DISSERTATIONES
KINESIOLOGIAE
UNIVERSITATIS
TARTUENSIS
39

ARTŪRS IVUŠKĀNS

Bone mineral parameters in 11–13-year-old boys: associations with body composition and physical activity

DISSERTATIONES KINESIOLOGIAE UNIVERSITATIS TARTUENSIS
39

DISSERTATIONES KINESIOLOGIAE UNIVERSITATIS TARTUENSIS
39

ARTŪRS IVUŠKĀNS

Bone mineral parameters in 11–13-year-old boys: associations with body composition and physical activity

UNIVERSITY OF TARTU
PRESS
1632

Institute of Sport Pedagogy and Coaching Sciences, Faculty of Exercise and Sport Sciences, Centre of Behavioural, Social, and Health Sciences, University of Tartu, Tartu, Estonia

Dissertation was accepted for the commencement of the Degree of Doctor of Philosophy in Exercise and Sport Sciences on 21 of May, 2014 by the Council of the Faculty of Exercise and Sport Sciences, University of Tartu, Tartu, Estonia.

Supervisors: Senior Lecturer Jarek Mäestu
Professor Jaak Jürimäe
Professor Toivo Jürimäe

Opponent: Assistant Professor Arunas Emeljanovas,
Lithuanian Sport University

Commencement: 27th of June at 12.00 in the Senate room of University of Tartu

Publication of this dissertation is granted by the Institute of Exercise and Sport Sciences, University of Tartu and by the Doctoral School of Behavioral, Social and Health Sciences created under the auspices of European Social Fund

This research was also supported by European Social Fund's Doctoral Studies and Internationalisation Programme DoRa, which was carried out by Foundation Archimedes.

ISSN 1406-1058
ISBN 978-9949-32-603-7 (print)
ISBN 978-9949-32-604-4 (pdf)

University of Tartu Press
www.tyk.ee

CONTENTS

LIST OF ABBREVIATIONS 7

LIST OF ORIGINAL PUBLICATIONS 8

1. INTRODUCTION 9

2. REVIEW OF THE LITERATURE 11
   - 2.1. Bone development during growth and maturation 11
   - 2.2. Influence of body composition to bone mineral parameters in boys with different body weight status 13
   - 2.3. Influence of physical activity to bone mineral parameters in boys with different body weight status 14

3. AIM AND PURPOSES OF THE STUDY 18

4. MATERIALS AND METHODS 19
   - 4.1. Participants and study design 19
   - 4.2. Anthropometry and sexual maturation 19
   - 4.3. Bone mineral and body composition assessment 20
   - 4.4. Body composition indices 20
   - 4.5. Physical activity measurement 21
   - 4.6. Statistical analysis 21

5. RESULTS 23
   - 5.1. Body composition, bone mineral density and physical activity characteristics in overweight and normal weight boys 23
   - 5.2. Relationships between body composition and bone mineral density characteristics 26
   - 5.3. Relationships between physical activity and bone mineral density characteristics 29
   - 5.4. Changes in bone mineral density values after one year observation period in relation to changes in physical activity characteristics 31

6. DISCUSSION 34
   - 6.1. Bone mineral density values and their relationships with different body composition values in normal weight and overweight 11–13-year-old boys 34
   - 6.2. Relationships between bone mineral density and physical activity parameters in normal weight and overweight 11–13-year-old boys 37
   - 6.3. Changes in bone mineral density values after one year observation period in relation to changes in physical activity parameters in 11–13-year-old boys 39

7. CONCLUSIONS 43

8. REFERENCES 44

SUMMARY IN ESTONIAN 51

ACKNOWLEDGEMENTS 53
PUBLICATIONS 55
CURRICULUM VITAE 91
ELULOOKIRJELDUS 92

LIST OF ABBREVIATIONS

BA	bone area
BMAD	bone mineral apperent density
BMC	bone mineral content
BMD	bone mineral density
BMI	body mass index
Body fat%	body fat percent
DXA	dual-energy X-ray absorptiometry
FFM	fat free mass
FFMI	fat free mass index
FM	fat mass
FMI	fat mass index
FN	femoral neck
LS	lumbar spine
MVPA	moderate-to-vigorous physical activity
NW	normal weight
PA	physical activity
OW	overweight
TF:LF ratio	trunk fat/leg fat ratio
WB	whole body

LIST OF ORIGINAL PUBLICATIONS

I. Ivuškāns A, Lätt E, Mäestu J, Saar M, Purge P, Maasalu K, Jürimäe T, Jürimäe J. Bone mineral density in 11–13-year-old boys: relative importance of the weight status and body composition factors. *Rheumatology International* 2013; 33: 1681–1687

II. Ivuškāns A, Jürimäe T, Lätt E, Jürimäe J, Purge P, Saar M, Maasalu K, Rääsk T, Mäestu J. The role of physical activity in bone health in peripubertal boys. *Pediatrics International* 2014 (In Press)

III. Ivuškāns A, Mäestu J, Jürimäe T, Lätt E, Purge P, Saar M, Maasalu K, Jürimäe J. Sedentary time has a negative influence on bone mineral parameters in peripubertal boys: one-year prospective study. *Journal of Bone Mineral Metabolism* 2014 (In Press)

In all papers, Artūrs Ivuškāns had primary responsibility for protocol development, subjects´ screening, performing measurements, data analysis, and writing the manuscripts.

I. INTRODUCTION

Puberty is one of the most dynamic periods of growth and development, and is characterized by rapid changes in body morphology and composition, all of which are sexually dimorphic. Puberty begins by a growth spurt characterized by an important increase in body fat tissue. The pubertal growth spurt and the appearance of secondary sex characteristics are the most visible manifestations of puberty (Abbassi 1998). Timing of puberty differs according to gender by a skeletal or biological age of approximately 11 years in girls and 13 years in boys (Abbassi 1998). Puberty is a complex physiologic event in human growth and biologic maturation characterized by landmarks such as changes in hormonal levels and body composition, growth spurt and the appearance of secondary sex characteristics. Body proportions define gender differences in body composition. It is important to understand in more complex way the influence of potential environmental and genetic factors on growth and maturation during pubertal period as they both have strong influence on health in future life. Accordingly, the time period between 11 to 13 years of age appears to be important in terms of overall health, and further development and biological maturation of children.

Pubertal years are also an important period for linear growth and bone mineral accrual. Bone mineralization increases with age, height, and body mass throughout childhood, with a most significant gain during pubertal development. Early puberty is also a period of increased bone adaptation to mechanical loading due to the velocity of bone growth and endocrine changes at this time (Hind and Burrows 2007). In contrast, this is also a period when physical activity in children seems to decrease significantly, which may cause marked changes in body composition that mostly are related to increases in body fat amount. However, this decrease in physical activity, especially decreases in vigorous physical activity may also have an impact on further bone mineralization.

Physical activity, according to World Health Organization (WHO), is defined as any bodily movement produced by skeletal muscles that require energy expenditure. By WHO regular moderate intensity refers to physical activity such as walking, cycling, or participating in different sports. Physical activity benefits for health are possibly through the improvement of bone and functional health; to reduce risk of serious diseases such as cardiovascular disease, diabetes, obesity, etc. The influence of increased sedentary time might also have negative impact on bone mineral accrual, since it partly eliminates the loading effect of the body. As there are differences in timing of puberty, in the rate of the changes in body composition and differences in physical activity, it would be advantageous to focus the investigation to subjects from one sex only. To date, there are few data on specific bone mineral accrual in pubertal boys with different body composition values and the results in the literature concerning the exact influence are equivocal. Furthermore, to our best of knowledge, there have been no longitudinal data investigating the influence of

changes in physical activity on further bone mineralization in these age group boys. Accordingly, the main aim of the present thesis was to investigate possible associations between specific body composition and physical activity values with bone mineral parameters, and the contribution of measured body composition and physical activity values to the bone mineral accrual during one-year investigation period in boys entering puberty.

2. REVIEW OF THE LITERATURE

2.1. Bone development during growth and maturation

Bone is a metabolically active tissue with continuous remodeling occurring across the lifespan. In healthy adolescent males and females, bone mass and bone mineral density (BMD) at skeletal maturity are inversely related to the timing of puberty (Gilsanz et al. 2011). Bone mineral density and bone mineral content (BMC) measurements are widely used to characterize bone mineralization in children during growth and maturation. Bone mineral density is the result of the dynamic process of bone formation and bone resorption (Creighton et al. 2001).

Bone mineralization is a complex, multifactorial process, which is influenced by genetic, hormonal and nutritional factors during the entire lifespan. It is well known that puberty has a key role in bone development. Bone mineralization increases with age, height and body mass throughout childhood (Gordon et al. 1991), and maximal BMD accrual occurs in years surrounding puberty (Ausili et al. 2012; Gordon et al. 1991). Early puberty is also a period of increased bone adaptation to mechanical loading due to the velocity of bone growth and endocrine changes at this time (Hind and Burrows 2007). The dramatic accumulation of BMD during puberty is caused by changes in both modeling and remodeling during this period of life (Ausili et al. 2012), however, peak BMD usually occurs later. Using the longitudinal and cross-sectional data in range of 4 to 30 years, studies have concluded that peak BMD were attained between 18 and 23 years in males (Boot et al. 2010).

Bone metabolism in children differs from that in adults, and reflects both skeletal growth and remodeling. Bone remodeling refers to the process where mature bone tissue is removed from the skeleton (also called resorption) and new bone tissue is formed (also called formation). During skeletal growth, new bone is formed at a site different from that of bone resorption (Csakvary et al. 2013). Puberty is characterized by a rapid growth of bone mass within a relativity short period, since skeletal mass approximately doubles at the end of adolescence (Saggese et al. 2002). In fact, maximum bone enhancement occurs between 11 and 14 years, corresponding to pubertal stages 3–5 according to Tanner classification (Bonjour et al. 1991; Theintz et al. 1992). Vigilant assessment of the factors associated with the increase in bone mass during this phase may be important for the prevention of osteoporosis in later life (Arabia et al. 2004; Javaid and Cooper 2002,). Since physical exercise and the amount of fat free mass (FFM) have been recommended to influence maximal increases in bone tissue at some stage in puberty, the intensity and the amount of physical activity (PA) appear to be the important predictors of peak bone mass (Daly et al. 2004). Specifically, there is a growing evidence to suggest that osteoporosis has some origins in childhood. Assuming that 60% of the risk of osteoporosis can be explained by the amount of bone mass accrued in early adulthood, maximizing peak bone mass might be an important goal for the prevention of

osteoporosis (Rizzoli et al. 2010). Peak bone mineral mass, a major determinant of the future risk of fractures in the elderly, is largely achieved by the end of sexual and skeletal maturity (Gilsanz et al. 2011). The greatest accretion of bone occurs during puberty, and low peak bone mineral mass may result from clinical states associated with abnormal pubertal development (Gilsanz et al. 1998, 2011; Matkovic et al. 1994).

Bone mineralization increases significantly during pubertal development, resulting in an increase of BMD of about 40% during this period (Gordon et al. 1991). The pubertal increases in BMD are comparable in girls and boys but are significantly greater in black compared with white adolescents (Gilsanz et al. 1991, 1997, 1998; Han et al. 1996; Leonard 2007). However, the pubertal growth spurt varies considerably in timing, tempo and duration among individuals (Malina et al. 2004). Bone mineral accrual during puberty is more dependent on sexual maturation than on chronological age, and both androgens and estrogens promote the deposition of bone mineral into bone tissue (Yilmaz et al. 2005). Estrogen levels make the greatest contribution to BMD accretion during puberty in boys and girls, while testosterone level makes only a modest contribution to the increase of BMD in boys (Yilmaz et al. 2005). The important determinants of bone mineral accrual during puberty are also optimal nutritional status (Misra et al. 2005), body composition parameters (Christo et al. 2008) and PA pattern (Gruodyte et al. 2010). However, each of these determinants are related to the state of energy balance, and peripheral indicators of energy balance, such as different adipocytokines, may also have a positive influence on the BMD of the growing skeleton (Garnett et al. 2004). The inadequate caloric intake that is often associated with adolescent PA exposes to several health risks including negative effects on the development of BMD (Gruodyte et al. 2010).

It appears that bone mineral accrual in adolescents is dependent on multiple mechanical and biochemical factors, which may impact on bone modeling of the growing bone (Gruodyte et al. 2010). Bone strength is regulated by mechanical loads, especially by muscle forces. In children and adolescents, body composition and BMC are highly related (Crabtree et al. 2004; Creighton et al. 2001; Schoenau et al. 2002). All these factors need to be considered when assessing bone development during puberty. Because building up an adequate bone mass and stimulate its growth during childhood is essential for preventing osteoporosis, it is important to investigate bone mineral parameters during the period of maximized growth.

The concept of growth, development and maturation is of particular importance in the context of children and adolescents who participate in sport and PA, as exercise is one of key factors with the potential to influence growth and maturation (Baxter-Jones 2008; Hills et al. 2007), as well as changes in health and health-related fitness (Hills et al. 2007).

In conclusion, bone is a metabolically active tissue with continuous remodeling across the lifespan and time of puberty has a key role in bone development. Bone mineral accrual during puberty is dependent on multiple mechanical factors including different body composition and PA values.

2.2. Influence of body composition to bone mineral parameters in boys with different body weight status

There are two different types of bone in the human body. Lumbar spine is mainly composed of trabecular bone, whereas the whole body is composed at about 80% of cortical bone (Martin et al. 1988). It has been shown that trabecular and cortical bone tissues do not have the same sensitivity to mechanical stress, which could be the consequence of different metabolic activities on bone tissue (Bakker et al. 2003). This can result in different bone site-specific effects of body composition parameters. It is well established that during early puberty human body adopts to mechanical loads due to the velocity of bone growth and changes in endocrine system (Hind and Burrows 2007). Childhood obesity is an increasing problem all around the world and in addition to overall health problems, obesity might also have the influence on bone mineral accrual during puberty. It may be expected that in general, being overweight is associated with a protective effect on osteoporosis because of the increased BMD (Bakker et al. 2003; Rocher et al. 2008). Overweight children have not only higher FM, but also higher FFM values (El Hage et al. 2009a; Goulding et al. 2000). In overweight boys, the skeleton must be stronger than in normal weight boys to support their higher body mass during everyday activities (Rocher et al. 2008). Similarly, it has been suggested that body mass might improve bone mineralization in obese children by increasing the mechanical load of increased body mass, especially in weight-bearing bones (El Hage et al. 2009a; Ellis et al. 2003; Rocher et al. 2008).

