motor Performance School
(The CHAMPS) study, Denmark

Malene Heidemann, René Holst, Anders J. Schou, Heidi Klakk, Steffen Husby, Niels Wedderkopp, Christian Mølgaard

1Hans Christian Andersen Children’s Hospital, Odense University Hospital, Denmark
2Institute of Regional Health Research, Department of Biostatistics, University of Southern Denmark
3RICH, Centre of Research in Childhood Health, University of Southern Denmark
4Spine Centre of Southern Denmark, Hospital Lillebaelt, Middelfart
5Department of Nutrition, Exercise and Sport, Faculty of Science, University of Copenhagen

Corresponding author:
Malene Heidemann M.D., PhD student
Hans Christian Andersen Children’s Hospital at Odense University Hospital, Sdr. Boulevard 29,
5000 Odense C, Denmark
+45 40 16 12 24
E-mail: msheidemann@dadlnet.dk

Keywords: Dual Energy X-ray Absorptiometry, Bone mass, Bone density, Lean mass, Fat mass, Body composition.

Disclosures: All authors state that they have no conflicts of interest and no disclosures.

Mini Abstract: Effects of growth on bone health in childhood was investigated. Height and BMI was considered in a simple model and lean mass and body fat percentage in a more complex model. Height and lean mass are both precise predictors of bone accrualent and predict bone accrualent in equal patterns.
Abstract

Overweight, physical inactivity and sedentary behaviour have become increasing problems during the past decade. Increased sedentary behaviour may change the body composition (BC) by increasing the fat mass relative to the lean mass (LM). These changes may influence bone health. **Purpose:** to describe how anthropometry and BC predict the development of the bone accruement and to explore differences in their potentials for predicting bone accruement. **Methods:** The longitudinal study is a part of The CHAMPS study- DK. Children were DXA scanned at baseline and at two-year follow up. BC (LM, BF%) and BMC, BMD, and BA were measured. The relationship between bone traits, anthropometry and BC were analysed by multi level regression analyses. **Results:** Of the invited children, 742/800 (93%) accepted to participate. Of these, 682/742 (92%) participated at follow up. Mean (range) of age at baseline was 9.5 years (7.7-12.1). Height, BMI, LM and BF% predicted bone mineral accruement and bone size positively and independently. **Conclusions:** Height and BMI are both positive predictors of bone accruement. LM is a more precise predictor of bone traits than BF% in both genders. The effects of height and BMI and LM on bone accruement are similar in the two genders while changes in BF% have different effects on bone health in boys and girls.
Introduction

The measurement of body composition (BC) may play an important role in understanding the relationship between body fat percentage (BF%), lean mass (LM) and changes in bone mass. Furthermore these relations may vary with age, pubertal stages and between genders.

The accumulation of bone mass in childhood is crucial in the prevention of osteoporosis since peak bone mass (PBM) achieved during the mid twenties is influenced by lifestyle exposures such as physical activity and diet in childhood as well as changes in BC [1,2]. These exposures help to achieve an optimal bone acquisition during childhood and adolescence. The achievement of a high PBM is considered an essential preventive strategy against the development of bone fragility and osteoporosis later in life [3].

The mechanisms behind the effect of adipose tissue on bone mass and bone mineral density are considered diverse. In adulthood, adiposity serves as a protective factor against fractures, whereas some reports suggest an opposite effect in childhood [4,5]. Several studies have demonstrated a higher incidence rate of fractures in adipose and obese children compared to children with normal weight and height [6-8]. This leads to an increased concern for future bone health since adiposity among otherwise healthy children has become an increasing problem in developed countries [9].

LM is defined as the total body weight less FM and BMC. It has an impact on bone accrualment, especially during puberty. The muscle force incurs a large load on the bone and several studies have reported a close relationship between LM and bone development [10,11]. Thus, the muscle mass or LM is considered a key stimulant of bone development throughout lifespan [12].

