DISSERTATIONES KINESIOLOGIAE UNIVERSITATIS TARTUENSIS
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Bone mineralization in rhythmic gymnasts before puberty: associations with selected anthropometrical, body compositional and hormonal parameters

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CONTENTS

ABBREVIATIONS

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
FM	fat mass
FN	femoral neck
L2-L4	antero-posterior lumbar spine
LS	lumbar spine
SD	standard deviation

LIST OF ORIGINAL PUBLICATIONS

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II. Parm AL, Jürimäe J, Saar M, Pärna K, Tillmann V, Maasalu K, Neissaar I, Jürimäe T. Plasma adipocytokine and ghrelin levels in relation to bone mineral density in prepubertal rhythmic gymnasts. *Journal of Bone and Mineral Metabolism*, 2011; 29: 717–24.

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1. INTRODUCTION

Rhythmic gymnastics, like ballet and figure skating, is a sport discipline that begins early in childhood and poses high demands on the performer`s functional and energy capacities, which may influence the development of the body`s physique and composition (Misigoj-Durakovic et al., 2005). Rhythmic gymnasts are subjected to high levels of physical and psychological stress due to the intensive physical training and to psychological stress imposed by athletic competitions (Theodoropoulou et al., 2005). Participation in aesthetic sports, which requires a lean body shape to fulfill aesthetic standards and to facilitate flexibility, is a very common disordered eating risk factor (Krentz & Warschburger, 2011; Martinsen et al., 2010; Thompson & Sherman, 2010).

Selected anthropometric characteristics (e.g., arm span, mid-thigh circumference, body mass), aerobic power, flexibility, and explosive strength are important determinants of successful performance and talent identification in rhythmic gymnastics (Douda et al., 2008). Atletes are usually very thin and have low fat mass (FM) because rhythmic gymnastics requires this kind of aesthetic ideal (Courteix et al., 2007). This is also a reflection of not only specific sport requirements but also a self-selection bias by rhythmic gymnasts and coaches who favour participation to each sport of the athlete with the appropriate body shape (Theodoropoulou et al., 2005). Elite rhythmic gymnasts compared to less successful rhythmic gymnasts and also untrained controls are taller, have longer leg length and sitting height, and have lower body mass and fat free mass (FFM), while there appears to be no differences in body mass index (BMI) (Di Cagno et al., 2008).

Rhythmic gymnasts exhibit a moderate but significant delay in skeletal maturation (delayed bone age), which is related to the type, intensity, frequency, and duration of exercise (Theodoropoulou et al., 2005). As bone maturation progresses slower also in ballet dancers, similarly to rhythmic gymnasts, over a long period of time, adequate compensatory catch-up growth is explained by the recovery of growth potential as puberty progresses (Donoso et al., 2010). Georgopoulos et al. (2001) demonstrated that rhythmic gymnasts compensate for their loss of the pubertal growth spurt by late acceleration of linear growth. There is also a delay in pubertal development in rhythmic gymnasts as menarche could be delayed by as much as 1.5–2.0 years (Klentrou & Plyley, 2003). In rhythmic gymnasts, prepubertal stage could be prolonged and pubertal development could be entirely shifted to a later age, following bone maturation rather than chronological age (Georgopoulos et al., 2010). The effects of these factors on growth, skeletal maturation, and pubertal development in rhythmic gymnasts have been studied before (Georgopoulos et al., 2010, 2012; Helge & Kanstrup, 2002; Tournis et al., 2010) and the late maturation of rhythmic gymnasts contributes to a girl's decision to continue participating in this sport rather than intensive training delaying menarche (Baxter-Jones et al., 1994).

Rhythmic gymnastics is known as a high-impact bone loading sport because gymnasts perform many jumps during their everyday trainings (Tournis et al., 2010). High-impact exercise (jumping) has a strong impact on the development of bone mineral density (BMD) and bone mineral content (BMC) (Fuchs et al., 2001; Ward et al., 2005). Young gymnasts have already higher bone mineral values than children participating in other sport disciplines or untrained controls. Rhythmic gymnasts start to train with rather heavy loads and compete already at relatively young ages before puberty (Misigoj-Durakovic et al., 2005; Tournis et al., 2010). This may lead to a relatively low body FM and probable low energy balance already before puberty. Accordingly, it is important to study selected hormonal markers of body energy balance in addition to anthropometric and body composition parameters in rhythmic gymnasts before puberty. It is well known that adipose tissue produces different adipocytokines such as leptin and adiponectin, which participate in energy homeostasis and contribute to the relationship between FM and BMD in addition to mechanical loading (Biver et al., 2011; Huang et al., 2004; Misra, 2008). In addition to adipocytokines, the gut hormone ghrelin may also influence bone mineralization in children (Pacifico et al., 2009).

To date, there are relatively few data on specific bone mineral accrual during the prepubertal years close to puberty in rhythmic gymnasts who start to exercise with relatively high training loads at a relatively early age. This time period has been found to be very sensitive for bone mineral accumulation in children (MacKelvie et al., 2002). To our best of knowledge, there have been no longitudinal studies about the influence of adipocytokines (leptin and adiponectin) and ghrelin together with body composition parameters on whole body and areal bone mineral density values in a specific group of prepubertal rhythmic gymnasts. Therefore, the main aim of the present thesis was to investigate possible associations between specific anthropometric, body composition and hormonal parameters with bone mineral values and the prediction of bone mineral development by baseline measured hormone variables during one year in prepubertal rhythmic gymnasts and untrained controls.

2. REVIEW OF THE LITERATURE

2.1. Bone development in childhood

Osteoporosis is a major global public health concern and it is characterized by microarchitectural deterioration and loss of bone tissue that consequently increases susceptibility to fracture (Cooper, 1999). Osteoporosis is a condition of bone fragility that can lead to pain, disability, and reduced quality of life (Peck, 1993). Individuals who achieve a higher peak bone mass during childhood might prevent or delay osteoporosis and have decreased fracture risk in later life (Bass et al., 1998, 1999). Fractures are also common during growth, occurring in approximately one third of children who are otherwise healthy (Cooper et al., 2004), although Goulding et al. (2005) found that fracture incidence increases from 16% in normal weight children to 33% in overweight and obese children. Maximal incidence of fractures in girls occurs between 11–12 years. This period corresponds to the age of peak height velocity in both sexes and preceeds by nearly one year the time of peak bone mineral content velocity (Kawalilak et al., 2010).