Studies that have investigated BMD and BMC values in obese children have reported controversial results. It has been argued that obese children have either lower (Goulding et al. 2000, 2002), equivalent (Hasanoglu et al. 2000) or higher (Ellis et al. 2003) BMC compared to normal weight children. Accordingly, there is a significant disagreement in the literature regarding the relative contributions of fat and fat-free body components to bone mineral values in growing children (El Hage et al. 2009a). Although it has been demonstrated that the effects of body mass on bone mineral parameters are the result of the applied load to the skeleton, this effect is also comparable to the influence of current PA level (Boot et al. 1997). Many studies have demonstrated a positive effect of FM on bone mineral values (Clark et al. 2006; El Hage et al. 2009a; Ellis et al. 2003). Further, it has been demonstrated that if bone values were adjusted for FM, no differences in bone mineral values were found between 9–12-year-old obese and normal weight children (Rocher et al. 2008). Similar results were recently found by Gracia-Marco et al. (2012), who demonstrated that adolescents with higher levels of adiposity have greater bone mass, yet this relationship was fully explained by their higher FFM values, and the amount of FM and PA level did not have any confounding role in these associations. In addition, it has not been clarified whether this is the result of the additional loading by body FM, FFM or their combination in pubertal children, as this is the crucial period for bone growth. For example, obese children seemed to have higher bone

mineral values compared to normal weight children (Ellis et al. 2003; Rocher et al. 2008), but there are also studies showing that body fat may be a negative determinant of BMD in children (Hrafnkelsson et al. 2010; Specker et al. 2001). Therefore, the effect of body composition values on bone mineral parameters is still under debate. Accordingly, increasing peak bone mass is an important focus to the protection against fracture risk (Clark et al. 2006), and it is important to evaluate the relative importance of FM and FFM on bone growth in children with different body mass values during pubertal development as the body mass increases the mechanical load on the growing bones (Boot et al. 1997; Ho and Kung 2005).

In conclusion, different body composition values influence bone development during growth and biological maturation. It appears that overweight children have higher FM and FFM values and both of them can influence bone health. It is important to evaluate the role of FM and FFM on bone mineralization in boys with different body weight status entering into puberty.

2.3. Influence of physical activity to bone mineral parameters in boys with different body weight status

Nowadays in general, children and adolescents spend less time participating in sports or different PA, while they devote more time to activities such as watching television and playing video games (Ruiz and Ortega 2009; Steel et al. 2009). This shift to more sedentary lifestyle is supposed to predispose to obesity and may attenuate bone mass accrual, thus increasing the risk for a low BMC, and perhaps leading to a low peak bone mass (Ho and Kung 2005; Vicente-Rodríguez et al. 2008. Functional model of bone development indicates that forces from muscle contractions are the main mechanical challenges to which bones adapt (Farr et al. 2011). In contrast, the influence of mechanical loading activity on bone is vitally important for skeletal strength and development (Baptista et al. 2012; Welten et al. 1994). Several cohort studies have indicated that physical training before and during puberty is associated with increased bone acquisition in children and young adults (Bass et al. 1998; Lorentzon et al. 2005; Nilsson et al. 2009; Tobias et al. 2007). Mechanical loading factors such as weight-bearing and muscle forces play an important role in bone mass acquisition and maintenance in children (Baptista et al. 2012; Ho and Kung 2005). Physical activity before and during puberty has been associated with an altered cortical bone geometry, especially attributed to periosteal augmentation (Bass et al. 2002; Lorentzon et al. 2005; Specker et al. 2004). However, it has, not been established for how long this cortical bone geometry alteration after physical activity will remain when the level of physical activity is decreased (Nilsson et al. 2009).

Although adaptive responses of bone are affected by the amplitude, frequency, distribution, and duration of the bone loading history, it is commonly believed that maximum strains during vigorous PA have the greatest influence

on bone development (Ehrlich and Lanyon 2002). Mechanical strain is an important determinant of skeletal growth and modeling, and therefore, PA has also been considered as the key factor affecting the achievement of optimum peak bone mass that may reduce later fracture risk (Baxter-Jones et al. 2008; Sardinha et al. 2008). Bone seems to adapt to the level of exercise intensity required depending on the mechanical stress generated by exercise. Therefore, the main influence of physical exercise on bones depends on the type, intensity, and duration of the stimulus (Vicente-Rodriguez et al. 2008). However, it can be argued that hands and legs respond differently to mechanical loading as for legs apply both forces mechanical strain and gravity, but for hands only gravity, although it has also been shown that for specific exercises (ie tennis) there is an increase also in bone minerals in dominant arm (Sanchis-Moysi et al., 2010).

Everyday PA is a multidimensional and complex behavior that is difficult to assess (Heaney et al. 2000). To date, many researchers have used questionnaires and interviews to assess PA level (Corder et al. 2007; Ott 1991; Sardinha et al. 2008). The use of questionnaires to assess PA could be imprecise in children, causing in some cases under- or overreporting, whereas accelerometers provide more valid and objective information on the frequency, intensity, and duration of PA (Heaney et al. 2000; Jones et al. 2010; McCarthy et al. 2006). Results from PA interventions in children aimed at increasing bone mass were effective in increasing BMC and bone strength in the range of 1 to 8% (Hind and Burrows 2007). Longitudinal data show that physically active children maintained their higher bone mass compared to less active peers into early adulthood, even independently of their actual PA level (Baxter-Jones et al. 2008; Kemper 2000), indicating clearly the importance of PA on bone health. However, it remains unclear, whether the effects of a more general PA intervention not exclusively targeting bone health would also be maintained over a prolonged period (Meyer et al. 2013).

One common strategy to increase peak bone mass is through regular, weight-bearing exercise (Rutherford 1999). Although the most suitable sporting activities remain unknown, participation in weight-bearing physical exercises generating high ground reaction forces, mainly if they include jumps, sprints, and rapid changes of directions, seem to have the most evident osteogenic effect on bone development during growth (Vicente-Rodriguez 2006, 2008). Weight-bearing exercises can include aerobics, circuit training, jogging, jumping, volleyball and other sports that generate impact to the skeleton. There is an evidence to suggest that the years of childhood and adolescence represent an opportune period during which bone adapts particularly efficiently to such loading (Bass et al. 2002; Hind and Burrows 2007).

Accordingly, lots of studies have been investigating the role of PA to BMD and BMC development, and have found positive influence on bone mineralization in pre- and early pubertal children (Baxter-Jones et al. 2008; Hind and Burrows 2007; Sardinha et al. 2008; Tobias et al. 2007). For example, exercise during growth seems to increase the peak BMD by between 10% and 20% in the loading bones of active adolescents compared with sedentary controls (Bass

et al. 1998). This effect could be even greater if exercise starts during the years preceding puberty. The proportion of BMC attained between ages 11 to 13 years in girls and 12 to 14 years in boys is around 25% of the adult BMC (Bailey et al. 2000). It is also likely that exercise during this period acts in a synergic way, with the growth-related bone development leading to a higher bone mass at the end of the pubertal period (Bailey et al. 2000; Vicente-Rodriguez et al. 2008). Therefore, the results are in agreement with those reported by Haapasalo et al. (1998), who found that the beneficial effect of PA during the growth spurt or biological maturation at Tanner stages 3–4. In a large part, this benefit can be explained by the specific characteristics of the sport itself (Nebigh et al. 2009). However, most studies so far have used heterogeneous groups of subjects with relatively broad range of body composition values that might have confounding effects regarding the influence of PA levels (Baxter-Jones et al. 2008; Ginty et al. 2005; Steel et al. 2009;), or have focusing on investigating the differences between boys and girls (Ausili et al. 2012; Baptista et al. 2012; Baxter-Jones et al. 2008; Steel et al. 2009; Tobias et al. 2007) in regards of PA association to bone mineral parameters. There is less data available concerning the influence of objectively measured PA levels to bone mineral parameters in peripubertal boys with different weight status.

Several studies have described and longitudinally confirmed the association between PA and bone mass acquisition in children and adolescents (Baptista et al. 2012; Farr et al. 2011; Gracia-Marco et al. 2012; Marshall et al. 1970; Sardinha et al. 2008; Sayers et al. 2011). In their review, Hind and Burrows (2007) reported positive effects of weight-bearing exercise intervention trials on bone mineral accrual in children and adolescents. In addition, high-volume and high-impact loading training such as gymnastics also promotes bone mineral acquisition (Courtiex et al. 1999). This together clearly suggests that there is a role of PA on bone mass acquisition during growth and biological maturation. However, the amount and intensity of PA needed to influence bone development in childhood is still unclear (Sardinha et al. 2008, Sayers et al. 2011).

Studies evaluating the relationship of objectively measured PA by accelerometer and bone mass are limited and mainly cross-sectional in children and adolescents (Ausili et al. 2012; Ginty et al. 2005; Heaney et al. 2000; Jones et al. 2010; McCarthy et al. 2006). For example, a positive association between cortical bone mass and the time spent (min/day) in vigorous PA was found, whereas light or moderate PA had no detectable association with bone mineral parameters in 15.5-year-old boys (Ausili et al. 2012). Another investigation concluded that vigorous PA for at least 25 min seems to improve femoral neck bone health in 9.7-year-old boys and girls (Heaney et al. 2000). In contrast, whole body bone mineral content (BMC) values were not different in 14.8-year-old adolescents participating less than 30 min/day with those participating 90 min/day or more in moderate and vigorous PA (Jones et al. 2010). There is a need for longitudinal studies to further clarify the role of different PA levels measured by accelerometers in the development of bone mass during the beginning of puberty.

To the best of our knowledge, no studies have been performed to observe longitudinal associations between objectively estimated different amounts and intensity categories of PA with bone mass acquisition over a one-year observation period in peripubertal boys. Furthermore, nothing is known about the longitudinal influence of sedentary behavior on bone mass acquisition. It is not entirely clear whether the sensitivity of bone mass accrual to PA may vary during further growth and biological maturation.

In conclusion, during puberty bone mass significantly increases and the rate of increase reaches its peak. During this period PA plays important role in bone development. Effects of exercise on bones depend on the type of PA, intensity, and duration of the PA stimulus. However, it is not fully clear, which type and duration the PA must be in order to have positive impact for bone development during the puberty. Therefore, the current investigation focuses on examination of PA exposure to bone mass accrual during a longer observation period.

3. AIM AND PURPOSES OF THE STUDY

The general aim of the present study was to evaluate bone mineral parameters in normal and overweight boys aged 11–13 years in associations with body composition and physical activity.

According to the general aim, the specific aims of the present investigation were to:

1) investigate different bone mineral density values between overweight and normal weight boys (Study I);
2) investigate the relationships of different body composition parameters on whole body and areal bone mineral density values in overweight and normal weight boys (Study I);
3) investigate the relationships of different physical activity levels on whole body and areal bone mineral density values in overweight and normal weight boys (Study II);
4) investigate the physical activity changes to whole body and areal bone mineral accrual during one year period in 11–13-year-old boys (Study III).

4. MATERIALS AND METHODS

4.1. Participants and study design

In total, 264 boys aged between 11 to 13 years from different schools in Tartu participated in the first year cross-sectional study (Studies I and II). The participants were divided into normal weight (body fat% <20.7–22.8) and overweight (body fat% ≥21.3–22.8) groups according to the age adjusted cutoffs described by McCarthy et al. (2006). Participants in the present study performed the same tests with one-year interval, and those tests included anthropometry, sexual maturation and bone age assessment, bone mineral and body composition, and physical activity measurements. Only those subjects were entered into one-year longitudinal analysis (Study III), who had all the measurement data from the second tests (Figure 1). The study protocol was reviewed and approved by the Human Ethical Committee of the University of Tartu, Estonia. All the subjects and their parents received a full written description of the nature of the study and signed written informed consent was obtained before entering the study. The subjects of the present study were part of a larger longitudinal study where boys were followed until pubertal maturity.

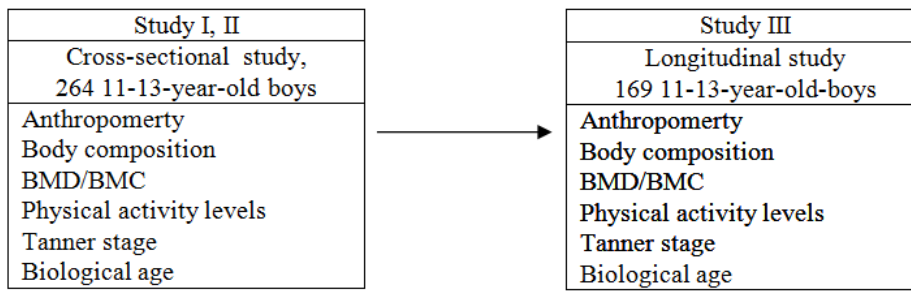

Figure 1. Tracking of the study population (11–13-year-old boys from different schools in Tartu).

4.2. Anthropometry and sexual maturation

Body height was measured to the nearest 0.1 cm using Martin`s metal anthropometer. Body mass was measured to the nearest 0.05 kg using medical scales (A&D Instruments Ltd; Abingdon; UK). The boys were dressed in light clothing and were wearing no shoes. Pubertal development of the participants was assessed according to the self-assessment using an illustrated questionnaire of pubertal stages according Tanner classification method (Tanner 1962). The pubertal development assessment according to Tanner method, which uses the self-assessment of genitalia and pubic hair stages, has been previously validated (Duke et al. 1980; Saito 1984). The boys were given photographs, figures, and

descriptions, and asked to choose the one that most accurately reflected their appearance. In case of discrepancies between the two variables, a greater emphasis for the determination of the Tanner stage was placed on the degree of genitalia development (Duke et al. 1980). The self-assessment of pubertal development in boys has previously been assessed in our laboratory (Lätt et al. 2009; Pomerants et al. 2006). In addition, bone age was assessed with an X-ray of the left hand and wrist, and determined according to the method of Greulich and Pyle (1959).

4.3. Bone mineral and body composition assessment

Bone mineral density (g/cm^2) of the whole body (WB), lumbar spine (L2-L4) (LS), femoral neck (FN), and the WB BMC (g) were measured by dual-energy X-ray absorptiometry (DXA) using the DPX-IQ densitometer (Lunar Corporation, Madison, WI, USA) equipped with proprietary software, version 3.6. Bone mineral apparent density (BMAD) (g/cm^3), an estimate of volumetric bone mineral density, was calculated as previously described (Katzman et al. 1991). For WB, the formula WB BMAD = WB BMC/(WB bone $area^2$/ height) was used. For LS, the formula LS BMAD = LS BMC/ LS bone area, and for FN, the formula FN BMAD = FN BMC/ FN bone $area^2$ were used (Katzman et al. 1991). The expression of WB BMC/height was calculated to adjust for WB bone size (Bachrach et al. 1999). Whole body fat%, FM, FFM, trunk fat and leg fat were also measured via DXA device. Participants were scanned in light clothing while lying flat on the back, with arms at the sides. The medium scan mode and standard subject positioning were used for total body measurements, and analyzed using the extended analysis option. DXA measurements and results were evaluated by the same examiner. Coefficients of variations for bone mineral and body composition measurements were less than 2% in our laboratory.

4.4. Body composition indices

Body mass index (BMI) (kg/m^2) was calculated as body mass (kg) divided by height squared (m^2) and was used as an indicator of obesity (Haslam and James 2005). However, the significance of the BMI is not clear as body mass is composed of two distinct components (i.e., FFM and FM). Therefore, FFM index (FFMI) (kg/m^2) and FM index (FMI) (kg/m^2) were also calculated (Gaba et al. 2012; van Itallie et al. 1990). These indices should better reflect obesity (Kelly et al. 2009). In addition, trunk fat:leg fat ratio was calculated as an indicator of body fat distribution (Jürimäe et al. 2009).