During the past decade children have become less physically active with a tendency of increased polarization of physically activity behaviour [13,14]. These changes may lead to a group of sedentary children at increased risk of adiposity due to reduced energy consumption with a negative influence on their bone acquisition.

Many parameters affect the bone accrualment and the development in BMC, BMD and BA. Among these are growth and changes in BC. This provides an opportunity to describe the bone development by different sets of parameters. In this longitudinal study the purpose was to describe the parameters of growth that predict the development of the bone accrualment in a cohort of healthy Danish children. We approached this by introducing two models that both
contain key measures of growth. Model I used simple anthropometry (height and body mass index (BMI)), which are easily measured in any physical examination of a child, in combination with, age, gender and puberty and allowed for gender specific effects of the anthropometric measures. Model II was based on more detailed and complex BC information from DXA scans in terms of LM and BF%. This model also included potential effects of age, gender and puberty as well as gender specific effects of the BC information.

Materials and Methods

Study design

The study is a sub study of the CHAMPS study, DK, a natural experiment. Ten public schools in the municipality of Svendborg, Denmark, participated in the study. Six schools chose to implement four additional lessons of physical education (PE) weekly to their usual PE program (“sports schools”). These were matched to four schools based on school size and location. The children in the “normal schools” continued with two mandatory PE lessons per week. The study has been described in details elsewhere [15,16].

The population in the CHAMPS study comprised children from the pre-school year to 4th grade. Children attending 2nd to 4th grade (7.2-12 years) at baseline in year 2008 were invited to participate in DXA scans at baseline and at two-year follow-up. Examinations of the children took place at The Hans Christian Andersen Children’s Hospital, Odense, Denmark.

Ethical considerations

Children and parents received verbal and written information about the study. Participation in the study was at any time voluntary. The parents signed informed consent forms. Permission to conduct The CHAMPS Study–DK was granted by the Regional Scientific Ethics Committee (Project number: S-20080047).

Data collection

Anthropometrical data
Height was measured to the nearest 0.5 cm using a portable stadiometer, SECA 214. Body weight was measured to the nearest 0.1 kg on an electronic scale, SECA 861 (both Seca Corporation, Hannover, MD). Height and weight measurements were both conducted barefoot and BW was measured in a T-shirt and underwear.

Dual Energy X ray Absorptiometry (DXA scans):
Whole body DXA scans were performed with the GE Lunar Prodigy (GE Medical Systems, Madison, WI), ENCORE software (version 12.3, Prodigy; Lunar Corp, Madison, WI). The DXA scans provided estimates of bone mineral content (BMC), bone area (BA) and bone mineral density (BMD) as well as lean mass (LM) and fat mass (FM). The body fat percentage (BF%) was calculated as \( \frac{FM}{FM + LM + BMC} \times 100\% \). The total body less head (TBLH) values were used in this study. DXA scans were performed at study baseline (fall 2008) and at two-year follow-up (fall 2010). The participants were scanned wearing underwear, a thin T-shirt, stockings and a blanket. The DXA scanner automatically altered scan depth depending on the size of the subject. The DXA scanner was reset every day following standardized procedures. The coefficient of variation (CV) for BMD was 0.28%- 0.33% during baseline and follow-up examinations. Previous reports have demonstrated that GE Lunar Prodigy has reproducibility with precision errors (1 SD) of approximately 0.75% CV for BMC, 2.01% for LM and 1.29% for BF% in children and adolescents with a mean age 11.4 years (5-17 years) [17]

Pubertal self-assessment
The Tanner pubertal stages self-assessment questionnaire (SAQ) used in this study consists of line drawings of the 5 Tanner stages [18] supported by explanatory text. The children were asked to indicate which stage (Tanner stage 1-5) best referred to their own pubertal stage. Boys were presented with pictures and text of Tanner staging for pubic hair development, whereas girls were presented with pictures and text representing breast development and pubic hair [19]. The information about puberty was handled as a continuous variable in the analyses. The procedure took place in a private setting with sufficient time for self-assessment.