There are different opinions concerning the exact period when the peak bone mass is reached, but the general agreement is that the maximal accrual of bone mineral density is acquired in the years surrounding puberty (Heaney et al., 2000). Prepubertal time period is a very sensitive period for bone mineral accumulation in children (MacKelvie et al., 2002) and about 90% of adult skeletal mass is acquired during childhood (Heaney et al., 2000). Longitudinal growth of the human skeleton during the entire childhood until early adult age is the result of endochondal ossification (Kalkwarf et al., 2010). As children`s lifestyle affects growth of the skeleton and development of BMD, it is important to understand mechanisms regulating bone mineral accrual (Tubić et al., 2011) with the goal of optimizing the achievement of peak bone mass and minimizing the risk of osteoporotic fractures in adulthood (Berger et al., 2010). Factors predicting BMD in children are not yet fully known but studies indicate that genetic factors (about 50–70%) (Duren et al., 2007; Ferrari et al., 1998; Kelly et al., 1995), endocrine status (Rhie et al., 2010; Wit & Camacho-Hübner, 2011), timing of pubertal maturation (Ausili et al., 2012; Chevalley et al., 2011; Jackowski et al., 2011), body composition (Baptista et al., 2012; Rhie et al., 2010), lifestyle – diet (Vatanparast et al., 2007; Wosje et al., 2010), including vitamin D (Harkness & Bonny, 2005), and physical activity (Janz et al., 2001; Marwaha et al., 2011; Rizolli et al., 2010) during early childhood may contribute to optimal bone development in children.

Wosje et al. (2010) identified dietary patterns related to body FM and bone mineral accrual in 3.8–7.8-year-old children and found that increasing intakes of dark-green and deep-yellow vegetables (eg. spinach, romaine lettuce, broccoli, carrots and sweet potatoes) and limiting fried-food intake may promote healthy FM and bone mineral accrual. The main reason of this remains unclear but may

related to their high content of alkalizing minerals such as potassium. Accordingly, it is very important to have healthy diet for bone development in children.

Another main factor for skeletal strength and bone development is mechanical loading activity. Mechanical loading during growth has the potential to optimize bone structure and produce worth-while gains in bending, compressive, and torsional strength (Seeman, 2003). The type, intensity, frequency, load, and especially duration of the physical activity are all affecting children`s bone development (Farr et al., 2011a; Rizolli et al., 2010; Scarpella et al., 2003), probably even more than dietary calcium intake (Bonjour et al., 1997; Dowthwaite et al., 2011; Welten et al., 1994). Participation in vigorous physical activity, but not in moderate or light physical activites, may have a long-term benefits for skeletal health (Lazcano-Ponce et al., 2003; Sayers et al., 2011). Furthermore, it could be suggested that regular high-impact weight-bearing physical activity during growth and development plays an important role in maximising bone mineral mass gain and might prevent osteoporosis in later life (Baxter-Jones et al., 2008; Erlandson et al., 2012; Rizolli et al., 2010). Children who are less physically active at an early age may loose the opportunity to obtain the highest bone parameters possibly later in life when they are likely to be less physically active (Janz et al., 2010).

Rhythmic gymnastics seems to be especially osteogenic in children (Gruodyté et al., 2010; Munoz et al. 2004) and young adults (Sööt et al., 2005), probably due to high-volume, high impact training and the involvement at a relatively early age (Tournis et al., 2010). It has been found that BMD values are significantly higher in early pubertal (Munoz et al., 2004), adolescent (Gruodyté et al., 2010; Munoz et al., 2004) and adult (Helge & Kanstrup, 2002; Sööt et al., 2005) rhythmic gymnasts compared to untrained controls. Intensive rhythmic gymnastics trainings in premenarcheal girls are associated with positive effects on the skeleton, especially in cortical bone, characterized by increased bone mineral mass and improved geometric properties (Tournis et al., 2010). Bareither et al. (2008) found that habitual gymnastics trainings significantly increase distal radius strength also in older women, thereby reducing the incidence of fracture. Skeletal adaptations to mechanical loading have site-specific impact through higher muscle mass and strength because muscle and bone are biomechanically linked (Courteix et al., 1999; Daly et al., 2004; Dowthwaite et al., 2012; Heinonen et al., 2001). Rhythmic gymnastics stimulates bone resorption activity and bone turnover in 11.9±2yr girls (Jaffré et al., 2001), and higher rate of bone turnover in rhythmic gymnasts compared with untrained controls induces also a higher BMC (Courteix et al., 2007).

Whether the advantage of an active lifestyle during childhood is maintained through the young adult years or affects later fracture risk is not still clear (Georgopoulos et al., 2004), but the encouraging evidence does exist (Baxter-Jones et al., 2008; Kontulainen et al., 1999). It is suggested that the benefits in bone mineral development accrued from participating in high impact activities

during childhood and adolescence may be retained into adulthood (Ducher et al., 2006; Erlandson et al., 2012; Gunter et al., 2008; Kontulainen et al., 2002), while other researchers have suggested that increases in bone mineral development are lost when these activities cease (Karlsson et al., 2000; Khan et al., 1996; Nordstrom et al., 2005; Valdimarsson et al., 2005) because bone formed early in life is completely replaced during growth due to skeletal modeling and remodeling (Gafni & Baron, 2007). It is reported that physically active children and adolescents (8–15-year-old) had 8 to 10% more hip BMC in young adulthood (ages 23 to 30) when compared to less active peers, even after controlling for their adult physical activity levels. This provides intriguing evidence of the long-term benefits of childhood physical activity on adolescent and adult BMC and supports conjecture that the promotion of physical activity in children could help prevent osteoporosis in later life (Baxter-Jones et al., 2008). Similar results were found by Janz et al. (2010) who claimed that early childhood may be an important developmental period for the promotion of physical activity to optimize peak bone mass during young adulthood and therefore it is possible to prevent osteoporosis during later years. It has also been found that mechanical loading during rhythmic gymnastics training in childhood yields skeletal advantages that persist at least 24 months after loading cessation and menarche (Scarpella et al., 2010). In addition, other study from same researchers showed that despite of brief de-training losses in BMD and BMC values, significant benefits appear to persist for at least four years beyond rhythmic gymnastics activity cessation into early adulthood (Scarpella et al., 2011).

In summary, it is known that several factors including high impact activity affect bone mineral parameters in childhood. Several studies have shown that rhythmic gymnastics training beneficially affects the development of BMD during puberty. However, there are less data on specific bone mineral accrual during prepubertal years close to puberty in rhythmic gymnasts who start to exercise with relatively high training loads at a relatively early age. In girls, greater attention should be paid to pre- and also perimenarcheal period, when large amount of lumbar adult peak bone mineral mass is acquired (Heaney et al., 2000).

2.2. Anthropometry, body composition and bone mineral parameters

Body mass has been considered as one of the strongest predictors of BMD (Reid, 2002; Rhie et al., 2010). Although bone mineral mass in obese individuals compared with lean subjects contributes only ~0.5 kg (1%) of total body mass (Cifuentes et al., 2003), it is about 20% of total mineral content, thus making a substantial contribution to the higher risk of osteoporosis in lean individuals compared with overweight subjects (Shapses & Riedt, 2006). The benefits of higher BMD disappear during weight loss (Ensrud et al., 2005). Who

loose the most weight tended to loose the most BMD (Salamone et al., 1999) and in normal-weight individuals the bone loss is greater (>1%) than in overweight or obese people (<1%) (Nguyen et al., 1998). So it may assume that higher body mass (FM and FFM) has beneficial effect on bone mass.