4.5. Physical activity measurement

Physical activity (PA) was measured with the Actigraph accelerometer (model GT1M ActiGraph, Monrovia, CA, USA). The accelerometer is small (3.8 x 3.7 x 1.8 cm) and light-weight (~27 g) uniaxial monitor instrument. This Actigraph has been validated previously in children and adolescents under free-living conditions against PA. The accelerometer is designed to detect and measure vertical accelerations ranging from 0.05 to 2.00 G with frequency response of 0.25 – 2.50 Hz (Freedson et al. 2005; Sardinha et al. 2008). All subjects were asked to wear the accelerometer on the right hip for seven consecutive days during the wake up time. For the analyses of accelerometer data, all night activity (24:00–6:00 hours), and all sequences of 10 min or more of consecutive zero counts were excluded from each individual's recording. Physical activity was included for further analyses if the subject had accumulated a minimum of eight hours of activity data per day for at least two weekdays and one weekend day (Freedson et al. 2005; Lätt et al. 2013). The accelerometer counts the movements from vibrations of the body, and was programmed to record data in one-minute epochs (Lätt et al. 2013; Ruiz et al. 2006).

The total amount of PA (total PA) was expressed as total number of counts divided by the registered time (counts/min) (Lätt et al. 2013; Sardinha et al. 2008). The time spent in the moderate PA (3–6 METs) and in the vigorous PA (>6 METs) was calculated based upon cut-offs of 2000, and 4000 counts per minute (Lätt et al. 2013; Martinez-Gomez et al. 2010). The time spent in at least moderate-intensity PA (>3 METs) was calculated as the sum of time spent in moderate and in vigorous PA (MVPA). Each minute over the specific cut-off was summarized in the corresponding intensity level group (Lätt et al. 2013; Sardinha et al. 2008).

4.6. Statistical analysis

Data analysis was performed using SPSS 15.0 for Windows (Chicago, IL, USA). Standard statistical methods were used to calculate means and standard deviations (±SD). Evaluation of normality was performed with the Shapiro-Wilks statistical method and variables that were not normally distributed were log transformed. Statistical comparisons between groups were performed with parametric unpaired t-tests. In addition, bone parameters between groups were also compared after adjustment for body mass, FM and FFM using a one-way analysis of covariance (ANCOVA) (El Hage et al. 2009a; Rocher et al. 2008). Paired t-tests were performed to determine the changes in measured variables over the 12-month study period. Relationships between body composition variables and bone data were analyzed using partial correlation analysis after controlling for age and pubertal status, or after controlling for body mass, bone age and pubertal status (Lätt et al. 2009; Pomerants et al. 2006). Partial correlation analysis was also used to assess the relationships between changes in BMD and BMC values during a 12-month study period with changes in PA variables

after controlling for age, pubertal status and FFM. Backward linear regression analysis was performed to determine the relationship between bone parameters and PA. Bone parameters (WB BMD, WB BMC, FN BMD and LS BMD) were inserted as dependent variables and PA levels were entered as independent variables, adjustments were made for body mass, bone age and pubertal status. Stepwise multiple linear regression analysis was used to determine most significant parameters that contribute to changes in different bone parameters with those changes in body composition and PA parameters that were significantly correlated to changes in bone parameters were included together in regression models. Baseline PA values, baseline body mass, baseline pubertal status, and changes in age, pubertal status, and body mass were included as covariates using the enter method. Statistical significance was set at $P < 0.05$.

5. RESULTS

5.1. Body composition, bone mineral density and physical activity characteristics in overweight and normal weight boys

The descriptive characteristics in normal weight and overweight boys are presented in Table 1. Age and body height were not different between groups ($P > 0.05$). Bone age was significantly higher in overweight group ($P < 0.05$). Similarly, overweight boys had significantly higher ($P < 0.05$) values for body fat%, body mass, FM, trunk fat, leg fat, trunk fat/leg fat ratio, FFM, BMI, FMI and FFMI compared to normal weight controls.

Table 1. Main physical and body composition characteristics in normal and overweight boys (mean ± SD).

Variable	Normal (n=154)	Overweight (n=110)
Age (yrs)	12.08 ± 0.77	11.96 ± 0.76
Bone age (yrs)	11.74 ± 1.20	12.21 ± 1.08*
Body height (cm)	153.6 ± 8.7	155.6 ± 7.6
Body mass (kg)	41.75 ± 8.19	56.57 ± 15.36*
Body fat %	16.0 ± 4.1	33.9 ± 7.9*
FM (kg)	6.27 ± 2.15	19.02 ± 9.57*
Trunk fat (kg)	2.20 ± 0.84	8.04 ± 4.57*
Leg fat (kg)	3.11 ± 1.09	8.34 ± 4.12*
FFM (kg)	32.75 ± 6.24	34.51 ± 6.37*
TF/LF ratio	0.71 ± 0.14	0.95 ± 0.21*
BMI (kg/m^2)	17.5 ± 2.2	23.1 ± 4.6*
FMI (kg/m^2)	2.64 ± 0.82	7.69 ± 3.34*
FFMI (kg/m^2)	13.73 ± 1.36	14.13 ± 1.57*
Tanner (1/2/3/4/5)	4/57/76/16/1	4/41/54/11/0

FM – fat mass, FFM – fat free mass, TF/LF ratio – trunk fat/leg fat ratio, BMI – body mass index, FMI – fat mass index, FFMI – fat free mass index
* Significant difference between the groups; $P < 0.05$.

Bone mineral measurements expressed as crude values are displayed in Table 2. WB BMD, LS BMD, WB BMC, and WB BMC/height were significantly higher and WB BMAD significantly lower in overweight boys compared to the respective values in normal weight boys ($P < 0.05$). There were no differences ($P > 0.05$) in FN BMD, LS BMAD and FN BMAD values between studied groups.

Table 2. Bone mineral measurements expressed as crude values in normal and overweight boys (mean ± SD).

Variable	Normal (n=154)	Overweight (n=110)
WB BMD (g/cm^2)	0.962 ± 0.065	1.007 ± 0.066*
LS BMD (g/cm^2)	0.815 ± 0.103	0.839 ± 0.092*
FN BMD (g/cm^2)	0.895 ± 0.101	0.904 ± 0.095
WB BMC (g)	1623.4 ± 332.0	1850.3 ± 374.7*
WB BMAD (g/cm^3)	0.089 ± 0.006	0.087 ± 0.006*
LS BMAD (g/cm^3)	0.144 ± 0.015	0.147 ± 0.012
FN BMAD (g/cm^3)	0.202 ± 0.023	0.197 ± 0.023
BMC/height (g/cm)	10.50 ± 1.62	11.82 ± 1.89*

WB – whole body, BMD – bone mineral density, LS – lumbar spine, FN – femoral neck, BMC – bone mineral content, BMAD – bone mineral apparent density
* Significant difference between the groups; $P < 0.05$.

Bone mineral values adjusted for body mass, FFM and FM are shown in Table 3. Whole body BMD and BMAD values were significantly higher and lower, respectively, between normal weight and overweight groups when adjusted for FFM ($P < 0.05$), but not when adjusted for body mass or FM values ($P > 0.05$). When measurements were adjusted for body mass or FM, WB BMC or WB BMC/height were significantly lower in overweight boys in comparison with normal weight boys ($P < 0.05$). Femoral neck BMD and LS BMD were significantly lower in overweight boys when adjusted for body mass or FM, while no differences in these values between groups were seen when adjusted for FFM ($P > 0.05$). Finally, when measurements were adjusted for body mass, FM or FFM, FN BMAD and LS BMAD were not different between the two groups.

Table 3. Bone mineral values adjusted for body mass, fat free mass and fat mass in normal (n=154) and overweight (n=110) boys (mean±SD).

	Adjusted for body mass		Adjusted for fat free mass		Adjusted for fat mass	
	Normal	Overweight	Normal	Overweight	Normal	Overweight
WB BMD (g/cm^2)	0.985 ± 0.004	0.974 ± 0.005	0.968 ± 0.004	0.999 ± 0.004*	0.988 ± 0.006	0.971 ± 0.007
WB BMAD (g/cm^3)	0.088 ± 0.001	0.089 ± 0.001	0.089 ± 0.001	0.087 ± 0.001*	0.088 ± 0.001	0.089 ± 0.001
WB BMC (g/cm^2)	1777.1 ± 16.9	1635.2 ± 20.6*	1660.5 ± 11.7	1798.3 ± 13.9*	1789.0 ± 27.6	1618.5 ± 34.5*
BMC/height (g/cm)	11.24 ± 0.09	10.79 ± 0.11*	10.67 ± 0.07	11.58 ± 0.08*	11.33 ± 0.14	10.67 ± 0.17*
FN BMD (g/cm^2)	0.920 ± 0.008	0.870 ± 0.009*	0.902 ± 0.007	0.895 ± 0.008	0.915 ± 0.009	0.876 ± 0.011*
FN BMAD (g/cm^3)	0.201 ± 0.002	0.198 ± 0.002	0.202 ± 0.002	0.197 ± 0.002	0.200 ± 0.002	0.200 ± 0.003
LS BMD (g/cm^2)	0.843 ± 0.007	0.799 ± 0.009*	0.822 ± 0.006	0.829 ± 0.007	0.842 ± 0.009	0.801 ± 0.011*
LS BMAD (g/cm^3)	0.145 ± 0.001	0.145 ± 0.001	0.144 ± 0.001	0.147 ± 0.001	0.145 ± 0.001	0.145 ± 0.002

WB – whole body, BMD – bone mineral density, BMC – bone mineral content, LS – lumbar spine, FN – femoral neck, BMAD – bone mineral apparent density

* Significant difference between the groups; $P < 0.05$.

In PA levels, overweight boys had significantly lower values in moderate, vigorous, MVPA and total PA compared to normal weight boys (Table 4). There were no significant differences in sedentary time and light PA levels.

Table 4. Physical activity parameters in normal and overweight boys (mean ± SD).

Physical activity parameter	Normal (n=154)	Overweight (n=110)
Sedentary time (min/day)	399 ± 67	411 ± 73
Light PA (min/day)	316 ± 63	310 ± 57
Moderate PA (min/day)	51 ± 21	43 ± 19*
Vigorous PA (min/day)	14 ± 13	8 ± 8*
MVPA (min/day)	65 ± 29	51 ± 24*
Total PA (counts/day)	528 ± 155	461 ± 132*

PA – physical activity, MVPA – moderate-to-vigorous physical activity.
* Significant difference between groups; $P < 0.05$.

5.2. Relationships between body composition and bone mineral density characteristics

Body mass, FM, FFM, trunk fat, trunk fat:leg fat ratio, BMI, FMI and FFMI were all positively related ($P < 0.05$) to WB BMD, WB BMC and WB BMC/ height in both groups (Table 5). All body composition variables were negatively related ($P < 0.05$) to WB BMAD in overweight group, while body mass, FM, FFM, trunk fat and FFMI were negatively correlated with WB BMAD in normal weight boys. Almost all presented body composition variables were positively related to LS BMD and LS BMAD ($P < 0.05$), except FMI for LS BMD, and FM and FMI for LS BMAD ($P > 0.05$) in normal weight boys (Table 6). All body composition variables except FMI and FFMI were correlated with LS BMD, while only trunk fat and FMI were significantly related to LS BMAD in overweight boys. All measured body composition variables were significantly correlated with FN BMD in both groups ($P < 0.05$), except trunk fat:leg fat ratio in normal weight boys. Finally, no relationships between measured and calculated body composition values with FN BMAD were observed in both groups ($P > 0.05$) (Table 6).

Table 5. Correlations between whole body (WB) bone mineral values and body composition indices in normal (n=154) and overweight (n=110) boys controlled for age and pubertal status.

	WB BMD (g/cm^2)		WB BMAD (g/cm^3)		WB BMC (g/cm)		WB BMC/height (g/cm)	
	Normal	Overweight	Normal	Overweight	Normal	Overweight	Normal	Overweight
Body mass (kg)	0.607*	0.705*	–0.421*	–0.502*	0.818*	0.856*	0.774*	0.839*
FM (kg)	0.381*	0.615*	–0.190*	–0.489*	0.454*	0.756*	0.459*	0.756*
FFM (kg)	0.622*	0.735*	–0.532*	–0.431*	0.895*	0.876*	0.834*	0.839*
Trunk fat (kg)	0.422*	0.610*	–0.213*	–0.485*	0.495*	0.737*	0.510*	0.746*
TF/LF ratio	0.199*	0.230*	–0.111	–0.214*	0.211*	0.259*	0.246*	0.290*
BMI (kg/m^2)	0.465*	0.635*	–0.159	–0.427*	0.474*	0.725*	0.534*	0.745*
FMI (kg/m^2)	0.253*	0.551*	–0.032	–0.464*	0.224*	0.668*	0.273*	0.689*
FFMI (kg/m^2)	0.531*	0.586*	–0.347*	–0.406*	0.630*	0.663*	0.680*	0.693*

FM – fat mass, FFM – fat free mass, TF/LF ratio – trunk fat/leg fat ratio, BMI – body mass index, FMI – fat mass index, FFMI – fat free mass index, LS – lumbar spine, FM – femoral neck, BMD – bone mineral density, BMAD - bone mineral apparent density

* Statistically significant; $P < 0.05$.

Table 6. Correlations between areal bone mineral values and body composition indices in normal (n=154) and overweight (n=110) boys controlled for age and pubertal status.

	LS BMD (g/cm^2)		LS BMAD (g/cm^3)		FN BMD (g/cm^2)		FN BMAD (g/cm^3)	
	Normal	Overweight	Normal	Overweight	Normal	Overweight	Normal	Overweight
Body mass (kg)	0.558*	0.532*	0.201*	0.161	0.451*	0.412*	–0.053	–0.110
FM (kg)	0.279*	0.455*	0.126	0.187	0.160*	0.322*	–0.128	–0.113
FFM (kg)	0.634*	0.563*	0.199*	0.103	0.486*	0.480*	–0.048	–0.078
Trunk fat (kg)	0.359*	0.457*	0.197*	0.200*	0.165*	0.344*	–0.122	–0.079
TF/LF ratio	0.280*	0.182	0.241*	0.078	0.012	0.273*	–0.035	0.145
BMI (kg/m^2)	0.360*	0.438*	0.245*	0.171	0.299*	0.357*	0.040	–0.064
FMI (kg/m^2)	0.131	0.391	0.118	0.192*	0.038	0.278*	–0.097	–0.090
FFMI (kg/m^2)	0.520*	0.414	0.286*	0.082	0.384*	0.429*	0.066	0.011

FM – fat mass, FFM – fat free mass, TF/LF ratio – trunk fat/leg fat ratio, BMI – body mass index, FMI – fat mass index, FFMI – fat free mass index, LS – lumbar spine, FM – femoral neck, BMD – bone mineral density, BMAD - bone mineral apparent density

* Statistically significant; $P < 0.05$.

5.3. Relationships between physical activity and bone mineral density characteristics

Relationships between bone mineral parameters and PA levels are shown in Table 7. In normal weight group, there were significant relationships between FN BMD and vigorous PA (r = 0.289), MVPA (r = 0.216) and total PA (r = 0.259). In overweight group, moderate PA was significantly related to FN BMD (r = 0.321), vigorous PA was significantly related to WB BMD (r = 0.273), WB BMC (r = 0.272) and FN BMD (r = 0.228). MVPA was significantly related with WB BMD (r = 0.201), total BMC (r = 0.199) and FN BMD (r = 0.331) and total PA were significantly related to WB BMD (r = 0.208), WB BMC (r = 0.203) and FN BMD (r = 0.320) in overweight subjects. All the correlations were adjusted to pubertal stage, body mass and bone age.