Statistical analyses
Descriptive statistics for all dependent and independent variables at baseline and follow up are presented as means, range
and standard deviation (SD). Frequencies by pubertal stages were stratified by gender.

Two different models were designed to describe the relationship between each of the outcome variables BMC and BA and the explanatory variables. The first set of models described how the outcome variables depend on anthropometric measures (height, BMI) in combination with age, gender and puberty. The second set of models was designed to describe the same variables by BC variables (LM, BF%) and age, gender and puberty. In all cases gender specific roles of anthropometric and BC related measures were accommodated by interaction terms in the regressions models. The variance inflation factor was used to assess potential multi collinearity between explanatory variables. Variations causing conflicts of independence were removed from the models. Box-Cox analyses were used to identify transformations that would ensure normality of the residuals. BMC was transformed by \((BMC)^{0.2}\) and BA was transformed by \((BA)^{0.4}\) in model I. BMC was transformed by \((BMC)^{0.5}\) in model II. The Box-Cox analyses indicated no need for transformation of BA in model II. Linearity between the transformed responses and the explanatory variables were assessed graphically. None of the explanatory variables were transformed.

Data were sampled in a hierarchical structure (schools, classes, children). Each level in this structure added to the random variation in the data. A multilevel random effects model was used for proper modelling of the variance. This model was also used to assess the relationships between each of BMC and BA accretion and different explanatory variables. The models were reduced by backward elimination. The regression analyses were stratified by gender to find the most precise predictor for BMC and BA by the Akaike’s information criteria in each of the two models.

BMD is defined as a BMC/ BA and hence assessed by the regression models for these two quantities. Data (BMC and BA) were transformed to meet the requirements of normality. The usual interpretations of the parameters therefore apply on the transformed scale. The fitted models for the impact on bone health of each of the outcome variables are presented on the ordinary scale in Figure 2 for the explanatory variables of particular interest.

Residual plots; histograms and qq-plots were used to check the assumptions of normality of the residuals. The level of significance for all statistical tests was 0.05. All statistical calculations were performed using STATA (version 12, Stata Corp LP, College Station, TX)

Results
Children from the preschool year to 4\textsuperscript{th} grade were invited to participate in The CHAMPS study-DK from baseline in September 2008, of which 1218/1500 (81 \%) accepted. Children attending 2\textsuperscript{nd} to 4\textsuperscript{th} grade (7.7 -12 years) at baseline were invited for the DXA scans. Of these 742/800 (93\%) accepted to participate. At baseline 717/742 (97\%) children participated and 682/742 (92\%) participated at follow up (Figure 1). The characteristics of the participants at baseline and at follow up regarding all explanatory variables are reported in Table 1.

The accretions of BMC and BA were assessed by two different sets of predictors using multilevel regression models with schools, classes and children as random effects. Model I used simple anthropometric variables whereas model II used BC variables. Results are listed in Table 2 and Table 3 respectively. Except for BA in model II, the outcome variables were transformed by suitable power transformations to ensure normality. The estimated effects described below are therefore only linear on the scale of the transformed outcomes. The effects do however retain the same directions when back transformed, as the transformations are monotonic.

Model I

The relationship between BMC accretion and anthropometry was assessed. Of the examined explanatory variables; height ($\hat{\beta}_{\text{Height}} =0.022, p<0.001$), BMI ($\hat{\beta}_{\text{BMI}} =0.019, p<0.001$), gender ($\hat{\beta}_{\text{Gender}} =0.029, p<0.001$), and puberty in girls ($\hat{\beta}_{\text{Puberty girls}} = 0.012, p<0.001$) predicted the accrual of BMC positively. The effect of puberty in boys was insignificant (Table 2). Gender interactions with height and BMI were removed from the model due to multi collinearity.

The relationship between BA accretion and anthropometry was assessed. From the initial model all examined explanatory variables, height ($\hat{\beta}_{\text{Height}} =0.113, p<0.001$), BMI ($\hat{\beta}_{\text{BMI}} =0.128, p<0.001$), predicted the accrual of BA positively. The effect of gender and puberty was insignificant (Table 2). Gender interactions with height and BMI were removed from the model due to multi collinearity.