Recent reports indicate that FM and FFM are related to BMD values also in prepubertal girls (Rhie et al., 2010). A significant relationship between FFM and BMD has been found in healthy prepubertal (Hrafnkelsson et al., 2010; Rhie et al, 2010) and adolescent (Fonseca et al., 2008) children, and also in adult females (Högler et al., 2003) including rhythmic gymnasts (Courteix et al., 1999; Vicente-Rodriguez et al., 2007). Fonseca et al. (2003) claimed that FFM is the main predictor of bone mass at least during the adolescence, regardless of gender. There is also a significant relationship between FFM and BMC values. Ashby et al. (2011) found that females (5–18-year-old) accrue more BMC in relation to FFM, in comparison to males, at most skeletal sites.

Studies in children have yielded conflicting results with regard to the relationships between FM and BMD (Ackerman et al., 2006; Janicka et al., 2007). Fat mass is an independent risk factor for osteoporosis but has also a positive effect on the development of bone mass through the weight-bearing-loading (Hsu et al., 2006) and by the adipose-modulated biochemical signals of appetite regulation and energy homeostasis (Garnett et al., 2004; Pacifico et al., 2009). It has been found that adipose tissue is a positive independent determinant of bone mass (Ackerman et al., 2006; Clark et al., 2006; Thomas et al., 2001), and a negative predictor of BMD (Janicka et al., 2007; Lazcano-Ponce et al., 2003; Pollock et al., 2007; Young et al., 2001) and also of BMC (Hrafnkelsson et al., 2010; Lazcano-Ponce et al., 2003; Skaggs et al., 2001; Weiler et al., 2000) in young healthy females. Some authors claim that FM is not related to bone development outcomes (Petit et al., 2005). Furthermore, Cole et al. (2012) claimed that FM is negatively associated with volumetric BMD in children, independent of FFM, despite positive associations with bone size. Although childhood obesity seems to be slightly associated with trabecular bone in adulthood in both weight-bearing and nonweight-bearing bones among obese premenopausal women, obesity is not associated with cortical density, neither is childhood obesity associated with total cross-sectional bone area (Uusi-Rasi et al., 2010). Farr et al. (2010) found significant correlations between FM and volumetric BMD, bone geometry, and indices of bone strength, while these relationships were no significant after adjustment for muscle cross-sectional area in 8–13-year-old girls. Weeks and Beck (2008) suggested that while obesity is represented by increased body mass, and thus the mass needed to move during habitual activities, the incident muscular contractions, particularly the magnitude of force, the rate of force production, and the total amount of contractions play a more important role than body mass. Although body mass alone imposes relatively small and static load on bones (corresponding to Earth`s gravity), the load can be substantially amplified in different activities by muscle actions.

The advantage of higher body mass to bone mass during childhood is probably maintained through the young adult years. Javaid et al. (2011) found in a study of 6370 Finnish women that reduction in body mass index gain between 1–12 years of age is associated with an increased risk of hip fracture in later life. Potential explanations discussed by the authors were firstly, a difference in pubertal timing and secondly, a slowing of growth in response to adverse environmental influences. In conclusion the authors claimed that thinness in childhood is a risk factor for hip fracture in later life by a direct effect of low FM on bone mineralization. Viljakainen et al. (2011) found also that besides high body fat content, also a low body fat content has adverse effects on bone development.

The relationships between different body composition and BMD variables seem to be site-specific in young female gymnasts (Dowthwaite et al., 2012). One of the reason might be because of positive correlations between BMD and maximal muscle strength (Helge & Kanstrup, 2002), because bone is a dynamic tissue that adapts its mass and architecture to best the physical forces to which it is regularly subjected (Lanyon & Rubin, 1984). Accordingly, it could be suggested that there is a relationship between body mass and BMD regardless whether it is FM or FFM (Rhie et al., 2010; Van Langendonck et al., 2004). Still it is not clear how lower body mass affects bone development in children who are physically very active and have probably lower energy intake. Michopoulou et al. (2011) compared 10–12-year-old rhythmic gymnasts and sedentary age-matched females and found that although both groups demonstrated comparable daily energy intake, rhythmic gymnasts exhibited a higher daily energy expenditure resulting in a daily energy deficit.

In prepubertal children, BMD is inversely correlated with body fat percentage (Specker et al., 2001). Similarly, studies in adults have shown positive correlations between BMI and BMD, and body fat percent and BMD (Reid et al., 1992). In prepubertal children, waist circumference and subscapular skinfold are positively correlated with bone mass parameters (Tubić et al., 2011). Slameda et al. (1990) found that in adult females each of the anthropometric measurements (subscapular skinfold, calf circumference and biacromial width) were independent significant predictors of bone mineral mass, even when height, body mass and age were included in the models. Arm span has not been significantly correlated with BMD in young adults and postmenopausal women, but together with height low correlation with bone mineral density occured (Trivitayaratana et al., 2001). Bone mineral density appeared to be related to trunk skeletal parameters and leg skinfolds in endurance- and strength trained sedentary normal-weight and overweight young females (Jürimäe et al., 2005).

In summary, it has been found that body composition parameters and different skinfold thicknesses, circumferences and widths are correlated with bone mineral parameters at least in adult females. However, to our best of knowledge, there is not available any information about the relationship of specific anthropometric (skinfolds, girths, lengths, breadths) and body composition para-

meters with different bone mineral values in a specific group of prepubertal rhythmic gymnasts.

2.3. Adipocytokines, ghrelin and bone mineral parameters

Adipose tissue is a metabolically active tissue, secreting a variety of adipocytokines that modulate biological functions. Among numerous adipose-modulated biochemical signals that may participate in energy homeostasis and contribute to the relationship between FM and BMD in normal weight children and adolescents are leptin (Garnett et al., 2004; Gruodyté et al., 2010), adiponectin (Huang et al., 2004; Misra et al., 2007) and ghrelin (Pacifico et al., 2009). Leptin and adiponectin are cytokine-like hormones, secreted by adipocytes, and carry signals from adipose tissue to bone tissue and contribute to the relationship between FM and BMD (Huang et al., 2004; Jürimäe & Jürimäe, 2007; Misra, 2008). For example, leptin receptors may be present in osteoblasts, allowing a direct effect of leptin on these cells (Thomas et al., 1999). Chronic physical exercise decreases leptin (Jürimäe et al., 2011; Plonka et al., 2011) and increases adiponectin (Jürimäe et al., 2011; Kriketos et al., 2004; Simpson & Singh, 2008) concentrations, and the expression of adiponectin receptors in muscle (Blüher et al., 2007). Accordingly, these findings may suggest that adipocytokines and also ghrelin may have beneficial effects on bone mineral parameters.