Table 7. Correlations between bone parameters and PA levels adjusted for body mass, bone age and pubertal stage in normal (n=154) and overweight (OW) (n=110) boys.

	WB BMD (g/cm^2)		WB BMC (g/cm)		FN BMD (g/cm^2)		LS BMD (g/cm^2)	
	Normal	OW	Normal	OW	Normal	OW	Normal	OW
Sedentary PA (min/day)	–0.107	–0.121	–0.079	–0.156	–0.101	–0.106	–0.045	–0.041
Light PA (min/day)	–0.106	–0.123	–0.070	–0.176	0.027	0.034	–0.175	–0.083
Moderate PA (min/day)	0.010	0.169	–0.012	0.179	0.137	0.321*	–0.072	0.090
Vigorous PA (min/day)	0.049	0.273*	0.035	0.272*	0.289*	0.228*	–0.061	0.131
MVPA (min/day)	0.026	0.201*	0.023	0.199*	0.216*	0.331*	–0.067	0.080
Total PA (counts/day)	0.073	0.208*	0.061	0.203*	0.259*	0.320*	–0.052	0.077

WB – whole body, BMD – bone mineral density, BMC – bone mineral content, LS – lumbar spine, PA – physical activity, MVPA – moderate-to-vigorous physical activity
* Statistically significant; $P < 0.05$

The backward regression models (adjusted for pubertal stage, body mass and bone age) for PA level associations with bone mineral parameters indicated that in normal weight subjects, vigorous PA was the most important PA parameter to predict FN BMD (R^2 x 100 = 41.7; $P < 0.001$) (Table 8). No more PA parameters were associated with different bone parameters in normal weight subjects. In overweight subjects, vigorous PA was the best predictor for WB BMD (R^2 x 100 = 59.6, $P = 0.0049$) and for WB BMC (R^2 x 100 = 78.6, $P = 0.005$). For FN BMD the most important predictor of PA levels was MVPA (R^2 x 100 = 29.7, $P < 0.001$) in overweight subjects.

Bone mineral parameters in normal weight subjects according to tertiles of MVPA are presented in Table 9. Only the highest tertile of MVPA was

associated with significantly higher FN BMD compared to the lowest MVPA tertile (0.92 ± 0.1 vs 0.86 ± 0.1). No further differences in bone parameters were found between MVPA tertiles.

Table 8. Backward linear regression coefficients of determination (R^2), standardized regression coefficients (β) and level of significance (P) examining the association between physical activity levels and bone parameters, after controlling for body mass, bone age and pubertal stage in normal and overweight boys.

	Normal (n=154)			Overweight (n=110)		
	R^2x100	β	P	R^2x100	β	P
WB BMD (g/cm^2)						
Vigorous PA (min/day)	–	–	–	59.6	0.188	0.004
MVPA (min/day)	–	–	–	–	–	–
Total PA (min/day)	–	–	–	–	–	–
WB BMC (g)						
Vigorous PA (min/day)	–	–	–	78.6	0.136	0.005
MVPA (min/day)	–	–	–	–	–	–
Total PA (min/day)	–	–	–	–	–	–
FN BMD (g/cm^2)						
Moderate PA (min/day)	–	–	–	–	–	–
Vigorous PA (min/day)	41.7	0.214	0.040	–	–	–
MVPA (min/day)	–	–	–	29.7	0.305	<0.001
Total PA (counts/day)	–	–	–	–	–	–

PA levels were inserted in the model together using backward mode. Bone parameters were entered in the model as dependent variables. Only those PA levels that had significant correlations with bone parameters were entered in the model.

Table 9. Bone mineral parameters for boys in each tertile of moderate to vigorous physical activity in normal weight boys (mean ± SD).

Variable	Low tertile	Average tertile	High tertile
MVPA (min/day)	37.47 ± 10.36	61.22 ± 5.62*	97.77 ± 24.1*#
WB BMC (g)	1209.6 ± 327.6	1312.2 ± 341.8	1235.4 ± 261.7
WB BMD (g/cm^2)	0.95 ± 0.07	0.97 ± 0.06	0.97 ± 0.06
FN BMD (g/cm^2)	0.86 ± 0.1	0.91 ± 0.1	0.92 ± 0.1*
LS BMD (g/cm^2)	0.82 ± 0.1	0.81 ± 0.1	0.81 ± 0.09

*– Significantly different from low tertile; $P < 0.05$; # – Significantly different from average tertile; $P < 0.05$.

5.4 Changes in bone mineral density values after one year observation period in relation to changes in physical activity characteristics

The subjects reported to the laboratory after the initial baseline measurements again in 376.4 ± 10.4 days. Mean chronological age, height (+4.9%), body mass (+12.5%), BMI (+3%), FM (+8.2%) and FFM (+14.6%) were significantly increased ($P < 0.05$) after one-year study period (Table 10).

Table 10. Changes in measured characteristics at baseline and after one-year period in boys (n=169) (mean ± SD).

Variable	Baseline	One year	Absolute value change in %
Anthropometry			
Age (y)	12.06 ± 0.71	13.06 ± 0.72*	
Tanner	2.72 ± 0.63	3.39 ± 0.84*	
(1/2/3/4/5)	(0/64/89/16/0)	(0/20/82/48/19)	
Height (cm)	154.3 ± 7.90	162.3 ± 9.0*	4.9%
Body mass (kg)	47.68 ± 14.05	54.47 ± 15.68*	12.5%
BMI (kg/m^2)	19.77 ± 4.51	20.38 ± 4.61*	3.0%
FM (kg)	11.81 ± 9.23	12.86 ± 9.90*	8.2%
FFM (kg)	33.27 ± 6.14	38.93 ± 8.07*	14.6%
Physical Activity			
Sedentary time (min/d)	409.61 ± 64.0	432.3 ± 71.6*	5.4%
Light PA (min/d)	314.8 ± 60.5	271.5 ± 55.1*	–13.8%
Moderate PA (min/d)	46.1 ± 19.4	41.4 ± 18.6*	–10.9%
Vigorous PA (min/d)	11.4 ± 11.5	13.9 ± 12.4*	18.0%
MVPA (min/d)	57.6 ± 27.0	55.3 ± 27.2	–4.0%
Total PA (counts/min)	488.4 ± 141.4	459.9 ± 157.0*	–5.9%
Bone mineral parameters			
WB BMD (g/cm^2)	0.979 ± 0.069	1.015 ± 0.081*	3.6%
WB BMC (g)	1706.6 ± 367.6	1980.9 ± 458.8*	14.0%
WB BA (cm^2)	1727.9 ± 263.4	1933.3 ± 305.5*	10,7%
LS BMD (g/cm^2)	0.825 ± 0.096	0.888 ± 0.119*	7.1%
LS BMC (g)	27.0 ± 6.5	32.1 ± 8.9*	15,9%
LS BA (cm^2)	32.7 ± 4.7	35.7 ± 5.8*	8.5%
FN BMD (g/cm^2)	0.897 ± 0.094	0.942 ± 0.112*	4.8%
FN BMC (g)	4.1 ± 0.7	4.5 ± 0.8*	9%
FN BA (cm^2)	4.5 ± 0.4	4.7 ± 0.5*	4.3%

BMI – body mass index, FM – fat masss, FFM – fat free mass, PA – physical activity, MVPA – moderate-to-vigorous physical activity, WB – whole body, BMD – bone mineral density, BA – bone area, LS – lumbar spine (L2-L4), FN – femoral neck, BMC – bone mineral content.
*Significantly different from Baseline; $P < 0.05$.

The valid times for accelerometer data were 822.7 ± 69.8 min/d and 759.1 ± 79.2 min/d for the baseline and after 12-month period, respectively. The accelerometry-derived levels of PA demonstrated that sedentary time (+5.4%) was significantly increased, whereas light (–13.8%) and moderate (-10.9%) PA significantly decreased as a result of 1-year study period (Table 10). In contrast, significant increases ($P < 0.05$) in vigorous PA (+18.0%) and a trend to decrease ($P < 0.10$) in MVPA (-4.0%) were observed after 12-month study period. Total PA (counts/min/day) was also significantly decreased (–5.9%; $P < 0.05$) during one-year growth and maturation in 11–13-year-old boys. Additionally, all measured bone parameters increased significantly with the smallest increase was observed in WB BMD (+3.6%) and the largest in WB BMC (+14.0%) (Table 10).

Partial correlation coefficients between changes in bone mineral parameters and PA levels are presented in Table 11. All correlations were controlled for changes in age, pubertal status and body mass. Changes in sedentary time were negatively related to changes in WB BMD, LS BMD, LS BA, FN BMD and FN BMC ($r > –0.157$; $P < 0.05$). Changes in vigorous PA were related to changes in FN BMD and FN BMC ($r > 0.163$; $P < 0.05$), and finally changes in total PA were related to changes in WB BMD, LS BMC, FN BMD and FN BMC ($r > 0.155$; $P < 0.05$).

Table 11. Partial correlation coefficients of change (Δ scores) in bone mineral density and content values during 12-months study period with the change of physical activity variables after controlled for Δ age, Δ pubertal status and Δ body mass in boys (n=169).

	Δ Sedentary time (min/d)	Δ Light PA (min/d)	Δ Moderate PA (min/d)	Δ Vigorous PA (min/d)	Δ MVPA (min/d)	Δ Total PA (counts/min)
ΔWB BMD (g/cm^2)	**–0.157***	–0.011	0.080	0.109	0.111	**0.169***
ΔWB BMC (g)	–0.147	–0.057	–0.057	0.074	–0.005	0.096
ΔWB BA (cm^2)	–0.051	–0.066	–0.053	–0.001	–0.147	–0.030
ΔLS BMD (g/cm^2)	–0.129	–0.004	0.044	0.052	0.060	0.116
ΔLS BMC (g)	**–0.182***	–0.029	0.044	0.078	0.072	**0.155***
ΔLS BA (cm^2)	**–0.183'**	–0.073	–0.010	0.100	0.041	0.143
ΔFN BMD (g/cm^2)	**–0.252***	–0.003	0.082	**0.205***	**0.154***	**0.244***
ΔFN BMC (g)	**–0.222***	0.057	0.053	**0.163***	0.115	**0.216***
ΔFN BA (cm^2)	**–0.**037	0.094	–0.016	0.023	0.001	0.054

PA – physical activity, MVPA – moderate-to-vigorous physical activity, WB – whole body; BMD – bone mineral density; BMC – bone mineral component; BA – bone area; LS – lumbar spine; FN – femoral neck.
* – Statistically significant; $P < 0.05$.

Linear stepwise regression models to identify PA parameters that contribute most to the changes in bone mineral parameters are presented in Table 12. Those models were corrected for changes in age, pubertal stage, body mass, changes in body mass and pubertal stage and baseline PA levels (sedentary,

light, moderate, vigorous). Changes in total PA were selected as the only PA parameter to explain changes in WB BMD (R^2 x 100 = 2.9%; $P = 0.047$), while the whole model predicted changes in WB BMD by 37.9% ($F_{(12,156)} = 7.942$, $P < 0.001$). The model characterizing changes in LS BMC also included sedentary time as the most important from PA levels in the whole model (R^2 x 100 = 48.9%, $F_{(12,156)} = 12.427$, $P < 0.001$), however, the contribution of sedentary time in the model was non-significant ($P = 0.150$). Changes in sedentary time and vigorous PA were selected as the most important PA parameters to describe changes in FN BMD (R^2 x 100 = 8.6%, $F_{(13,155)} = 5.426$); and both components were also significant (P=0.024 and P=0.001 for sedentary time and vigorous PA, respectively). Changes in sedentary time had a significant prediction (R^2 x 100 = 4.7%, $P = 0.027$) on changes in FN BMC, while the total model predicted changes in FN BMC by 39.4% ($F_{(12,156)} = 8.443$, $P < 0.001$). In all models the most significant covariates were baseline body mass ($P < 0.001$ in all models) and changes in pubertal stage (P<0.007 in all models) (data not shown). The additional inclusion of FFM as a covariate did not significantly influence the results, except for loosing PA associations with WB BMD. If running the regression models with only cross-sectional physical activity data (sedentary, light, moderate, vigorous), no significant associations ($P > 0.072$) were seen with any of the PA levels with changes in bone mineral parameters (data not shown).

Table 12. Stepwise regression models to determine variables that predict bone mineral parameters controlling for Δ chronological age, Δ pubertal stage and Δ body mass and baseline physical activity levels, body mass and pubertal stage in boys (n=169).

	B (SE)	Beta	P
Δ WB BMD (g/cm^2)		$F_{(12,156)} = 7.942$	<0.001
Δ Total PA (counts/min)	0.000 (0.000)	0.140	0.047
Δ WB BMC (g)	–	–	–
Δ WB BA (cm^2)	–	–	–
Δ LS BMD (g/cm^2)	–	–	–
Δ LS BMC (g)		$F_{(12,156)} = 12.427$	<0.001
Δ Sed time (min/day)	–0.004 (0.003)	–0.099	0.150
Δ LS BA (cm^2)	–	–	–
Δ FN BMD (g/cm^2)		$F_{(13,155)} = 5.426$	<0.001
Δ Sed time (min/day)	0.000 (0.000)	–0.185	0.024
Δ Vig PA (min/day)	0.001 (0.000)	0.249	0.001
Δ FN BMC (g)		$F_{(12,156)} = 8.443$	<0.001
Δ Sed time (min/day)	–0.001 (0.000)	–0.167	0.027
Δ FN BA (cm^2)	–	–	–

WB – whole body; BMD – bone mineral density; PA – physical activity; BMC – bone mineral content; BA – bone area; LS – lumbar spine; FN – femoral neck.

6. DISCUSSION

6.1 Bone mineral density values and their relationships with different body composition values in normal weight and overweight 11–13-year-old boys

The main findings of the present study were that overweight boys displayed similar values for areal BMAD values and lower WB BMAD values, despite significantly higher values for more widely used WB and LS BMD, WB BMC and also WB BMC/height values in comparison with normal weight peers. These results suggest that BMAD values should be computed and adjusted for different body mass values when assessing bone development in boys reaching puberty. Furthermore, FFMI characterizes better than more widely used BMI bone development in normal weight boys reaching puberty. In contrast, BMI and FMI are better determinants of bone mineral values than FFMI in overweight boys of the present study.

This study also demonstrated no significant differences in LS BMAD and FN BMAD crude values and also when these values were adjusted for body mass, FFM and FM in studied normal weight and overweight boys. Similarly, WB BMAD was not significantly different between studied boys after adjustment for body mass and FM values, while overweight boys had significantly lower crude WB BMAD values in comparison with normal weight boys. In addition, the FFMI had the highest correlation coefficients from the calculated body composition indices with all bone mineral values in normal weight boys. In overweight boys, the FFMI had the highest correlation only with FN BMD, while other measured bone mineral values had highest correlations with either BMI or FMI values.

In our study, overweight boys had higher WB BMD, LS BMD, WB BMC and also BMC/height ratio than normal weight controls, which is in accordance with other studies conducted in prepubertal children (Leonard et al. 2004, 2007; Petit et al. 2005; Rocher et al. 2008) and also in adolescents (El Hage et al 2009a,b). It has been suggested that body mass might improve bone mineralization in obese children by increasing the mechanical load of increased body weight especially in weight-bearing bones (El Hage et al. 2009b; Ellis et al. 2003; Rocher et al. 2008). Therefore, overweight children should have greater bone strength because of the greater muscle force required to move the increased body weight (El Hage et al. 2009a,b; Leonard et al. 2004). Overweight children have not only more FM but also FFM (El Hage et al. 2009a; Goulding et al. 2000) and this was also the case in our study (see Table 1). In overweight boys, the skeleton must be stronger than in normal weight boys to support their higher body mass (Rocher et al. 2008).