Model II

The relationship between BMC accretion and BC was assessed in model two (Table 3). From the initial model the examined explanatory variables, LM ($\hat{\beta}_{\text{LM}} =6.03e-04, p<0.001$), BF\% ($\hat{\beta}_{\text{BF\%}} =0.103, p<0.001$), age ($\hat{\beta}_{\text{Age}} =0.684, p<0.001$), puberty ($\hat{\beta}_{\text{Puberty girls}} =0.337, \hat{\beta}_{\text{Puberty boys}} =-0.122$, both $p<0.02$) predicted the BMC accretion. There was an interaction between gender and BF\% with a higher effect ($\hat{\beta}_{\text{BF\% boys}} =-0.015, p=0.08$) for boys than for girls (Table 3).
The relationship between BA accretion and BC was assessed. There were positive associations between LM, BF%, age and an interaction between gender and puberty with a positive impact of puberty in girls.

BMD was described as a function of BMC and BA it was therefore redundant to perform a regression for the outcome variable BMD. The result of the functional description of BMD is displayed in Figure 2.

In model I height was the most precise predictor of BMC compared to BMI (lowest AIC value) in both genders. In model II LM was the most precise predictor of BMC in both genders (Table 4).

Discussion

This study aimed to describe the influence of anthropometry and BC on bone accrual in a large cohort of healthy Danish children. The main finding of the study is that height, BMI, LM and BF% are all independently positive predictors of bone accrual in children with height being a more precise predictor of bone accrual than BMI and LM being a more precise predictor of bone accrual than BF% in both genders.

Understanding the impact of growth and BC on bone health and particular gender differences is important when planning prevention strategies towards osteoporosis. Approximately 700 children were examined at baseline and at two-year follow-up with the intention to achieve more knowledge about the complexity of bone accrual during growth in childhood. We therefore evaluated the impact of changes in simple anthropometry, which can easily be evaluated in every child compared to the more complex measurements of BC by DXA on bone health parameters and the gender differences of these effects.

The graphs in Figure 2 display the expected values of BMC and BA as estimated from the regressions. Regression on BMD was considered redundant as BMD=BMC/BA and can therefore be assessed as a function of these two outcome variables. In model I both height and BMI predicts BMC, BA and BMD positively. The overall conclusion of model I is that no gender differences in the effect of height and BMI on BMC, BA and BMD are observed. In model II the effect of LM on BMC, BA and BMD is the same in boys and girls and the curves show similar patterns of dependency with height and BMI (Figure 2). Gender differences are observed for BF% on BMC, BA and BMD. Girls reach higher values of BMC at lower BF% values than boys with the same BF%. Girls reach higher BA than boys with the same BF% although with parallel curves. The effect on BF% on BMD shows two different curves with girls having higher BMD values at low BF% whereas this relationship is opposite at the higher BF% values (Figure 2).
A cross-sectional study [20] of 267 students (8-18 years) concluded that there was a correlation between bone mass and BC in both genders with LM and fat mass index being the predictor of bone mass in males and females, respectively. In this longitudinal study, all regression analyses were stratified by gender and LM was found to be a more precise predictor of bone traits than BF% in both boys and girls. Height, which is a cheap and an easy accessible measurement, predicts bone mineral accrualment in childhood equally in boys and girls. In addition, height seems to be a more precise predictor of bone mineral accrualment than BMI (Table 4).

The two models reflect two different approaches to explain the development in bone traits in a longitudinal perspective. The two models explain gender specific differences in the impacts of anthropometry and BC on bone accrualment in a simple easy assessable and cheap model (anthropometry) and a more complex model (BC). Both models predict bone accrualment in similar patterns although only a gender difference is observed with BF% as the predictor of bone accrualment.