Circulating leptin concentrations steadily increase with increasing pubertal stages in girls (Garcia-Mayor et al., 1997), and decrease when energy intake is restricted and increases when body fat is increased (Ambroszkiewicz et al., 2011; Jürimäe et al., 2010; Hassink et al., 1996). Leptin may regulate bone mineral mass concurrently with changes in body mass (Zanker & Hind, 2007). Leptin has direct effect on bone tissue by stimulating proliferation and differentiation of osteoblasts at least in adults (Thomas et al., 1999; Yamauchi et al., 2001) and inhibiting osteoclastogenesis (Holloway et al., 2002) in vitro. In vivo studies no correlations between circulating leptin and BMD in middle-aged and older women have been found (Iida et al., 2011; Martini et al., 2001), while Rhie et al. (2010) found leptin as an independent predictor of femoral BMD in prepubertal girls. Accordingly, the relationships between leptin and BMD may be age dependent (Sherk et al., 2011). Leptin also affects bone development through its actions on the central nervous system, in particular the hypothalamus (Elefteriou et al., 2005). Leptin is directly related to FM as well as to BMD in normal weight prepubertal girls (Garnett et al., 2004; Rhie et al., 2010), while the impact of lowered leptin concentrations on bone mineral mass acquisition remains still questionable in prepubertal and pubertal female rhythmic gymnasts in the presence of elevated energy expenditure and reduced FM (Courteix et al., 2007; Munoz et al., 2004). Gruodyté et al. (2010) found independent

relationship of circulating leptin with lumbar spine (LS) and femoral neck (FN) BMD values in pubertal rhythmic gymnasts, while Maimoun et al. (2010b) found no direct role of leptin on bone mineral mass acquisition during pubertal development in rhythmic gymnasts. It has been suggested that the hypoleptinemia induced by intensive and stressful trainings in the presence of elevated energy expenditure and reduced FM do not affect bone development in rhythmic gymnasts during puberty (Courteix et al., 2007; Lou et al., 2006). However, the role of leptin on bone development in the conditions of chronic elevated energy expenditure and lower FM in rhythmic gymnasts during prepuberty is not known.

Adiponectin may be a hormone linking bone and fat metabolism because adiponectin and its receptors are expressed in human osteoblasts and the receptors have been identified also in osteoclasts (Berner et al., 2004). Adiponectin has been suggested to promote osteogenesis, increase osteogenetic markers and augment osteoblast differentation (Lee et al., 2009; Lou et al., 2006), suggesting that adiponectin could be therapeutically beneficial for patients with osteopenia (Oshima et al., 2005). It is also suggested that higher adiponectin concentrations may cause increased osteoclastic activity and low BMD values (Luo et al., 2006). Adiponectin might be inversely related to FM and BMI in overweight and obese children (Cambuli et al., 2008), in amenorrheic physically active women (O'Donnell & De Souza, 2011) and in obese and non-obese adults (Arita et al., 1999;); to BMD in healthy and anorectic adolescents (Misra et al., 2007), pre- and postmenopausal women (Biver et al., 2011; Iida et al., 2011; Richards et al., 2007) and in elderly men (Basurto et al., 2009), while different studies have reported that adiponectin probably do not predict BMD among prepubertal girls (Rhie et al., 2010), female athletes (Gruodyté et al., 2010; Russell et al., 2009) and perimenoausal women (Kontogianni et al., 2004). Barbour et al. (2012) found that in women, higher adiponectin levels predicted greater hip whole body areal bone mineral density loss independent of age, race, BMI, diabetes and weight change. In another study, Donoso et al. (2010) found positive relationship between adiponectin and BMD in 10.5±1.4 yrs ballet dancers, with high circulating adiponectin levels and normal BMD, possibly because of intense exercise. Specifically, it was suggested that intense exercise increased adiponectin levels and this maintained a normal BMD in young ballet dancers. Recently, similar results were found in a group of amenorrheic physically active women (O`Donnell & De Souza, 2011).

Ghrelin is mainly secreted in the stomach (Kojima et al., 1999) and in smaller amounts from renal, pituitary, and hypothalamus cells (van der Lely et al., 2004). Ghrelin regulates a large array of endocrine and also non-endocrine functions, regulates food intake, energy balance, and has a control of adiposity (Tena-Sempere, 2005). Ghrelin levels increase with weight loss (Ackerman et al., 2012; Harada et al., 2008; Kojima et al., 2005; Misra et al., 2005b), and decrease with increasing age over childhood and from prepuberty to puberty

(Kletter et al., 2002; Whatmore et al., 2003). Ghrelin may also influence bone mineralization in children and adolescents (Pacifico et al., 2009), and also in women (Napoli et al., 2011). It has been found that ghrelin secretion predicted lumbar spine and hip BMD independently of body composition, growth hormone-insulin-like growth factor-I (GH-IGF-I) axis or sex steroids in adolescent girls (Misra et al., 2005b), while other studies have demonstrated that ghrelin concentration has no direct influence on the development of BMD values in adolescent female athletes (Jürimäe et al., 2010), in healthy postmenopausal women (Jürimäe et al., 2008) and also in middle-aged men (Oh et al., 2005). Studies have showed that ghrelin had a direct effect on BMD promoting bone resorption by osteoclasts (Costa et al., 2011), and osteoblast proliferation, differentiation and calcified accumulation (Fukushima et al., 2005; Kim et al., 2005; Maccarinelli et al., 2005). It is also found that ghrelin is negatively associated with bone age in adolescent boys (El-Eshmawy et al., 2010). Accordingly, to date, the reports on the relationship between circulating ghrelin concentration and bone mineralization remains contradictory.

In summary, there are several contradicting results about adipocytokines, ghrelin and bone mineral density in childhood and adulthood. Nevertheless, the possible relationships of leptin, adiponectin and ghrelin with the development of BMD remain questionable in a specific group of prepubertal rhythmic gymnasts. Since different adipocytokines are produced by adipose tissue, and FM is a strong determinant of BMD in healthy girls (El Hage et al., 2009), it could be suggested that adipocytokines and ghrelin may be related to bone development in prepubertal girls.

3. AIM AND PURPOSES OF THE STUDY

The general aim of the current thesis was to investigate possible associations between specific anthropometric, body composition and hormonal values with bone mineral parameters and the prediction of bone mineral development by baseline measured hormone and body composition variables during one year in prepubertal rhythmic gymnasts and untrained controls.

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

1. evaluate relationships of specific anthropometric (skinfolds, girths, lenghts and breadths) and body composition (fat mass and fat free mass) parameters with bone mineral (BMD and BMC) values in prepubertal rhythmic gymnasts and age-matched untrained controls (Study I);
2. determine relationships of adipocytokine (adiponectin and leptin) and ghrelin concentrations with BMD parameters in prepubertal rhythmic gymnasts and age-matched untrained controls (Study II);
3. investigate relationships between a normal increase in BMD values after 12-month study period with the baseline adipocytokine (adiponectin and leptin) and ghrelin concentrations and body composition (fat mass and fat free mass) parameters in prepubertal rhythmic gymnasts and age-matched untrained controls (Study III).

4. METHODS

4.1. Participants and experimental design

In total, 89 7–9-year-old prepubertal girls from different schools and sport clubs in Tartu (Estonia) participated in the first year cross-sectional study (Studies I and II). They were divided into rhythmic gymnasts (RG; n = 46) and untrained controls (UC, n = 43). In the 12-month prospective study (Study III), the number of participants was cut to 68 (RG = 33; UC = 35) due to the fact that some rhythmic gymnasts finished their gymnastic trainings, and some of the untrained controls started regular trainings in different sport disciplines (e.g., volleyball) during the 12-month study period (Figure 1).