It is interesting to note that while significant relationships between measured and calculated body FM and FFM values were also seen in both groups (see Tables 5–6), FFM values were better determinants of measured bone mineral values than FM measures in normal weight boys. This is in accordance with the

results of other studies conducted in normal weight boys (El Hage et al. 2009a, 2011; Hrafnkelsson et al. 2010; Rocher et al. 2008). In contrast, measured and calculated FM indices were better determinants of measured bone mineral values than FFM measures in overweight boys. To date, there is a significant disagreement in the literature regarding the relative contributions of fat and fat free body components to bone mineral values in growing children (El Hage et al. 2009a). While many studies have demonstrated a positive effect of FM on bone mineral values (Clark et al. 2006; El Hage et al. 2009a; Ellis et al. 2003; Leonard et al. 2004), there are also studies showing that body fat may be a negative determinant of BMD in children (Hrafnkelsson et al. 2010; Specker et al. 2001). However, it has been suggested that increased FM may be related to bone maturation (El Hage et al. 2009a) and bone mass gain accelerates earlier than bone mineral accrual (Bass et al. 1999). This was also supported by the findings of present study, where crude values of WB BMC and WB BMC/ height ratio were significantly higher in overweight boys in comparison with normal weight boys (see Table 2). During puberty, bone maturation may be mediated by the increasing synthesis of estrogen in the adipose tissue that promotes bone mass accrual (Cobayashi et al. 2005; Schoenau et al. 2001). In addition, overweight and excessive adiposity are associated with increased secretion of bone active hormones from the pancreatic beta cells and the adipocytes (Artz et al. 2005; Jürimäe et al. 2009). These factors may explain the strong relationship between increased FM and bone mineral values in our overweight peripubertal boys. In agreement with our results, it has been suggested that the relationships between FM and FFM values with measures of BMD and/or BMC could be dependent on the weight status of the studied population (El Hage et al. 2009a,b).

It appears that there might be a positive site-specific effect of increased adiposity on bone mineral values during puberty as LS BMD values were significantly higher in overweight boys, while no differences were seen in FN BMD values between studied groups (see Table 2). The FN is mainly composed of cortical bones whereas LS is mainly composed of trabecular bones (Martin et al. 1988). In addition, trabecular bone is known to be more metabolically active than cortical bone tissue (Martin et al. 1988). It has been suggested that in response to mechanical loading, cortical bone mainly enhances its size, while trabecular bone mainly increases its density (Ducher et al. 2004). Furthermore, Rocher et al. (2008) argued that WB BMC, which is composed of 80% of cortical bone would adapt to increased body weight by increasing both BMC and bone area, while LS would react by improving BMC only in obese prepubertal children. However, to minimize the contributions of bone dimensions on BMD values, different equations have been proposed to calculate volumetric BMD in growing children (Katzman et al. 1991; Kroger et al. 1995). In our study, LS and FN BMAD values were not different when expressed as crude values (see Table 2) and also when adjusted for body mass, FFM and FM values (see Table 3) between normal weight and overweight peripubertal boys. These results are in accordance with other studies (Rocher et al. 2008) and would suggest that

overweight does not have a protective effect on BMAD values at the specific sites of the skeleton in boys during puberty. In contrast, one could argue that the site-specific effect of mechanical loading and bone metabolic activity on bone mineral development has been demonstrated by the fact that in contrast to LS BMAD, no relationship between FN BMAD with measured and calculated body composition values were seen in both groups (see Table 6). Consequently, further studies are needed before any conclusions can be drawn.

The results of present investigation indicate that adipose tissue may even have negative effect on bone mineral values during puberty as body mass, FM and FFM values were negatively associated with WB BMAD in both groups of studied boys. In general, these relationships appeared to be stronger in overweight boys (see Table 5). These results are in accordance with the results obtained in adolescent boys and girls (El Hage et al. 2009b, 2011). In addition, crude WB BMAD and WB BMAD values adjusted for FFM but not when adjusted for body mass or FM were significantly lower in our overweight peripubertal boys when compared with normal weight boys (see Table 3). These results are in line with those observed in prepubertal boys and girls (Rocher et al. 2008) and in contrast to adolescent girls (El Hage et al. 2009a). Therefore, El Hage et al. (2009a) suggested that the relation between bone development and obesity may be sex specific. Accordingly, it could be argued that WB adiposity plays a negative role in bone development at least in boys reaching puberty. In addition, the mismatch between body weight and bone mineralization in overweight children in comparison with normal weight peers increases their propensity to sustain fractures (Goulding et al. 2000). Therefore, Rocher et al. (2008) argued that it is not clear whether the association between fracture occurrence and obesity is a consequence of weaker bones or greater forces applied on the skeleton when a fall occurs.

Our findings partly support the recommendations to use FFMI and FMI in determining the deeper meaning of BMI (Eissa et al. 2009; Kelly et al. 2009) as FFMI were higher correlated with bone mineral values in normal-weight boys, while FMI and also BMI were better determinants of measured and calculated bone measures in overweight boys (see Tables 4 and 5). It has been suggested that calculation of FFMI and FMI in the context of BMI enables to identify children with normal BMI and excess adiposity to initiate possible intervention (Eissa et al. 2009). For example, only FMI was correlated with LS BMAD in overweight boys, while FFMI and also BMI were related to LS BMAD in normal weight boys.

In conclusion, the results of present investigation demonstrate that overweight boys have higher crude WB BMD, BMC and BMC/height ratio values in comparison with normal weight boys. However, this bone growth appears to be insufficient to compensate for the higher mechanical load applied on the bone by higher FM and also FFM values in overweight boys. Specifically, excessive adiposity does not have a protective effect on the development of BMAD in growing boys reaching puberty. Moreover, this study suggests that measured and calculated body composition values were negative determinants

of WB BMAD in peripubertal boys. However, BMAD was calculated from the DXA measurements and not assessed by computed tomography, which measures volumetric BMD directly. Another limitation was that diet and especially calcium intake was not measured in this study. Accordingly, further studies are needed to better understand bone growth in boys during puberty.

6.2 Relationships between bone mineral density and physical activity parameters in normal weight and overweight 11–13-year-old boys

The main aim of this study was to investigate the association of different PA levels to bone mineral parameters in normal weight and overweight peripubertal boys. After adjusting for pubertal stage, bone age and body mass, only FN BMD site was associated with vigorous PA and MVPA in normal weight boys, while WB BMD, FN BMD, and WB BMC were associated with MVPA levels in overweight subjects. Furthermore, moderate PA was associated with FN BMD in overweight subjects. No effect of PA was seen on LS BMD in normal and overweight peripubertal boys. These results of the present investigation clearly indicate that the impact of objectively measured PA levels are significantly different in peripubertal boys with different weight categories, and already moderate PA was associated with bone mineral values in overweight peripubertal boys.

Throughout the growing years, particularly early in puberty, the ability of bone to adapt to mechanical loading seems to be better than after puberty (Khan et al. 2000). Increased bone mass in response to PA in childhood may reduce the risk of osteoporotic fracture in later life, particularly if associated with improvements in bone geometry (Tobias et al. 2007). Therefore, the possible impact of PA during this period is important. Previous studies have indicated positive associations between bone mass with participation in sport or other vigorous PA in boys (Baptista et al. 2012; Högström et al. 2007) or that the bones are more responsive to PA in boys compared to girls (Baxter-Jones et al. 2008; Ginty et al. 2005; Martinez-Gomez et al. 2010; Sardinha et al. 2008; Vincent-Rodriguez et al. 2008).

Majority of the studies focusing on the associations between PA and bone parameters have used relatively heterogeneous groups of subjects. In this study we divided the subjects into normal- and overweight boys in order to study the influence of PA to bone mineral parameters in subjects with different weight categories. In PA levels, there were no differences between sedentary and light PA, while normal weight subjects spent significantly more time in moderate and vigorous PA (see Table 4). In overweight subjects, we found that MVPA levels were significantly associated with all the measured bone values except for LS BMD (see Table 7). This, in general, is in agreement with other studies that have found similar associations with PA and bone mineral parameters. However, in overweight subjects, moderate PA alone was associated with

higher BMD in FN site. This finding, concerning moderate PA is far less reported in the literature. To our best knowledge, only Tobias et al. (2007) found that moderate PA was related to bone size and BMD in 11-year-old children. However, in that study moderate PA was defined differently compared to our study. Specifically, moderate PA in Tobias et al. (2007) study was similar to vigorous PA in our study. This indicates that in overweight subjects, despite significantly less activity in moderate PA compared to normal weight subjects, already benefit from moderate PA. This is probably related to their additional body mass, which effect becomes significant already at moderate PA level. Overweight subjects in our study showed also significantly higher values in all measured bone mineral parameters ($P < 0.05$), except for FN ($P > 0.05$).

Physical activity did not predict bone mineral parameters in normal weight group, except for FN site. Several studies have reported the same positive associations between bone mass and/or bone area of FN with participation in sport or in vigorous or high-impact PA in boys (Baptista et al. 2012; Högström et al. 2007; Kriemler et al. 2008; Sardinha et al. 2008, Tobias et al. 2007). This skeletal site seems to be considerably affected by mechanical factors, LS, however, is more influenced by metabolic hormonal factors (Sardinha et al. 2008). Femoral neck has emerged as the site as one of the most responsive to physical activity and loading (Baptista et al. 2012; Ginty et al. 2005; Högström et al. 2007). Strong associations between vigorous PA and FN BMD measures may be attributable, in part, to the objective evaluation of PA by means of hip displacement, but also by the fact that this skeletal site seems to be considerably affected by the mechanical factors during the peak bone mass accrual (Sardinha et al. 2008). During puberty, all cross-sectional studies dealing with impact load sports in male and female adolescents (running, gymnastics) have shown increased BMD in weight-bearing bones, particularly at the FN site, in comparison with untrained controls (Zouch et al. 2008).

Contrary, despite significantly higher body mass and FFM, but fewer vigorous PA and MVPA, WB BMD and WB BMC were significantly predicted by MVPA levels in overweight boys (see Table 7). Our study indicates that the beneficial effects of vigorous PA are not similar if different weight categories of the peripubertal children are taken into account. This indicates that the effect of PA to bone mineral parameters is significantly modified by different body composition parameters and already moderate PA levels are advantageous in overweight subjects to increase BMD at FN site. Previous studies have mainly indicated that high impact types of PA are needed to promote bone health (Högström et al. 2007; Martinez-Gomez et al. 2010). In the current study we measured PA by accelerometry without any additional PA diary or questionnaire. Therefore, the reported moderate and vigorous activities include only those activities that have weight-bearing effects and activities with no or low impact (swimming and cycling) do not contribute to MVPA because accelerometer does not track them reliably. Objective assessments of PA using accelerometers provide valid information on the intensity and duration of PA. As accelerometer measures ground-reaction forces during various speeds and inten-

sities of weight-bearing activities, they are particularly beneficial in studying the dimensions of PA that are directly related to structural properties of bone (Sardinha et al. 2008).

To investigate the association between MVPA and bone mineral parameters in more detail and to find weather there exist any difference also in total BMC or BMD parameters in normal-weight subjects, we further divided the normal weight group into tertiles according to MVPA level. Unfortunately we were not able to find any differences in WB BMC between the highest MVPA (1209.6 ± 327.6 g), average MVPA (1312.2 ± 341.8 g) and lowest MVPA (1235.4 ± 261.7 g) group in normalweight boys. Many studies in the literature have indicated that the activity has to be at least vigorous in order to have the significant effect on bone mineral parameters (Baptista et al. 2012; Baxter-Jones et al. 2008; Ginty et al. 2005; Sardinha et al. 2008). However, in our normal weight subjects, the positive influence of body mass could have been too small, as almost three times higher MVPA group in the highest tertile compared to the lowest tertile group did not result in higher WB BMC or BMD values.

There are some limitations in the study that should be considered. Firstly, the wearing of an accelerometer may cause some changes in PA towards higher activity, however, as the wearing time was one week and the average of workdays and weekend days was used in the study. Further, it should be noted that the cross-sectional design of the study does not take into account the historical PA. However, it has been shown that present and historical PA levels are strongly interrelated (Caspersen et al. 2000). Nevertheless, it would be interesting to study the longitudinal influence of PA on bone mineral parameters. Furthermore, the possible influence of sex steroids (ie testosterone) to BMD was not directly considered. As the models were corrected for bone age and pubertal stage as parameters for maturation probably reduces the effect of testosterone on bone mineral parameters and does have significant influence on the conclusions that have been drawn.

In conclusion, different PA associations with bone mineral parameters were found in 11–13-year-old normal weight and overweight boys. The results indicate that in normal weight boys the effect of PA is smaller compared to overweight boys, probably because the weight-bearing effect of the body is smaller. However, this must be further studied in the future to clarify those associations.

6.3 Changes in bone mineral density values after one year observation period in relation to changes in physical activity parameters in 11–13-year-old boys

In the current study, the associations between changes in PA exposure to bone mass acquisition during growth and maturation in 11–13-year-old boys were investigated. This study indicated for the first time, that increases in sedentary time had a negative influence on bone mineral accrual that characterized 4.7%

of the changes in FN BMC ($P < 0.05$), while the combination of sedentary time and vigorous PA characterized 8.6% changes in FN BMD ($P < 0.05$).

One major preventive measure of bone health is the optimization of peak bone mass in the early years and during the puberty (Hind and Burrows 2007). The association of activity duration (minutes per session) has been found to be greater than that of the frequency and load in girls (Farr et al. 2011). The potential importance of PA duration indicates that prolonged loading sessions can continue to stimulate bone formation as long as the distribution of strain is altered throughout the session (Farr et al. 2011; Lanyon 1987). Despite using boys in the current study, it could be suggested that quantifying PA by the amount and the intensity of activity can be considered valid to study its influence on bone mineral parameters. Moreover, the duration of the exercise/loading is likely the case in everyday life concerning their activities and increasing the duration of those activities may be an important osteogenic stimulus. Lots of studies have indicated the beneficial effect of PA, especially weight-bearing PA, on bone mineral acquisition (Gracia-Marco et al. 2011b; Sardinha et al. 2008, Sayers et al. 2011). Our previous study also indicated that already moderate PA level has a positive effect on FN BMD in overweight boys (see Table 7), which is probably mediated by the body composition effect. Accordingly, it has been suggested that the effect of PA on bone mineral value is not mediated only by the amount of PA but the intensity, and more specifically by vigorous PA, in combination of body composition parameters (Gracia-Marco et al. 2011b; Sardinha et al. 2008; Sayers et al. 2011; Tobias et al. 2007). Farr et al. (2011) have also indicated that the effects of PA duration and frequency on bone development are independent and site specific. If those gains in bone mineral acquisition during puberty can be maintained and promoted, they have a potential to reduce the fracture risk in later life.