The BC changes through puberty. Both sexes experience increases in total body fat but the proportion of body fat decreases in boys as a result of simultaneously rapid increase in LM [21]. Girls reach higher BF% whereas boys reach higher levels of LM. These gender differences in the development in BC may be very relevant to consider as BF% seem to exert different effects on bone traits in boys and girls as demonstrated in this study.

The prevalence of overweight and adiposity, usually assessed by BMI, has increased in most developed countries over the past decades [9]. Obesity has become a topic of increased interest in relation to preventive strategies towards a wide variety of potential co-morbidities. Several studies have evaluated the effect of overweight and adiposity on bone traits in childhood [22,5-7] and there is an agreement that body mass and the relative changes in the different components of BC are important in the regulation of bone growth and accrualment of bone mineral. In the HELENA study 330 adolescents were examined. The study aimed to examine the independent association of FM and LM with bone mass. They concluded that a high bone mass in adipose adolescents is explained by their higher levels of LM [23]. In this longitudinal study analyses suggest that BF% exert different effects on bone accrualment in boys and girls and BF% and LM are both positive predictors with independent effect on bone outcome in boys and girls. This supports the results of Heidemann et al. that training and physical activity has positive effect on bone accrualment [16]. The effect could be mediated through an increase in lean mass that introduces a constant increased traction at the muscle insertions of the bones, inducing an increased bone accrualment or through a direct impact from physical activity.
The analyses did not consider potential confounders such as vitamin D status or nutritional habits. Nor does the study present data on the children’s physical activity. Data on the children’s socio-economic status were also not mentioned. However, we presented data obtained from a cohort of healthy children that show homogeneity in relation to anthropometry and BC at baseline.

In the statistical analyses we find significant interactions of gender and some of the parameters of interest. Although these effects are highly significant (p<0.001) they may not be of clinical relevance as demonstrated in Figure 2. The reason for this may be that this present study has a high statistical power and therefore we are able to detect small differences in the data.

An advantage of the longitudinal design is the ability to measure accrualment of bone health outcome. Another strength is the data collection including DXA as the reference standard method for the measurement of bone health parameters (BMC, BA and BMD) and body composition (LM and BF%).

Conclusions

In this longitudinal study the purpose was to describe the parameters of growth that predict the development of the bone accrualment in a cohort of healthy Danish children. Height and BMI were both independent positive predictors of BMC, BA and BMD in childhood. However, height, BMI and LM did not reveal gender differences in bone mineral accrual as well as BF%. Height is a more precise predictor of BMC, BA and BMD than BMI. LM and BF% are both positive predictors of bone accrualment with LM being a the most precise predictor of bone accrualment in both boys and girls, supporting increased physical activity and training for increased bone accrualment in children. From simple anthropometry measured in every physical examination of a child, valuable predictive information is offered and seems to predict bone accrualment in the same pattern as the more complex measurement of LM.

Acknowledgements

The authors gratefully acknowledge the work of numerous students and PhD students who have participated in the data collection for the CHAMPS study- DK. We thank children, parents and teachers in the schools involved in the project, and we are grateful for the cooperation with “Elitesekretariatet” in the municipality of Svendborg. The study had not been possible without financial support from The Nordea foundation, The TRYG foundation, The IMK foundation, The Region
of Southern Denmark, The Egmont Foundation, The A. J. Andersen Foundation, The Danish Rheumatism Association, and TEAM Denmark

Table 1: Participant characteristics at baseline (year 2008) and follow-up (year 2010). Results of anthropometrics and DXA values are reported as means (standard deviation; range), and pubertal stages as numbers (n)