All rhythmic gymnasts had trained usually 10–12 hrs per week (5–6 training sessions per week) for the past two years before starting the study. However, there were some easier periods in their trainings, when rhythmic gymnasts trained a mean six hours per week (three training sessions per week). All these rhythmic gymnasts were training in the same club and had very similar training lessons (ballet, acrobatics and rhythmic gymnastics trainings). Most training sessions lasted 2 hrs and consisted of a warm-up, routine training, and strength and stretching exercises. All rhythmic gymnasts were competing at the national level. Untrained controls had compulsory physical education classes 2–3 times a week at school. Participation only in physical education classes was inclusion criteria for untrained control subjects.

All participants were free from present or past diseases known to affect skeletal metabolism, and none of the girls were receiving medications known to affect bone. Throughout the study period, no restrictions were placed on dietary intake and participants consumed their usual everyday diet. All rhythmic gymnasts, untrained controls and their parents gave their written informed consent before entering the study. All prodecures were reviewed and approved by the Medical Ethics Committee of the University of Tartu (Estonia) and were explained to the children and their parents.

Participants were tested once a year at the same time during a 12-month study period. All testing at both times was completed during one visit to the laboratory. A venous blood sample was taken in the morning after an overnight fast. Anthropometric parameters were measured and a self-assessment of pubertal development was performed after a light breakfast. This was followed by the measurement of bone age by X-ray of the left hand. Finally, body composition and bone mineral assessments were performed by dual energy X-ray absorptiometry (DXA).

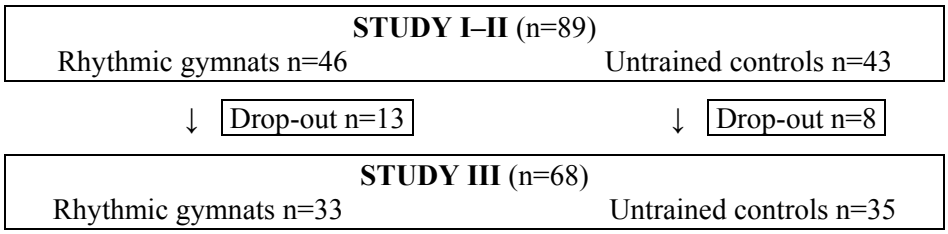

Figure 1. Tracking of the study population (7–9-year-old girls from different schools and one gymnastic club in Estonia).

4.2. Biological age

Biological age of the participants was assessed on the basis of self-assessment of breast and pubic hair stages using an illustrated questionnaire of pubertal stages according to the method of Tanner (1962). Pubertal development assessment according to the method of Tanner, which uses self-assessment of of breast and pubic hair stage in girls, has been previously validated (Leone & Comtois, 2007; Matsudo & Matsudo, 1994) and used in our previous laboratory studies with girls (Gruodyte et al., 2010; Jürimäe et al., 2007). The participants were given photographs, figures and descriptions of breast and pubic hair development stages, and asked to choose the one that most accurately reflected their appearance. In both measurement times, all girls were in Tanner stage 1. Bone age was assessed with an X-ray of the left hand and wrist, and determined according to the method of Greulish and Pyle (1959).

4.3. Anthropometric measurements

Body height was measured using a Martin metal anthropometer to the nearest 0.1 cm according to the standard technique, and body mass was measured with minimal clothing to the nearest 0.05 kg with a medical electronic scale (A&D Instruments, Ltd, Abingdon, UK). Body mass index (BMI; kg/m²) was calculated as body mass (kg) divided by square of body height (m²).

Anthropometric parameters were measured according to the protocol recommended by the International Society for Advancement of Kinanthropometry (ISAK) (Marfell-Jones et al., 2006). Nine skinfolds (*triceps, subscapular, biceps, iliac crest, supraspinale, abdominal, front thigh, medial calf* and *midaxilla*) were measured using Holtain (Crymmych, UK) skinfold caliper. Thirteen girths (*head, neck, arm relaxed, arm flexed and tensed, forearm, wrist, chest, waist, gluteal* [*hip*], *mid-thigh, thigh* [*mid trochanter-tibiale-laterale*], *calf and ankle*) and eight lengths (*acromiale-radiale, radiale-stylion, midstylion-dactylion, iliospinale height, trochanterion height, trochanterion-tibiale laterale, tibiale-laterale* height*, tibiale mediale-sphyrion tibiale*) and

eight breadths (*biacromial, biiliocristal*, *foot length, sitting height, transverse chest, anterior-posterior chest depth, biepicondylar humerus, biepicondylar femur*) were measured using the CENTURION KIT instrumentation (Rosscraft, Surrey, BC, Canada). All anthropometric measurements were performed by a well-trained anthropometrist (Level 1 ISAK anthropometrist) and the mean of three trials was used in the analysis.

4.4. Body composition and bone mineral parameters

Body composition (body fat %, FM, and FFM), and BMD (g/cm²) from whole body (WB), lumbar spine (LS; L2-L4) and femoral neck (FN) 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. Participants were scanned in light clothing while lying flat on their backs with arms on their sides. The fast scan mode and standard subject positioning were used for total body measurements, which were analyzed with the use of the extended analysis option. DXA measurements and results were evaluated by the same examiner. Coefficients of variations (CVs) for the DXA measurements were less than 2%.

4.5. Blood analysis

Venous blood samples were drawn in both years between 7:30 and 8:30 AM after an overnight fast from an antecubital vein with the participant sitting in the upright position. The plasma was separated and frozen at –20 ^{0}C for later analysis. Adiponectin was determined in duplicate via commercially available radio-immunoassay (RIA) kit (cat. No. HADP-61HK, Linco Research, St. Charles, MO, USA), the intra- and interassay CVs were <7%, and the least detection limit was 1 μg/ml. Leptin was determined in duplicate by RIA (Mediagnost GmbH, Reutlingen, Germany) and this assay has the intra- and inter-assay CV values less than 5%, and the least detection limit was 0.01 ng/ml. Ghrelin was also determined in duplicate using a commercially available RIA kit (Linco Research, St. Charles, MO, USA). The sensitivity of this kit was 93 pg/ml, and the intra- and inter-assay CVs were <10% and 14.7%, respectively.

4.6. Statistical analysis

Standard statistical methods were used to calculate the means (X) and standard deviations (±SD). Normality of parameters was controlled by the Kolmogorov-Smirnov test. Differences between groups were calculated using an independent t-test. Paired t-tests were performed to determine changes in measured variables over the 12-month study period. Comparisons between chronological age and

bone age were also performed using a paired t-test. The least significant change (LSC) for the measured BMD variables was also calculated (Leonard et al., 2009) resulting in an LSC of 3.1% at the measured sites. Correlation coefficients and stepwise multiple regression analysis were applied to examine relationships between measured variables. The level of significance was set at $p<0.05$ for all statistical analysis.