Most studies so far have investigated the possible association of PA with bone mineral parameters using cross-sectional design (Baptista et al. 2012; Gracia-Marco et al. 2011a; Vicente-Rodriguez et al. 2008; Sardinha et al. 2008). However, this design has only limited historical information over PA level (Farr et al. 2011). It has been shown that present and historical PA levels are strongly interrelated (Nilsson et al. 2010). It is also known that there is a sharp decrease in PA during pubertal development (Ruiz et al. 2006, US DHHS 2008), and therefore, cross-sectional PA data might have less reliability. This was also the case in our study, where in general, significant decreases in PA levels were seen, except for vigorous PA that was slightly, but significantly increased (see Table 10). Based on their results regarding low and moderate PA, Farr et al. (2011) have suggested that PA during growth may be detrimental for bone development, but the direct influence so far have not been indicated. Further, it is known that vigorous PA is important for bone mineral aqusition (Baptista et al. 2012; Sayers et al. 2011; Sundberg et al. 2002), however, the current study indicated that despite the positive effect of vigorous PA, the increases in sedentary time had a negative influence on bone development during one year observation period as indicated by the negative association with changes in

sedentary time and changes in measured bone mineral parameters (see Table 11). Sedentary time refers to movements that do not increase energy expenditure significantly higher above the resting level (ie. sleeping, sitting, television-watching, etc). All regression analyses in the current study were controlled for changes in body mass and baseline body mass due to their significant impact (Nilsson et al. 2010; Vicente-Rodriguez et al. 2008). In the current study, changes in sedentary time were selected as one of the most important predictor of the development in FN BMD (together with changes in vigorous PA) and FN BMC after controlling for changes in age, pubertal stage, body mass, and the baseline body mass, baseline pubertal stage and baseline PA levels (see Table 11). One of the explanations of our finding on sedentary time effect might be attributable to the longitudinal design that gives more reliable information regarding the change of both variables – PA and bone parameters during the investigation period. Moreover, we were able to track both variables reliably using accelerometry and DXA, respectively, in a relatively large sample of 11–13-year-old boys. We did also analyze data using cross-sectional data of PA to predict changes in bone mineral parameters, but we did not find any significant effect of PA levels to any of the measured bone mineral parameters ($P > 0.072$), suggesting that changes in PA pattern during puberty have a significant effect. Our study results also indicated that the effect of light and moderate PA on bone mineral parameters has no significant effect on bone mineral parameters. Despite the fact that total PA was selected in the regression model to be associated with changes in WB BMD, this effect was probably mediated by vigorous PA.

Physical activity effects of our study were mainly associated with FN site of the skeleton. This finding is in accordance with Baptista et al. (2012), who found that one-third of the total variation in FN BMC was determined by PA level. It has further been suggested that the intensity of PA has to be vigorous to influence the bone strength of FN (Sardinha et al. 2008). Femoral neck has be also considered as a skeletal site that has the most direct influence from the combination of ground reaction force and vigorous PA. In contrast, being sedentary eliminates the effect of both components (load and ground reaction force) and therefore might have a negative effect.

Physical Activity Guidelines for children and adolescents recommend that children and adolescents should engage at least 60 minutes or more MV PA per day and at least three days a week should include activities to improve bone health (US DHHS 2008). The mean of 58.0 min/day of MVPA in our studied boys in general, reaches the recommended levels at the beginning of the study, (see Table 10), and that increases in vigorous PA were selected as one of most important components to predict increases in FN BMD (see Table 11). Recently, it has also been argued that sedentary time predicts chronic diseases independently of total PA levels and should therefore be considered a separate construct (Ekelund et al. 2012). This is supported by finding that changes in sedentary time and total PA were related to almost similar bone mineral parameters, however, in the opposite direction (see Table 11). Although, the

contribution of sedentary behaviour was relatively small but significant in the models, it should be noted that those contributions reflect only the period of 12 months and if persisted for longer period the actual effect on bone health might be stronger. For example, the increase in sedentary time by 60 minutes a day predicted the decrease of FN BMC by approximately 0.06 g that might be considered biologically important. Therefore, it could be concluded that focusing to increases in MVPA and higher PA intensities as suggested before (Kriemler et al. 2008; Lanyon 1987; Sundberg et al. 2002; Tobias et al. 2007) and in the current study (see Table 3) are vital to stimulate bone mineral accrual in 11–13-year-old boys. However, at the same time, it is also important to avoid of being sedentary due to its negative contribution to bone health (see Table 11).

The strength of the current study is a 12-month longitudinal observation period, which allowed us to have more reliable data on the actual changes of PA levels and bone mineral accrual compared to cross-sectional design. The used longitudinal design indicated significant PA effects, especially for sedentary time on bone mineral parameters. It must be considered that the change in one unit on PA value is rather small in real life (i.e., 60 s), especially for sedentary activity, therefore, it would be expected that the b-values would be low. For comparison, the Beta-weights mirror the significance of the parameters better (see Table 12). It should also be considered that the bone itself is not a tissue that can be influenced to high rate within a short period. We were not able to track the nutrition of the subjects (which includes calcium and vitamin D intake) and their possible interactions with bone mineral acquisiton. It has also been suggested that in healthy children with adequate dietary intakes, PA has a greater osteogenic effect than calcium or protein intake (Iuliano-Burns et al. 2005). Nevertheless, not measuring dietary and especially calcium intakes should be considered as limitations of the present study. The use of 60 s epochs might have some negative effect on very short bouts of vigorous activities and could result in underestimation of the vigorous activity impact in the regression models. At the same time there might be some overestimation of sedentary time due to uniaxial accelerometer, therefore to be more valid in measuring PA, the 3-D accelerometers would be suggested. In the future, if planning studies on PA probably 24-hour recording and multiple testing per year taking into account the seasonal variability of PA level will produce even more reliable results. This investigation also used several confounders like chronological age, sexual maturation, height and body mass, which are important to study the associations between changes in PA and bone mineral acquisition during longitudinal study period.

In conclusion, our data demonstrate that increase in sedentary time emerged as one of the main predictors from physical activity levels to have a negative influence on bone mineral acquisition during a 12-month observation period in 11- to 13-year-old boys.

7. CONCLUSIONS

1) Overweight boys, aged 11–13 years had higher whole body, lumbar spine and femoral neck BMC values compared with normal weight boys. In contrast, no differences in lumbar spine and femoral neck body apparent (volumetric) BMD were seen in both groups. Despite higher whole body BMD, overweight boys had lower whole body apparent (volumetric) BMD compared with normal weight boys;
2) Fat free mass characterizes better than fat mass and BMI bone mineral values in normal weight boys, while fat mass and BMI are better determinants of bone mineral values in overweight boys;
3) Physical activity is more associated with bone mineral values in overweight boys compared with normal weight boys, and in addition to vigorous physical activity, already moderate physical activity has the significant impact on bone mineral values in overweight boys;
4) The increase in sedentary time emerged as one of the main predictors from physical activity to have the negative influence on bone mineral acquisition during a one-year observation period in 11–13-year-old boys.

8. REFERENCES

1. Abbassi V. Growth and normal puberty. Pediatrics 1998;102;507.
2. Arabia A, Nabulsib M, Maalouf J, Choucaira M, Khalife´ H, Viethc R, El-Hajj Fuleihana G. Bone mineral density by age, gender, pubertal stages, and socio-economic status in healthy Lebanese children and adolescents. Bone 2004;35: 1169–1179.
3. Artz E, Haqq A, Freemark M. Hormonal and metabolic consequences of childhood obesity. Endocrinol Metab Clin N Am 2005;34:643–658.
4. Ausili E, Rigante D, Salvaggio E, Focarelli B, Rendeli C, Ansuini V, Paolucci V, Triarico S. Determinants of bone mineral density, bone mineral content, and body composition in a cohort of healthy children: influence of sex, age, puberty, and physical activity. Rheumatol Int 2012;32:2737–2743.
5. Bachrach LK, Hastie T, wang MC, Narasimhan B, Marcus B. Bone mineral acquisition in healthy Asian, Hispanic, Black, and Caucasian youth: a longitudinal study. J Clin Endocrinol Metab 1999;84:4702–4712.
6. Bailey DA, Martin AD, McKay HA, Whiting S, Mirwald R. Calcium accretion in girls and boys during puberty: a longitudinal analysis. J Bone Miner Res 2000;15: 2245–2250.
7. Bakker I, Twisk JWR, Van Mechelen W, Kemper HCG. Fat-free body mass is the most important body composition determinant of 10-yr longitudinal development of lumbar bone in adult men and women. J Clin Endocrinol Metab 2003;88:2607–2613.
8. Baptista F, Barrigas C, Vieira F, The role of lean body mass and physical activity in bone health in children. J Bone Miner Metab 2012;30:100–108.
9. Bass S, Delmas PD, Pearce G, Hendrich E, Tabensky A, Seeman E. The differing tempo of growth in bone size, mass, and density in girls is region specific. J Clin Invest 1999;104:795–804.
10. Bass S, Pearce G, Bradney M, Hendrich E, Delmas PD, Harding A, Seeman E. Exercise before puberty may confer residual benefits in bone density in adulthood: Studies in active prepubertal and retired female gymnasts. J Bone Miner Res 1998;13:500–507.
11. Bass SL, Saxon L, Daly RM, Turner CH, Robling AG, Seeman E, Stuckey S. The effect of mechanical loading on the size and shape of bone in pre-, peri-, and post-pubertal girls: A study in tennis players. J Bone Miner Res 2002;17:2274–2280.
12. Baxter-Jones A: Growth and maturation; in Armstrong N, van Mechelen W (eds): Paediatric Exercise Science and Medicine, ed 2, revised. Oxford, Oxford University Press 2008;157–168.
13. Bonjour JF, Theintz G, Buchs B, Slosman D, Rizzoli R. Critical years and stages of puberty for spinal and femoral bone mass accumulation during adolescence. J Clin Endocrinol Metab 1991;73:555–563.
14. Boot AM, De Ridder MA, Pols HA, Krenning EP, De Muinck Keizer-Schrama SM. Bone mineral density in children and adolescents: relation to puberty, calcium intake, and physical activity. J Clin Endocrinol Metab 1997;82:57–62.
15. Boot AM, de Ridder MAJ, van der Sluis IM, van Slobbe I, Krenning EP, de Muinck Keizer-Schrama SMPF: Peak bone mineral density, lean body mass and fractures. Bone 2010;46:336–341.

16. Caspersen CJ, Pererira MA, Curran KM. Changes in physical activity paterns in the United States, by sex and cross.sectional age. Med Sci Sports Exerc 2000;32:1601–1609.
17. Christo K, Prabhakaran R, Lamparello B, Cord J, Miller KK, Goldstein MA, Gupta N, Herzog DB, Klibanski A, Misra M: Bone metabolism in adolescent athletes with eumenorrhea, and control subjects. Pediatrics 2008;121:1127–1136.
18. Clark EM, Ness AR, Bishop NJ, Tobias JH. Association between bone mass and fractures in children: a prospective cohort study. J Bone Miner Res 2006;21:1489–1495.
19. Cobayashi F, Lopez LA, Taddei JAAC. Bone mineral density in overweight and obese adolescents. J Pediatr 2005;81:337–342.
20. Corder K, Brage S, Ekelund U. Accelerometers and pedometers: methodology and clinical application. Curr Opin Clin Nutr Metab Care 2007;10:597–603.
21. Courteix D, Lespessailles E, Jaffre C, Obert P, Benhamou CL. Bone mineral acquisition and somatic development in highly trained girl gymnasts. Acta Paediatr 1999; 88:803–808.
22. Crabtree NJ, Kibirige MS, Fordham JN, Banks LM, Muntoni F, Chinn D. The relationship between lean body mass and bone mineral content in paediatric health and disease. Bone 2004;35:965–72.
23. Creighton DL, Morgan AL, Boardley D, Brolinson PG: Weight-bearing exercise and markers of bone turnover in female athletes. Med Sci Sports Exerc 2001;90: 565–570.
24. Csakvary V, Puskas T, Oroszlan G, Lakotos P, Kalman B, Kovacs GL, Toldy E. Hormonal and biochemical parameters correlated with bone densitometric markers in prepubertal Hungarian children. Bone 2013;54:106–112.
25. Daly RM, Saxon L, Turner CH, Robling AG, Bass SL. The relationship between muscle size and bone geometry during growth and in response to exercise. Bone 2004;34:281–287.
26. Ducher G, Proteau S, Courteix D, Benhamou CL. Cortical and trabecular bone at the forearm show different adaptation patterns in response to tennis playing. J Clin Densitom 2004;7:399–405.
27. Duke PM, Litt IF, Gross RT. Adolescents' self assessment of sexual maturation. Pediatrics 1980;66: 918–920.
28. Ehrlich PJ, Lanyon LE. Mechanical strain and bone cell function: a review: Osteoporos Int 2002;13:688–700.
29. Eissa MA, Dai S, Mihalopoulos NL, Day RS, Harrist RB, Labarthe DR. Trajectories of fat mass index, fat free-mass index, and waist circumference in children. Am J Prev Med 2009;37:S34-S39.
30. Ekelund U, Luan J, Sherar LB, Esliger DW, Griew P, Cooper A. Moderate to vigorous physical activity and sedentary time and cardiometabolic risk factors in children and adolescents. JAMA 2012;307:704–712.
31. El Hage RP, Courteix D, Benhamou CL, Jacob C, Jaffre C. Relative importance of lean and fat mass on bone mineral density in a group of adolescent girls and boys. Eur J Appl Physiol 2009a;105:759–764.
32. El Hage RP, Jacob C, Moussa E, Benhamou CL, Jaffre C. Total body, lumbar spine and hip bone mineral density in overweight adolescent girls: decreased or increased? J Bone Miner Metab 2009b;27:629–633.
33. El Hage R, Moussa E, El Hage Z, Theunynck D, Jacob C. Influence of age and morphological charecteristics on whole body, lumbar spine, femoral neck and 1/3