<table>
<thead>
<tr>
<th></th>
<th>Baseline, girls n=373</th>
<th>Baseline, boys n=344</th>
<th>2 year follow up, girls n=348</th>
<th>2 year follow-up, boys n=335</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs.)</td>
<td>9.5 (0.86; 7.8-11.6)</td>
<td>9.5 (0.88; 7.7-12.1)</td>
<td>11.5 (0.87; 9.8-13.5)</td>
<td>11.5 (0.90; 9.7-13.9)</td>
</tr>
<tr>
<td>Anthropometric measures</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>32.3 (6.1; 19.7-63.6)</td>
<td>33.1 (6.5; 21-62.7)</td>
<td>40.9 (8.6; 24.4-72.7)</td>
<td>40.8 (8.7; 25.4-81.8)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>138.2 (7.1; 122.9-164.6)</td>
<td>140.1 (7.8; 121.1-172.2)</td>
<td>150.7 (8.3; 131.5-174.6)</td>
<td>151.2 (8.9; 130.5-185.5)</td>
</tr>
<tr>
<td>BMI</td>
<td>16.82 (2.25; 12.36-28.57)</td>
<td>16.70 (2.6; 12.8-28.44)</td>
<td>17.86 (2.58; 12.38-28.01)</td>
<td>17.70 (2.46; 12.86-28.44)</td>
</tr>
<tr>
<td>DXA measures (TBLH)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>22.8 (3.1; 16.3-35.8)</td>
<td>25.3 (3.3; 17.8-40.1)</td>
<td>28.5 (4.9; 14.1-46.9)</td>
<td>30.7 (5.2; 21.1-62.9)</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>7.8 (3.8; 1.2-27.2)</td>
<td>6.2 (3.9; 15.6-24.6)</td>
<td>10.4 (4.9; 2.1-3.0)</td>
<td>8.3 (5.0; 17.5-32.9)</td>
</tr>
<tr>
<td>Fat %</td>
<td>23 (7.4; 6-43)</td>
<td>17 (7.8; 6-42)</td>
<td>25 (7; 8-46)</td>
<td>19 (7.8; 5-43)</td>
</tr>
<tr>
<td>BMC (g)</td>
<td>846 (208; 453-2005)</td>
<td>888 (191; 408-1771)</td>
<td>1184 (310; 576-2366)</td>
<td>1191 (281; 563-2893)</td>
</tr>
<tr>
<td>BMD (g/cm²)</td>
<td>0.75 (0.06; 0.6-1.1)</td>
<td>0.76 (0.05; 0.59-0.96)</td>
<td>0.83 (0.08; 0.67-1.15)</td>
<td>0.83 (0.06; 0.64-1.15)</td>
</tr>
<tr>
<td>BA (cm³)</td>
<td>1105 (181; 720-1883)</td>
<td>1149 (175; 686-1842)</td>
<td>1393 (238; 851-2164)</td>
<td>1405 (229; 874-2509)</td>
</tr>
<tr>
<td>Pubertal (Tanner) stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tanner stage</td>
<td>281/81/5/4/0</td>
<td>170/155/17/1/0</td>
<td>97/123/102/23/2</td>
<td>55/175/87/15/3</td>
</tr>
</tbody>
</table>
Table 2:
Model I. Coefficient estimates of the significant parameters for the regression of the Box Cox transformed BMC and BA. Backward elimination of insignificant parameters was performed.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Coefficient estimate for the regression of (BMC)^0.2</th>
<th>P Value</th>
<th>Coefficient estimate for the regression of (BA)^0.4</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height</td>
<td>0.022 (0.020-0.023)</td>
<td>&lt;0.001</td>
<td>0.113 (0.111-0.115)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body mass index (BMI)</td>
<td>0.0185 (0.0182-0.0187)</td>
<td>&lt;0.001</td>
<td>0.128 (0.119-0.137)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gender</td>
<td>0.029 (0.017-0.041)</td>
<td>&lt;0.001</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Puberty girls</td>
<td>0.012 (0.009-0.016)</td>
<td>&lt;0.001</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.89 (0.85-0.92)</td>
<td>&lt;0.001</td>
<td>-1.47 (-1.67- -1.28)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Note: In the initial model one (BMC)^0.2 and (BA)^0.4 were adjusted for height, BMI, gender, puberty and the interaction between gender and puberty.
Table 3:
Model II. Coefficient estimates of the significant parameters for the regression of the Box Cox transformed BMC and BA. Backward elimination of insignificant parameters was performed.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Coefficient estimate for the regression of $\frac{BMC}{\hat{\beta}}$ (95% CI)</th>
<th>$P$ Value</th>
<th>Coefficient estimate for the regression of $\frac{BA}{\hat{\beta}}$ (95% CI)</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lean mass (LM)</td>
<td>6.03e-04(5.8e-04-6.2e-04)</td>
<td>&lt;0.001</td>
<td>0.035(0.035-0.037)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fat percentage (BF%)</td>
<td>0.103((0.091-0.114)</td>
<td>&lt;0.001</td>
<td>6.475(5.893-7.056)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age</td>
<td>0.684(0.619-0.749)</td>
<td>&lt;0.001</td>
<td>30.974(27.270-34.678)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fat percentage boys</td>
<td>0.015(0.004-0.027)</td>
<td>0.008</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Puberty girls</td>
<td>0.337(0.246-0.428)</td>
<td>&lt;0.001</td>
<td>13.190(8.122-18.257)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Puberty boys</td>
<td>-0.122(-0.220-0.024)</td>
<td>0.014</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>5.815(5.386-6.243)</td>
<td>&lt;0.001</td>
<td>-168.253(-193.329- -143.178)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Note: In the initial model one $(BMC)^{0.5}$ and BA were adjusted for lean mass, fat percentage, the interaction between body fat percentage and gender (the interaction between body lean mass and gender was omitted from the model due to co-linearity), age, puberty and the interaction between gender and puberty.
Table 4: Predictors of model one and two estimated by Akaike’s Information Criteria.