5. RESULTS

5.1. Relationships between anthropometric, body composition and bone mineral parameters

Mean physical characteristics in rhythmic gymnasts and untrained controls are presented in Table 1. There were not any significant differences ($p>0.05$) between groups in mean age, body height, body mass, BMI, FFM and WB BMC. Body fat % and FM were significantly lower ($p<0.05$) in rhythmic gymnasts compared with untrained controls. Bone mineral density at WB, LS and FN were significantly higher in rhythmic gymnasts (Table 1). All measured skinfold thicknesses were significantly thinner in rhythmic gymnasts (Table 2). Neck, forearm and thigh girths (Table 3), acromiale-radiale length (Table 4) and sitting height (Table 5) from measured breadth parameters were lower ($p<0.05$) in rhythmic gymnasts compared with untrained controls. All other measured girth, length and breadth values were not different ($p>0.05$) between studied groups.

Table 1. Mean (±SD) physical and blood biochemical characteristics in prepubertal rhythmic gymnasts (RG) and untrained controls (UC).

Variable	RG (n=46)	UC (n=43)
Age (yr)	8.0±0.6	8.2±0.6
Bone age (yr)	7.9±1.3	7.8±1.2
Height (cm)	130.2±5.2	129.6±5.4
Body mass (kg)	27.2±3.3	27.9±5.1
BMI (kg/m^2)	16.0±1.3	16.5±2.2
Body fat (%)	19.8±5.5	24.3±7.0#
Fat mass (kg)	5.2±2.0	6.7±2.9#
Fat free mass (kg)	20.4±2.0	19.8±2.4
Lumbar spine BMD (g/cm^2)	0.75±0.07	0.69±0.08#
Femoral neck BMD (g/cm^2)	0.78±0.07	0.71±0.07#
Whole body BMD (g/cm^2)	0.87±0.04	0.84±0.05#
Whole body BMC (kg)	1.0±0.1	1.0±0.2
Leptin (ng/ml)	2.4±1.7	4.1±2.5#
Adiponectin (μg/ml)	9.9±3.9	10.1±4.0
Ghrelin (pg/ml)	1435.9±502.1	1190.4±375.2#

Significantly different from RG ($p<0.05$)

All measured skinfolds (Table 2) and girths (Table 3) were not related ($p>0.05$) to WB and areal BMD values in rhythmic gymnasts. In addition, supraspinale and abdominal skinfolds (Table 2) and neck, arm (relaxed), forearm, wrist, chest, gluteal, thigh, mid-thigh and calf girths (Table 3) were related ($p<0.05$) to WB BMC in rhythmic gymnasts. In contrast, all measured skinfolds (Table 2)

and girths (Table 3) were significantly correlated ($p<0.05$) with WB BMD (except iliac crest skinfold), WB BMD, WB BMC and LS BMD values, while no relationship ($p>0.05$) was observed between measured skinfolds and girths with FN BMD in untrained controls.

No relationships ($p>0.05$) were observed between measured lengths (Table 4) and breadths (except transverse chest) (Table 5) with WB BMD in rhythmic gymnasts. In untrained controls, almost all measured lengths (Table 4) and breadths (Table 5) were related to WB BMD and WB BMC values. In addition, radiale-stylon, midstylion-dactylion, iliospinale height, trochanterion height, trochanterion-tibiale laterale, tibiale laterale height and tibiale mediale-sphyrion tibiale from lengths (Table 4) and biacromial, foot length, transverse chest and femur from breadths (Table 5) were related ($p<0.05$) to WB BMC in rhythmic gymnasts. While iliospinale height, trochanterion height and tibiale-laterale height from length parameters were related ($p<0.05$) to LS BMD in rhythmic gymnasts, all length values (except trochanterion-tibiale laterale) were related ($p<0.05$) to LS BMD in untrained controls (Table 4). Midstylion-dactylion, iliospinale height, trochanterion height, trochanterion-tibiale laterale and tibiale-laterale height from length values were related ($p<0.05$) to FN BMD in rhythmic gymnasts. No relationships ($p>0.05$) between measured length values and FN BMD were observed in untrained controls (Table 4). Only transverse chest and foot length from breadth values were related ($p<0.05$) to LS and FN BMD values in rhythmic gymnasts, respectively (Table 5). In comparison, all breadth values were related ($p<0.05$) to LS BMD, while no relationships ($p>0.05$) were observed between measured breadths and FN BMD in untrained controls (Table 5).

Only body mass and FM were related ($p<0.05$) to WB BMD, height was related ($p<0.05$) to LS BMD and chronological age was related ($p<0.05$) to FN BMD from the basic anthropometric and body composition parameters in rhythmic gymnasts (Table 6). All measured basic anthropometric and body composition parameters were related ($p<0.05$) to WB BMC in rhythmic gymnasts. In untrained controls, all measured basic anthropometric and body composition characteristics were related to WB BMD, WB BMC and LS BMD values, while no relationship was observed with FN BMD value (Table 6).

Table 2. Mean (X±SD) skinfold thicknesses and relationships with lumbar spine (LS), femoral neck (FN) and whole body (WB) bone mineral density (BMD) and bone mineral content (BMC) in rhythmic gymnasts (RG) and untrained controls (UC)

Variables	RG (n = 46)	UC (n = 43)	LS BMD		FN BMD		WB BMD		WB BMC	
			RG	UC	RG	UC	RG	UC	RG	UC
Triceps (cm)	9.4 ± 2.8	11.9 ± 3.5#	0.080	0.500*	0.156	0.096	0.073	0.429*	0.157	0.578*
Subscapular (cm)	5.6 ± 1.9	7.5 ± 3.0#	0.020	0.529*	0.013	–0.010	0.053	0.388*	0.075	0.563*
Biceps (cm)	6.4 ± 2.4	7.7 ± 2.9#	0.037	0.460*	0.111	0.057	0.082	0.330*	0.096	0.513*
Iliac crest (cm)	7.7 ± 2.6	11.1 ± 4.4#	0.181	0.431*	–0.059	0.064	0.189	0.299	0.277	0.501*
Supraspinale (cm)	4.9 ± 1.7	7.0 ± 3.1#	0.157	0.560*	–0.170	0.002	0.171	0.366*	0.380*	0.606*
Abdominal (cm)	7.0 ± 3.0	9.3 ± 4.6#	0.126	0.519*	–0.112	–0.021	0.062	0.325*	0.339*	0.597*
Front thigh (cm)	13.1 ± 3.6	17.1 ± 5.5#	–0.034	0.505*	0.108	–0.058	0.007	0.475*	0.091	0.638*
Medial calf (cm)	8.6 ± 2.7	10.9 ± 3.7#	0.093	0.533*	0.043	–0.136	0.157	0.538*	0.157	0.605*
Mid-axilla (cm)	4.6 ± 1.3	7.0 ± 2.9#	–0.005	0.470*	–0.099	0.109	0.033	0.321*	0.197	0.549*

Significantly different from RG, $p<0.05$; *Statistically significant correlation, $p<0.05$

Table 3. Mean (X±SD) girths and relationships with lumbar spine (LS), femoral neck (FN) and whole body (WB) bone mineral density (BMD) and bone mineral content (BMC) in rhythmic gymnasts (RG) and untrained controls (UC)