radius bone mineral apparent density in a group of Lebanese adolescent boys. J Bone Miner Metab 2011;29:477–483.
34. Ellis KJ, Shypailo RJ, Wong WW, Abrams SA. Bone mineral mass in overweight and obese children: diminished or enhanced? Acta Diabetol 2003;40:274–277.
35. Farr JN, Blew RM, Lee VR, Lohman TG, Going SB. Associations of physical activity duration, frequency, and load with volumetric BMD, geometry, and bone strength in young girls. Osteoporos Int 2011;22:1419–1430.
36. Freedson P, Pober D, Janz KF. Calibration of accelerometer output for children. Med Sci Sports Exerc 2005;37:523−S530.
37. Gaba A, Kapuš O, Pelclova J, Riegerova J. The relationship between accelerometer-determined physical activity (PA) and body composition and bone mineral density (BMD) in postmenstrual women. Arch Gerontol Geriatr 2012; 54:315–321.
38. Garnett SP, Högler W, Blades B, Baur LA, Peat J, Lee J, Cowell CT. Relation between hormones and body composition, including bone, in prepubertal children. Am J Clin Nutr 2004;80:966–972.
39. Gilsanz V, Chalfant J, Kalkwarf H, Zemel B, Lappe J, Oberfield S, Shepherd J, Wren T, Winer K. Age at onset of puberty predicts bone mass in young adulthood. J Pediatrics 2011;158:100–105.
40. Gilsanz V, Kovanlikaya A, Costin G, Roe TF, Sayre J, Kaufman F. Differential effect of gender on the sizes of the bones in the axial and appendicular skeletons. J Clin Endocrinol Metab 1997;82:1603–1607.
41. Gilsanz V, Roe TF, Mora S, Costin G, Goodman WG. Changes in vertebral bone density in black girls and white girls during childhood and puberty. *N Engl J Med* 1991;325:1597–1600.
42. Gilsanz V, Skaggs DL, Kovanlikaya ADifferential effect of race on the axial and appendicular skeletons of children. *J Clin Endocrinol Metab.* 1998;83:1420–1427.
43. Ginty F, Rennie KL, Mills S, Stear S, Jones S, Prentice A. Positive, site-specific asociations between bone mineral status, fitness, and time spent at high-impact activities in 16- to 18-year-old boys. Bone 2005;36:101−110.
44. Gordon CL, Halton JM, Atkinson SA: The contributions of growth and puberty to peak bone mass. Growth Dev Aging 1991;55:257–262.
45. Goulding A, Taylor RW, Jones IE, McAuley KA, Manning PJ, Williams SM. Overweight and obese children have low bone mass and area mfor their weight. Int J Obes Relat Metab Disord 2000;24: 627–632.
46. Goulding A, Taylor RW, Jones IE, Manning P, Williams SM. Spinal overload: a concern for obese children and adolescents? Ostoporos Int 2002;13:835–840.
47. Gracia-Marco L, Vicente-Rodriguez G, Casajus JA, Molnar D, Castillo MJ, Moreno LA. Effect of fitness and physical activity on bone mass in adolescents: the HELENA study. Eur. J Appl Physiol 2011a;111: 2671–2680.
48. Gracia-Marco L, Moreno LA, Ortega FB, Leon F, Sioen I, Kafatos A, Martinez-Gomez D, Widhalm K, Castillo MJ, Vicente-Rodriguez G. Levels of physical activity that predict optimal bone mass in adolescents. The HELENA study. Am J Prev Med 2011b;40: 599–607.
49. Gracia-Marco L, Ortega FB, Jimenez-Pavon D, Rodriguez G, Castillo MJ, Vicente-Rodriguez G, Moreno LA. Adiposity and bone health in Spanish adolescents. The HELENA study. Osteoporos Int 2012;23:937–947.
50. Greulich WW, Pyle SI. Radiographic atlas of skeletal development of hand and wrist. 2nd Ed. Stanford University Press, Stanford 1959.

51. Gruodyte R, Jürimäe J, Saar M, Jürimäe T. The relationships among bone health, insulin-intake growth factor-1 and sex hormones in adolescent female athletes. J Bone Miner Metab 2010;28:306–313.
52. Haapasalo H, Kannus P, Sievanen H, Pasanen M, Uusi-Rasi K, Heinonen A. Effect of long-term unilateral activity on bone mineral density of female junior tennis players. J Bone Miner Res 1998;13:310–319.
53. Han ZH, Palnitkar S, Rao DS, Nelson D, Parfitt AM. Effect of ethnicity and age or menopause on the structure and geometry of iliac bone. J Bone Miner Res 1996; 11:1967–1975.
54. Hasangolu A, Bideci A, Cinaz P, Turner L, Unal S. Bone mineral density in childhood obesity. J Pediatr Endocrinol Metab 2000;13:307–311.
55. Haslam DW, James WPT. Obesity. Lancet 2005;366:1197–1209
56. Heaney RP, Abrams S, Dawson-Hughes B, Looker A, Marcus R, Matkovic V. Peak bone mass. Osteoporos Int 2000;11:985–1009.
57. Hills AP, King NA, Armstrong T: The contribution of physical activity and sedentary behaviours to the growth and development of children and adolescents. Sports Med 2007;37:533–545.
58. Hind K, Burrows M. Weight-bearing exercise and bone mineral accrual in children and adolescents: a review of controlled trials. Bone 2007;40:14–27.
59. Ho AYY, Kung AWC. Determinants of peak bone mineral density and bone area in young women. J Bone miner Metab 2005;23:470–475.
60. Högström M, Nordström A, Alfredson H, Lorentzon R, Thorsen K, Nordström P. Current physical activity is related to bone mineral density in males but not in females. Int J Sports Med 2007;28:431−436.
61. Hrafnkelsson H, Sigrudsson G, Magnusson KT, Johannsson E, Sigurdsson EL. Factors associated with bone mineral density and content in 7-year-old children. Bone 2010;46:1058–1062.
62. Iuliano-Burns S, Stone J, Hopper JL, Seeman E. Diet and exercise during growth have site-specific skeletal effects: a co-twin control study. Osteoporos Int 2005; 16:1225–1232.
63. Javaid MK, Cooper C: Prenatal and childhood influences on osteoporosis. Best Pract Res Clin Endocrinol Metab 2002;16:349– 367.
64. Jones AP, van Sluijs EMF, Ness AR, Haynes R, Riddoch CJ. Physical activity in children: Does how we define neighborhood matter? *Health Place* 2010;16: 236−241.
65. Jürimäe J, Jürimäe T, Ring-Dimitriou S, LeMura LM, Arciero PJ, Von Duvillard SP. Plasma adiponectin and insulin sensitivity in overweight and normal-weight middle-aged premenopausal women. Metabolism 2009;58:638–643.
66. Katzman DK, Bacrach LK, Carter DR, Marcus R. Clinical and anthropometric correlates of bone mineral acquisition in healthy adolescent girls. J Clin Endocrinol Metab 1991;73:1332–1339.
67. Kelly TL, Wilson KE, Heymsfield SB. Dual energy X-ray absorptiomerty body composition reference values from NHANES. PLoS ONE 2009;4:e7038.
68. Kemper HCG. Skeletal development during childhood and adolescence and the effects of physical activity. Pediatr Exerc Sci 2000;12:198–216.
69. Khan K, McKay HA, Haapasalo H, Does childhood and adolescence provide a unique opportunity for exercise to strengthen the skeleton? J Sci Med Sport 2000;3:150−164.

70. Kriemler S, Zahner L, Puder JJ, et al. Weight-bearing bones are more sensitive to physical exercise in boys than in girls during pre- and early puberty: a cross-sectional study. Osteoporos Int 2008;19:1749−1758.
71. Kroger H, Vainio P, Niminen J, Kotaniemi A. Comparison of different models for interpreting bone mineral density measurements using DXA and MRI technology. Bone 1995;17:157–159.
72. Lanyon LE Functional strain in bone tissue as an objective, and controlling stimulus for adaptive bone remodelling. J Biomech 1987;20:1083–1093.
73. Lätt E, Jürimäe J, Haljaste K, Cicchella A, Purge P, Jürimäe T. Longitudinal development of physical and performance parameters during biological maturation of young male swimmers. Percept Motor Skills 2009;108:297–307.
74. Lätt E, Mäestu J, Rääsk T, Purge P, Saar M, Maasalu K, Jürimäe J, Jürimäe T. Association of physical activity to cardiovascular fitness and fatness in 12–13 year old boys of different weight status. J Public Health 2013;21:231–239.
75. Leonard MB, Shultz J, Wilson BA, Tershakovec AM, Zemel BS. Obesity during childhood and adolescence aubments bone mass and bone dimensions. A J Clin Nutr 2004;80:514–523.
76. Leonard MB. Glucocorticoid-Induced Osteoporosis in Children: Impact of the Underlying Disease. Pediatrics 2007;119;S166:2006–2023.
77. Lorentzon M, Mellstrom D, Ohlsson C. Association of amount of physical activity with cortical bone size and trabecular volumetric BMD in young adult men: The GOOD study. J Bone Miner Res 2005;20:1936–1943.
78. Malina RM, Bouchard C, Bar-Or O. Growth, Maturation, and Physical Activity. Champaign, Human Kinetics 2004.
79. Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in boys. Arch Dis Child 1970;45:13–23.
80. Martin TJ, Ng KW, Nicholson GC. Cell biology ob bone. Baillieres Clin Endocrinol Metab 1988;2:1–29.
81. Martinez-Gomez D, Ruiz JR, Ortega FB, Casajús JA, Veiga OL, Widhalm K, Manios Y, Béghin L, González-Gross M, Kafatos A, España-Romero V, Molnar D, Moreno LA, Marcos A, Castillo MJ, Sjöström M; HELENA Study Group. Recommended levels and intensities of physical activity to avoid low-cardiorespiratory fitness in European adolescents: The HELENA Study. Am J Hum Biol 2010; 22:750−756.
82. Matkovic V, Jelic T, Wardlaw GM, Ilich JZ, Goel PK, Wright JKTiming of peak bone mass in Caucasian females and its implication for the prevention of osteoporosis. Inference from a cross-sectional model. J Clin Invest 1994;93:799–808.
83. McCarthy HD, Cole TJ, Fry T, Jebb SA, Prentice AM. Body fat reference curves for children. Int J Obes 2006;30:598–602.
84. Meyer U, Ernst D, Zahner L, Schindler C, Puder JJ, Kraenzlin M, Rizzoli R, Kriemler S. 3-year follow-up results of bone mineral content and density after a school-based activity randomized intervention trial. Bone 2013;55:16–22.
85. Misra M, Miller KK, Stewart V, Hunter E, Kou K, Herzog DB, Klibanski A. Ghrelin and bone metabolism in adolescent girls with anorexia nervosa and healthy adolescents. J Clin Endorcinol Metab 2005;90:5082–5087.
86. Nebigh A, Rebai H, Elloumi M, Bahlous A, Zouch M, Zaouali M, Alexandre C, Sellami S, Tabka Z. Bone mineral density of young soccer players at different pubertal stages: relationships with hormonal concentration. J Bone Spine 2009; 76:63–69.

87. Nilsson M, Ohlsson C, Mellström Dan, Lorentzon M. Previous sport activity during childhood and adolescence is associated with increased cortical bone size in young adult men. J Bone Miner Res 2009:24:125–133.
88. Nilsson M, Ohlsson C, Sundh D, Mellstrom D, Lorentzon M. Association of physical activity with trabecular microstructure and cortical bone at distal tibia and radius in young adult men. J Clin Endocinol Metab 2010;95:2917–2926.
89. Ott SM. Bone density in adolescents. N Eng J Med 1991;325:1646–1647.
90. Petit MA, Beck TJ, Shults J, Zemel BS, Foster BJ, Leonard MB. Proximal femur bone geometry is appropriately adapted to lean mass in overweight children and adolescents. Bone 2005;36:568–576.
91. Pomerants T, Tillmann V, Karelson K, Jürimäe J, Jürimäe T. Ghrelin response to acute aerobic exercise in boys at different stages of puberty. Horm Metab Res 2006;38:752–757.
92. Rizzoli R, Bianchi ML, Garabedian M, McKay HA, Moreno LA. Maximizing bone mineral mass gain during growth for the prevention of fractures in the adolescents and elderly. Bone 2010;46:294–305.
93. Rocher E, Chappard C, Jaffre C, Benhamou CL, Courteix D. Bone mineral density in prepubertal obese and control children: relation to body weight, lean mass, and fat mass. J Bone Miner Metab 2008;26:73–78.
94. Ruiz JR, Ortega FB. Physical activity and cardiovascular disease risk factors in children and adolescents. Curr Cardiovasc Risc Rep 2009;3:281–287.
95. Ruiz JR, Rizzo NS, Hurtig-Wennlof A, Ortega FB, Warnberg J, Sjomstrom M. Relations of total physical activity and intensity to fitness and fatness in children: The European Youth Heart Study. Am J Clin Nutr 2006;84:299–303.
96. Rutherford CM. Is there a role for exercise in the prevention of osteoporotic fractures? Br J Sports Med 1999;33:378–386.
97. Saggese G, Baroncelli GI, Bertelloni S. Puberty and bone development. Best Pract Res Clin Endocrinol Metab 2002;16:53–64.
98. Saito MT. Sexual maturation: self-evaluation of the adolescent. Pediatrica 1984; 6:111–115.
99. Sanchis-Moysi J, Dorado C, Olmedillas H, Serrano-Sanchez JA, Calbet JA. Bone mass in prepubertal tennis players. Int J Sports Med 2010;31:416–20.
100. Sardinha LB, Baptista F, Ekelund U. Objectively measured physical activity and bone strength in 9-year-old boys and girls. Pediatrics 2008;122:728–736.
101. Sayers A, Mattocks C, Deere K, Ness A, Riddoch C, Tobias JH. Habitual levels of vigorous, but not moderate or light, physical activity is positively related to cortical bone mass in adolescents. J Clin Endocrinol Metab 2011;96:793–802.
102. Schoenau E, Neu CM, Beck B, Manz F, Rauch F. Bone mineral content per muscle cross-sectional area as an index of the functional muscle–bone unit. J Bone Miner Res 2002;17:1095–101.
103. Schoenau E, Neu CM, Rauch F, Manz F. The development of bone strength at the proximal radius during childhood and adolescence. J Clin Endocrinol Metab 2001;86:613–618.
104. Specker B, Binkley T, Fahrenwald N. Increased periosteal circumference remains present 12 months after an exercise intervention in preschool children. Bone 2004;35:1383–1388.
105. Specker BL, Johannsen N, Binkley T, Finn K. Total body bone mineral content and tibial cortical bone measures in preschool children. J Bone Miner Res 2001;16: 2298–2305.

106. Steel RM, van Sluijs EMF, Cassidy A, Griffin SJ, Ekelund U. Targeting sedentary time or moderate- and vigorous-intensity activity: independent relations with adiposity in a population-based sample of 10-y-old British children. Am J Clin Nutr 2009;90:1185−1192.
107. Sundberg M, Gärdsell P, Johnell O, Karlsson MK, Ornstein E, Sandstedt B, Sernbo I. Physical activity increases bone size in prepubertal boys and bone mass in prepubertal girls: a combined cross-sectional and 3-year longitudinal study. Calcif Tissue Int 2002;71:406–415.
108. Tanner J. Growth at Adolescence. 2nd edition. United Kingdom: Blackwell Scientific Publications, Oxford 1962.
109. Theintz G, Buchs B, Rizzoli R, Slosman D, Clavien H, Sizonenko PC, Bonjour JP. Longitudinal monitoring of bone mass accumulation in healthy adolescnece: evidence for a marked reduction after 16 years of age at the levels of lumbar spine and femoral neck in female subjects. J Clin Endocrinol Metab 1992;75:1060–1065.
110. Tobias JH, Steer C, Mattocks CG, Riddoch C, Ness A. Habitual levels of physical activity influences on bone mass in 11-year old children from the United Kingdom: findings from a large population-based cohort. J Bone Miner Res 2007;22:101−109.
111. US DHHS. Key guidelines for children and adolescents. http://www.health.gov/PAGuidelines/ 2008.
112. van Itallie TB, Yang MU, Heymsfield SB, Funk RC, Boileau RA. Height-normalized indicies of the body's fat-free mass and fat mass: potentially useful indicators of nutritional status. Am J Clin Nutr 1990;52:953–959.
113. Vicente-Rodriguez. How does exercise effect bone health during the growth? Sports Med 2006;36:561−569.
114. Vicente-Rodriguez G, Urzanqui A, Mesana MI, et al. Physical fitness effect on bone mass is mediated by the independent association between lean mass and bone mass through adolescence: a cross-sectional study. J Bone Miner Metab 2008;26:288−294.
115. Welten DC, Kemper HCG, Post GB, van Mechelen W, Twisk J, Lips P, Teule GJ. Weight-bearing activity during youth is a more important factor for peak bone mass than calcium intake. J Bone Miner Res 1994;9:1089–1096.
116. Yilmaz D, Ersoy B, Bilgin E, Gümüser G, Onur E, Pinar ED. Bone mineral density in girls and boys at different pubertal stages: relation with gonadal steroids, bone formation markers, and growth parameters. J Bone Miner Metab 2005;23:476–482.
117. WHO. Current health reccommendations for physical activity. http://www.who.int/dietphysicalactivity/factsheet_young_people/en/
118. Zouch M, Jaffré C, Thomas T, Frère D, Courteix D, Vico L, Alexandre C. Long-term soccer practice increases bone mineral content gain in prepubescent boys. Joint Bone Spine 2008;75:41–49.