<table>
<thead>
<tr>
<th>Model Predictor</th>
<th>Boys</th>
<th>Girls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BMC</td>
<td>BA</td>
</tr>
<tr>
<td>Model I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height</td>
<td>-1828</td>
<td>28</td>
</tr>
<tr>
<td>BMI</td>
<td>-439</td>
<td>1845</td>
</tr>
<tr>
<td>Model II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lean mass</td>
<td>1809</td>
<td>7259</td>
</tr>
<tr>
<td>Body fat percentage</td>
<td>2619</td>
<td>7920</td>
</tr>
</tbody>
</table>

Note: The smallest value of Akaike’s information criteria (AIC) (the most precise predictors within each model) is marked by *italics*. 
Figure 1: Flowchart of the participants

Figure 2: Graphs representing the effect of the independent changes of height, body mass index, lean mass, body fat percentage on bone outcome (BMC, BA and BMD) stratified by gender.

Note: The values of age, puberty, height, BMI (model I) and age puberty, lean mass and body fat percentage (model II) were chosen at the mean values. The impact on bone outcome of a change in the parameters of interest (keeping the other explanatory variables constant) is shown above for each parameter of interest in model one (height, BMI) and model two (LM and BF%). The solid lines refer to the girls whereas the dotted lines refer to the boys.
References

10. Sudhagoni Rg Fau - Wey HE, Wey He Fau - Djira GD, Djira Gd Fau - Specker BL, Specker BL (2012) Longitudinal effects of fat and lean mass on bone accrual in infants. 50 (3):638-642
11. Zemel B (2013) Bone Mineral Accretion and Its Relationship to Growth, Sexual Maturation and Body Composition during Childhood and Adolescence. (1662-3975 (Electronic))


21. Siervogel Rm Fau - Maynard LM, Maynard Lm Fau - Wisemandle WA, Wisemandle Wa Fau - Roche AF, Roche Af Fau - Guo SS, Guo Ss Fau - Chumlea WC, Chumlea Wc Fau - Towne B, Towne B Annual changes in total body fat and fat-free mass in children from 8 to 18 years in relation to changes in body mass index. The Fels Longitudinal Study. (0077-8923 (Print))


800 children invited for DXA

742/800 (93%) Accepted

717/742 (97%) Participated at baseline (2008-2009)

682/742 (92%) Participated at follow-up (2010-2011)