Variables	RG	UC	LS BMD		FN BMD		WB BMD		WB BMC	
	(n = 46)	(n = 43)	RG	UC	RG	UC	RG	UC	RG	UC
Head (cm)	52.3 ± 1.5	52.8 ± 2.2	0.164	0.503*	0.147	–0.085	0.135	0.616*	0.164	0.625*
Neck (cm)	26.3 ± 1.0	27.7 ± 1.9#	0.216	0.502*	0.184	0.090	0.167	0.488*	0.338*	0.491*
Arm (relaxed) (cm)	18.6 ± 1.3	19.4 ± 2.4	0.118	0.651*	0.044	0.243	0.046	0.595*	0.381*	0.722*
Arm (flexed) (cm)	19.8 ± 1.3	20.5 ± 2.4	0.077	0.668*	–0.012	0.197	–0.012	0.598*	0.278	0.720*
Forearm (cm)	18.3 ± 0.8	18.9 ± 1.5#	0.045	0.652*	–0.042	0.112	–0.002	0.601*	0.435*	0.750*
Wrist (cm)	12.7 ± 0.7	12.9 ± 1.0	–0.073	0.662*	0.087	0.100	–0.123	0.617*	0.303*	0.756*
Chest (cm)	61.2 ± 3.2	61.3 ± 9.1	0.248	0.389*	–0.135	0.124	0.144	0.376*	0.562*	0.316*
Waist (cm)	54.8 ± 3.4	55.8 ± 5.1	0.012	0.535*	0.053	–0.047	–0.094	0.437*	0.171	0.617*
Gluteal (hip) (cm)	66.7 ±3.5	68.7 ± 6.3	0.161	0.693*	–0.093	0.159	0.212	0.570*	0.514*	0.763*
Thigh (cm)	39.4 ± 2.7	40.9 ± 4.5#	–0.023	0.682*	0.015	0.147	0.033	0.561*	0.428*	0.747*
Mid-thigh (cm)	36.3 ± 2.0	36.7 ± 3.9	0.040	0.676*	0.031	0.094	0.024	0.576*	0.429*	0.777*
Calf (cm)	26.3 ± 1.6	26.6 ± 2.4	0.097	0.723*	0.086	0.072	–0.058	0.610*	0.340*	0.744*
Ankle (cm)	17.8 ± 1.1	18.3 ± 1.5	–0.140	0.557*	0.095	0.112	–0.164	0.443*	0.253	0.644*

Significantly different from RG, $p<0.05$; *Statistically significant correlation, $p<0.05$

Table 4. Mean (X±SD) lengths and relationships with lumbar spine (LS), femoral neck (FN) and whole body (WB) bone mineral density (BMD) and bone mineral content (BMC) in rhythmic gymnasts (RG) and untrained controls (UC)

Variables	RG (n = 46)	UC (n = 43)	LS BMD		FN BMD		WB BMD		WB BMC	
			RG	UC	RG	UC	RG	UC	RG	UC
Acromiale-radiale (cm)	22.5 ± 1.6	23.4 ± 1.6#	–0.199	0.407*	0.052	0.051	–0.186	0.322*	0.134	0.625*
Radiale-stylion (cm)	19.2 ± 1.2	18.9 ± 1.0	0.095	0.325*	–0.203	0.074	0.043	0.191	0.295*	0.441*
Midstylion-dactylion (cm)	14.1 ± 1.1	14.2 ± 0.9	0.233	0.487*	–0.427*	–0.012	0.029	0.494*	0.486*	0.696*
Iliospinale height (cm)	72.5 ± 4.9	72.5 ± 3.8	0.326*	0.496*	–0.383*	0.031	0.157	0.477*	0.610*	0.693*
Trochanterion height (cm)	65.4 ± 4.2	65.4 ±3.6	0.423*	0.452*	–0.420*	–0.021	0.089	0.276	0.557*	0.461*
Trochanterion-tibiale laterale (cm)	28.0 ± 2.4	28.7 ± 2.2	0.206	0.280	–0.448*	–0.066	0.172	0.442*	0.642*	0.702*
Tibiale-laterale height (cm)	35.9 ± 3.1	37.1 ± 2.3	0.315*	0.547*	–0.431*	–0.100	0.213	0.539*	0.608*	0.695*
Tibiale mediale-sphyrion tibiale (cm)	26.6 ± 1.9	26.6 ± 1.7	0.156	0.531*	–0.124	–0.086	0.159	0.425*	0.430*	0.673*

Significantly different from RG, $p<0.05$; *Statistically significant correlation, $p<0.05$

Table 5. Mean (X±SD) breadths and relationships with lumbar spine (LS), femoral neck (FN) and whole body (WB) bone mineral density (BMD) and bone mineral content (BMC) in rhythmic gymnasts (RG) and untrained controls (UC)

Variables	RG	UC	LS BMD		FN BMD		WB BMD		WB BMC	
	(n = 46)	(n = 43)	RG	UC	RG	UC	RG	UC	RG	UC
Biacromial (cm)	29.8 ± 1.5	29.2 ± 1.9	0.226	0.686*	0.281	0.105	0.249	0.552*	0.339*	0.729*
Biiliocristal (cm)	20.5 ±1.5	20.5 ± 1.2	0.050	0.601*	0.031	–0.053	–0.051	0.479*	0.152	0.708*
Foot length (cm)	20.2 ± 1.0	20.4 ± 1.1	0.220	0.560*	–0.326*	0.020	0.091	0.526*	0.569*	0.768*
Sitting height (cm)	64.5 ± 5.2	68.4 ± 2.9#	–0.037	0.601*	–0.086	0.024	0.067	0.477*	0.191	0.632*
Transverse chest (cm)	19.4 ± 0.9	19.3 ± 1.1	0.434*	0.657*	0.094	–0.072	0.295*	0.455*	0.569*	0.595*
Anterior-posterior chest depth (cm)	33.1 ± 0.9	33.2 ±0.9	0.009	0.490*	0.130	0.000	–0.039	0.433*	0.263	0.586*
Humerus (cm)	5.3 ± 0.3	5.2 ± 0.4	–0.009	0.439*	0.075	–0.108	–0.067	0.355*	0.155	0.582*
Femur (cm)	7.6 ± 0.3	7.7 ± 0.5	–0.014	0.385*	–0.088	–0.155	0.012	0.433*	0.347*	0.577*

Significantly different from RG, $p<0.05$; *Statistically significant correlation, $p<0.05$

Table 6. Correlation coefficients between bone mineral density (BMD) with measured body composition and blood biochemical characteristics in prepubertal rhythmic gymnasts (RG; n=46) and untrained controls (UC; n=43).