SUMMARY IN ESTONIAN

Luutiheduse, keha koostise ja kehalise aktiivsuse vahelised seosed 11–13 aastastel poistel

Puberteediperioodi jooksul toimuvad inimese organismis mitmed hormonaalsed, morfoloogilised ja antropomeetrilised (kasvuspurt) muutused. Erinevad uuringud on leidnud, et nii positiivsed kui ka negatiivsed muutused, mis tekivad puberteediperioodi jooksul omavad suurt mõju inimese edasisele elukäigule.

Puberteediperioodi jooksul toimub ka väga intensiivne luukoe juurdekasv. Uuringutes on leitud, et umbes 40–50% luumassi juurdekasvust toimub just puberteediperioodil, saavutades tippmassi umbes 20–22 eluaastal, millest edasi algab luumassi vähenemine. Osteoporoos ehk luude hõrenemine on seisund, mille puhul on luumineraalide hulk vähenenud ning luud muutuvad hapraks ja suureneb luumurruoht. Seega, mida kõrgem on luumineraalide sisaldus, seda hiljem tinglikult avaldub osteoporoosi oht, millest järeldub, et puberteediea jooksul, mil luukude on kõige paremini mõjustatav tuleb luumineraalide suurenemisele pöörata olulist tähelepanu.

Uuringud on samuti näidanud, et luumineraalide hulga suurenemine (luutiheduse kasv) on väga tihedas seoses mehaanilise koormusega, mis avaldub luudele. Üheks selliseks faktoriks on inimese kehamassi suurus. On leitud, et suurema kehamassiga inimestel on suurem luutihedus võrreldes väiksema kehamassiga inimestega. Siiski esineb ka vasturääkivusi, millist mõju avaldab inimese keha rasvamass suurus võrreldes keha rasvavaba massi (kaasaarvatud keha lihasmassi) osakaaluga.

Samuti on leitud, et kehaline aktiivsus, eriti just tugev, löögilise iseloomuga kehaline aktiivsus omab positiivset seost luutiheduse suurenemisega. Samas ei ole teada, milline on selles mudelis keha koostise erinevate komponentide osakaal just erineva suurusega kehamassiga inimestel. Siiamaani tehtud uuringud on põhiliselt olnud ristlõikeuuringud, samas kui on teada, et just puberteediea jooksul toimub oluline kehalise aktiivsuse vähenemine. Seetõttu ei pruugi ristlõike uuringute tulemused anda usaldusväärset infot kehalise aktiivsuse muutuse kohta.

Käesoleva uurimistöö eesmärgiks oli uurida võimalikke seoseid keha koostise parameetrite, kehalise aktiivsuse ning luu parameetrite vahel puberteediealistel poistel.

Vastavalt uurimistöö eesmärgile püstitati järgmised konkreetsed ülesanded:

1) uurida erinevaid luutiheduse parameetreid ülekaalulistel ja normaalkaalulistel poistel;
2) uurida keha koostise ja keha erinevate luutiheduse näitajate vahelisi seoseid ülekaaluslistel ja normaalkaalulistel poistel;
3) uurida seoseid keha koostise ja erineva intensiivsusega kehalise aktiivsuse ning luutiheduse parameetrite vahel ülekaaluslistel ja normaalkaalulistel poistel;

4) uurida longituudselt erinevate kehalise aktiivsuse näitajate muutuste mõju luutiheduse näitajate arengule ülekaalulistel ja normaalkaalulistel poistel.

Antud uuringus osales 264 10–13 aasta vanust Tartu ja selle ümbruskonna koolide poissi. Vaatlusalused jaotati normaal- ja ülekaalulisteks kasutades McCarthy jt. (2006) metoodikat. Vaatlusalustel määrati keha koostis DXA meetodil, kehaline aktiivsus aktselerомeetri abil, bioloogiline vanus, kasutades röntgenülesvõtet labakäest. Sarnased testid viidi läbi ühe aasta pärast. Longitudinaalsesse analüüsi kaasati ainult need vaatlusalused, kellel olid olemas kõik andmed nii esimesest kui ka teisest mõõtmispunktist.

Käesoleva uuringu põhjal tehti järgmised järeldused:

1) Ülekaaluliste poiste luutiheduse näitajad olid oluliselt kõrgemad võrreldes normaalkaaluliste poiste vastavate näitajatega. Samas ei leitud usutavaid erinevusi selja lumbaarosa ja reieluukaela mahulises luutiheduse näitajas, kuid ülekaalulistel esines oluliselt madalam kogu keha mahuline luutihedus.
2) Keha rasvavaba mass kajastavad paremini luutiheduse näitajaid normaalkaalulistel poistel, ülekaalulistel poistel kajastavad luutiheduse parameetreid paremini keha rasvamass .
3) Kehaline aktiivsus omab suuremat mõju luutiheduse parameetritele ülekaalulistel poistel ning lisaks intensiivsele kehalisele aktiivsusele omab olulist mõju ka mõõdukas kehaline aktiivsus.
4) Kehalise inaktiivsuse suurenemine omab olulist negatiivset mõju määratud luuparameetrite arengule ühe aastase uuringuperioodi jooksul 11–13 aastastel poistel.

Kokkuvõttes näitasid antud uuringu tulemused, et erinevate keha koostise ja kehalise aktiivsuse näitajate mõju luuparameetrite arengule on puberteediperioodi jooksul erinev normal- ja ülekaaluliste poiste vahel.

ACKNOWLEDGEMENTS

My special thanks to:

- My academic supervisors Professor Dr. Jaak Jürimäe, Senior Lecturer Dr. Jarek Mäestu and Professor Dr. Toivo Jürimäe for all the support and advice throughout preperation my thesis;
- Researchers Dr. Evelin Lätt, Dr. Priit Purge and Dr. Meeli Saar for all support and helping to conduct the procedures;
- All the participants, who went through this demanding study;
- My family for all the support during my PhD studies.

PUBLICATIONS

CURRICULUM VITAE

Name: Artūrs Ivuškāns
Date of birth: 05.05.1983
Citizenship: Latvian
Phone: +371 2957 7587
E-mail: arturs.ivuskans@gmail.com
Languages: Latvian, English, Russian

Educational Career:

2012–	VOKS School of Applied Kinesiology Lietišķās
2009–	Doctoral study, Faculty of Exercise and Sport sciences, University of Tartu, Tartu, Estonia
2007–2009	MSc, Academic MSc in Sport Sciences, LASE (Latvian Academy of Sports Education), Riga, Latvia
2002–2007	BSc, Specialist of all level sport education, and physiotherapy, LASE, Riga, Latvia
1998–2002	Riga Gymnasium of food makers, Riga, Latvia

Professional Career:

2007	Physiotherapist at „Baltijas fizioterapija”
2006–2011	Physiotherapist at „Auxilia Prima”
2004–2006	Coach/instructor at gym „Georgs 5”

Education in addition:

2013	Cervical spine and shoulder joint dysfunction diagnosis and treatment
2012	Anatomy Trains for Manual Therapists
2009	Medical Taping for Physio therapists
2009	The Redcord Education Program, Neurac 1
2009	Latvian Association Sertificate Latvijas
2008	Latvian Association of Manual Therapy (functional treatment in joint rehabilitation)

ELULOOKIRJELDUS

Nimi: Artūrs Ivuškāns
Sünniaeg: 05.05.1983
Kodakondsus: Läti
Telefon: +371 2957 7587
E-mail: arturs.ivuskans@gmail.com
Keeled: läti, inglise, vene

Haridus:

2012–	VOKS School of Applied Kinesiology Lietišķās
2009–	Doktoriõpe Liikumis- ja sporditeadused, Kehakultuuriteaduskond, Tartu Ülikool, Tartu, Eesti
2007–2009	Magistriõpe, MSc Sporditeadustes, LASE (Läti Spordiakadeemia), Riia, Läti
2002–2007	Bakalaureuseõpe, Sporditeadused ja füsioteraapia, LASE, Riia, Läti
1998–2002	Riia Toitlustuskool, Riia, Läti

Töökogemus:

2007	Füsioterapeut ettevõttes „Baltijas fizioterapija”
2006–2011	Füsioterapeut ettevõttes „Auxilia Prima”
2004–2006	Treener/instruktor spordiklubis „Georgs 5”

Koolitused:

2013	Kaelapiirkonna ja õlaliigese düsfunktsiooni diagnoos ja ravi
2012	Anatoomia manuaalterapeutidele
2009	Teipimine füsioterapeutidele
2009	The Redcord Education Program, Neurac 1
2009	Latvian Association Sertificate Latvijas
2008	Läti manuaalterapeutide seltsi liige

DISSERTATIONES KINESIOLOGIAE UNIVERSITATIS TARTUENSIS

1. **Lennart Raudsepp.** Physical activity, somatic characteristics, fitness and motor skill development in prepubertal children. Tartu, 1996, 138 p.
2. **Vello Hein.** Joint mobility in trunk forward flexion: methods and evaluation. Tartu, 1998, 107 p.
3. **Leila Oja.** Physical development and school readiness of children in transition from preschool to school. Tartu, 2002, 147 p.
4. **Helena Gapeyeva.** Knee extensor muscle function after arthroscopic partial meniscectomy. Tartu, 2002, 113 p.
5. **Roomet Viira.** Physical activity, ecological system model determinants and physical self-perception profile in early adolescence. Tartu, 2003, 167 p.
6. **Ando Pehme.** Effect of mechanical loading and ageing on myosin heavy chain turnover rate in fast-twitch skeletal muscle. Tartu, 2004, 121 p.
7. **Priit Kaasik.** Composition and turnover of myofibrillar proteins in volume – overtrained and glucocorticoid caused myopathic skeletal muscle. Tartu, 2004, 123 p.
8. **Jarek Mäestu.** The perceived recovery-stress state and selected hormonal markers of training stress in highly trained male rowers. Tartu, 2004, 109 p.
9. **Karin Alev.** Difference between myosin light and heavy chain isoforms patterns in fast- and slow-twitch skeletal muscle: effect of endurance training. Tartu, 2005, 117 p.
10. **Kristjan Kais.** Precompetitive state anxiety, self-confidence and ahtletic performance in volleyball and basketball players. Tartu, 2005, 99 p.
11. **Aire Leppik.** Changes in anthropometry, somatotype and body composition during puberty: a longitudinal study. Tartu, 2005, 161 p.
12. **Jaan Ereline.** Contractile properties of human skeletal muscles: Association with sports training, fatigue and posttetanic potentiation. Tartu, 2006, 133 p.
13. **Andre Koka.** The role of perceived teacher feedback and perceived learning environment on intrinsic motivation in physical education. Tartu, 2006, 137 p.
14. **Priit Purge.** Performance, mood state and selected hormonal parameters during the rowing season in elite male rowers. Tartu, 2006, 101 p.
15. **Saima Kuu.** Age-related contractile changes in plantarflexor muscles in women: associations with postactivation potentiation and recreational physical activity. Tartu, 2006, 101 p.
16. **Raivo Puhke.** Adaptive changes of myosin isoforms in response to long-term strength training in skeletal muscle of middle-aged persons. Tartu, 2006, 99 p.

17. **Eva-Maria Riso.** The effect of glucocorticoid myopathy, unloading and reloading on the skeletal muscle contractile apparatus and extracellular matrix. Tartu, 2007, 114 p.
18. **Terje Sööt.** Bone mineral values in young females with different physical activity patterns: association with body composition, leg strength and selected hormonal parameters. Tartu, 2007, 94 p.
19. **Karin Tammik.** Neuromuscular function in children with spastic diplegic cerebral palsy. Tartu, 2007, 102 p.
20. **Meeli Saar.** The relationships between anthropometry, physical activity and motor ability in 10–17-year-olds. Tartu, 2008, 96 p.
21. **Triin Pomerants.** Ghrelin concentration in boys at different pubertal stages: relationships with growth factors, bone mineral density and physical activity. Tartu, 2008, 80 p.
22. **Tatjana Kums.** Musculo-skeletal function in young gymnasts: association with training loads and low-back pain. Tartu, 2008, 128 p.
23. **Maret Pihu.** The components of social-cognitive models of motivation in predicting physical activity behaviour among school students. Tartu, 2009, 116 p.
24. **Peep Päll.** Physical activity and motor skill development in children. Tartu, 2009, 102 p.
25. **Milvi Visnapuu.** Relationships of anthropometrical characteristics with basic and specific motor abilities in young handball players. Tartu, 2009, 114 p.
26. **Rita Gruodytė.** Relationships between bone parameters, jumping height and hormonal indices in adolescent female athletes. Tartu, 2010, 82 p.
27. **Ragnar Viir.** The effect of different body positions and of water immersion on the mechanical characteristics of passive skeletal muscle. Tartu, 2010, 142 p.
28. **Iti Müürsepp.** Sensorimotor and social functioning in children with developmental speech and language disorders. Tartu, 2011, 90 p.
29. **Ege Johanson.** Back extensor muscle fatigability and postural control in people with low back pain. Tartu, 2011, 106 p.
30. **Evelin Lätt.** Selected anthropometrical, physiological and biomechanical parameters as predictors of swimming performance in young swimmers. Tartu, 2011, 90 p.
31. **Raul Rämson.** Adaptation of selected blood biochemical stress and energy turnover markers to different training regimens in highly trained male rowers. Tartu, 2011, 84 p.
32. **Helen Jõesaar.** The effects of perceived peer motivational climate, autonomy support from coach, basic need satisfaction, and intrinsic motivation on persistence in sport. Tartu, 2012, 108 p.
33. **Sille Vaiksaar.** Effect of menstrual cycle phase and oral contraceptive use on selected performance parameters in female rowers. Tartu, 2012, 86 p.

34. **Anna-Liisa Parm.** Bone mineralization in rhythmic gymnasts before puberty: associations with selected anthropometrical, body compositional and hormonal parameters. Tartu, 2012, 96 p.
35. **Jelena Sokk.** Shoulder function in patients with frozen shoulder syndrome: the effect of conservative treatment and manipulation under general anaesthesia. Tartu, 2012, 125 p.
36. **Helena Liiv**. Anthropometry, body composition and aerobic capacity in elite DanceSport athletes compared with ballet and contemporary dancers. Tartu, 2014, 80 p.
37. **Liina Remmel**. Relationships between inflammatory markers, body composition, bone health and cardiorespiratory fitness in 10- to 11-year old overweight and normal weight boys. Tartu, 2014, 94 p.
38. **Doris Vahtrik.** Leg muscle function in relation to gait and standing balance following total knee arthroplasty in women. Tartu, 2014, 105 p.