	LS BMD		FN BMD		WB BMD		WB BMC	
Variables	RG	UC	RG	UC	RG	UC	RG	UC
Age (yr)	0.093	0.375*	–0.373*	0.032	0.122	0.454*	0.289	0.476*
Bone age (yr)	0.131	0.541*	0.290	0.233	0.013	0.578*	0.459*	0.662*
Height (cm)	0.310*	0.599*	–0.287	0.014	0.264	0.535*	0.796*	0.789*
Body mass (kg)	0.202	0.721*	–0.190	0.044	0.343*	0.617*	0.801*	0.853*
BMI (kg/m²)	0.003	0.583*	0.013	0.078	0.242	0.487*	0.412*	0.646*
Body fat (%)	0.051	0.437*	–0.064	0.064	0.277	0.367*	0.360*	0.559*
Fat mass (kg)	0.115	0.584*	–0.119	0.050	0.337*	0.490*	0.541*	0.713*
Fat free mass (kg)	0.209	0.746*	–0.168	0.036	0.208	0.640*	0.754*	0.876*
Leptin (ng/ml)	–0.021	0.522*	–0.070	0.011	0.198	0.418*	0.275	0.563*
Adiponectin (μg/ml)	0.177	–0.128	–0.114	0.220	0.051	–0.145	0.058	–0.153
Ghrelin (pg/ml)	–0.020	–0.112	0.009	–0.384*	0.056	0.002	0.118	–0.178

* Statistically significant correlation, $p<0.05$; LS – lumbar spine; FN – femoral neck; WB – whole body; BMD – bone mineral density; BMC – bone mineral content

5.2. Adipocytokine and ghrelin levels in relation to bone mineral density

Rhythmic gymnasts presented significantly ($p<0.05$) lower and higher values for leptin and ghrelin concentrations, respectively, in comparison with untrained control subjects (Table 1). No differences ($p>0.05$) were observed for adiponectin levels between studied groups. Whole-body and LS BMD values were significantly correlated ($p<0.05$) with leptin concentration in untrained control subjects (Table 6). Femoral neck BMD was only related ($p<0.05$) to ghrelin concentration in untrained control subjects (Table 6). No significant correlations ($p>0.05$) were found between measured BMD values and adiponectin in rhythmic gymnasts and untrained controls (Table 6).

In rhythmic gymnasts, stepwise multiple regression analysis revealed a significant association only between WB BMD and FM, where FM explained 11.3% of the variability in WB BMD (Table 7). In untrained controls, FFM was the most significant predictor of WB and LS BMD, explaining 40.9% and 55.6% of the variability in WB and LS BMD values, respectively. When the influence of bone age, BMI, FM, and FFM was excluded, leptin concentration was the most important hormonal predictor of WB and LS BMD in the control group, explaining 17.5% in WB BMD and 27.2% in LS BMD (Table 7). In addition, stepwise multiple regressioon analysis demonstrated that ghrelin was the most significant predictor of FN BMD, explaining 14.7% of the variability in the untrained controls (Table 7).

Table 7. Results of stepwise multiple regression analysis with whole-body (WB), lumbar spine (LS) and femoral neck (FN) bone mineral density (BMD) as dependent variables and bone age, body mass index (BMI), fat mass (FM), fat free mass (FFM), leptin, adiponectin and ghrelin as independent variables (R^2 x 100; $p<0.05$) in rhythmic gymnasts (RG) and untrained controls (UC).

	Independent variables	RG	UC
LS BMD	FFM	–	55.6
	Leptin*	–	27.2
FN BMD	Ghrelin	–	14.7
WB BMD	FM	11.3	–
	FFM	–	40.9
	Leptin*	–	17.5

* After excluding the influence of bone age, BMI, FM and FFM

5.3. Changes in bone mineral density values after 12-month period in relation to baseline body composition, and adipocytokine and ghrelin concentrations

Mean age, bone age, height (+ 4.8%), body mass (+ 8.5%), body fat % (+ 5.4%), FM (+ 5.8%), FFM (+ 9.8%), WB BMD (+ 3.4%), LS BMD

(+ 6.7%), FN BMD (+ 6.4%) and adiponectin (+ 14.1%) were significantly increased ($p<0.05$), while ghrelin (- 17.2%) was significantly decreased ($p<0.05$) after 12-month study period in rhythmic gymnasts (Table 8). No changes ($p>0.05$) in BMI and leptin were observed over 12-month study period in rhythmic gymnasts. In untrained controls, significant increases ($p<0.05$) in mean age, bone age, height (+ 4.9%), body mass (+ 12.9%), BMI (+ 2.4%), FM (+ 19.6%), FFM (+ 11.1%), WB BMD (+ 3.6%), LS BMD (+ 7.2%), FN BMD (+ 5.6%), leptin (+ 22.0%) and adiponectin (+ 17.8%) were observed. In addition, significant decreases ($p<0.05$) in ghrelin (- 30.3%) occurred over 12-month study period in untrained controls (Table 8). Therefore, the increases in bone age over 12-month study period were significantly lower ($p<0.05$) in rhythmic gymnasts compared with untrained controls, while the increases in LS BMD over 12-month study period were significantly higher ($p<0.05$) in rhythmic gymnasts when compared with untrained controls. Changes in all other measured variables over 12-month study period were not significantly different ($p>0.05$) between studied groups. In addition, the increases in measured BMD variables in both groups were higher with respect to the calculated LSC of 3.1%.

Changes in WB and LS BMD values were significantly related ($p<0.05$) to baseline age, while changes in FN BMD were significantly related to baseline height and body mass values in rhythmic gymnasts (Table 9). All other relationships between changes in BMD values with measured body composition and blood biochemical variables were not significant ($p>0.05$) in rhythmic gymnasts. In addition, stepwise multiple regression analysis revealed that measured baseline independent variables did not predict ($p>0.05$) increases in BMD values over 12-month study period in rhythmic gymnasts. Increases in WB BMD were significantly correlated ($p<0.05$) with baseline height, body mass, BMI, body fat percent, FM, FFM, leptin and ghrelin values in untrained controls (Table 9). No significant correlations were observed between increases in LS BMD and measured baseline body composition and blood biochemical variables, while increases in FN BMD were related to baseline height, body mass, FFM and adiponectin values in untrained controls (Table 9). In untrained controls, stepwise multiple regression analysis demonstrated that baseline FM and FFM values together were the most significant predictors of ΔWB BMD and ΔFN BMD values, explaining 25.2% and 15.7% of the variability in ΔWB BMD and ΔFN BMD values, respectively. When the influence of baseline bone age, BMI, FM and FFM was excluded, baseline ghrelin and adiponectin concentrations were the most important hormonal predictors of ΔWB BMD and ΔFN BMD values in the untrained control group, explaining 15.5% in ΔWB BMD and 15.3% in ΔFN BMD values, respectively. In addition, stepwise regression analysis demonstrated no association ($p>0.05$) between ΔLS BMD and measured baseline independent variables in untrained controls.

Table 8. Mean (X±SD) of subject characteristics before and after a 12-month study period. p shows the significance before and after a study period in rhythmic gymnasts (RG) and untrained controls (UC).

	RG (n=33)			UC (n=35)		
	Before	After	*p* value	Before	After	*p* value
Age (y)	8.0±0.6	9.0±0.6	0.001	8.2 ± 0.6	9.3±0.5	0.001
Bone age (y)	7.9±1.4	8.6±1.5#	0.001	8.0 ± 1.1	9.3±0.8	0.001
Height (cm)	130.2±5.1	136.4±6.5	0.001	129.9 ± 5.8	135.9±6.4	0.001
Body mass (kg)	27.2±3.3	29.5±3.3	0.001	28.1 ± 5.3	31.5±6.3	0.001
Body mass index (kg/m²)	15.7±1.1	15.9±1.3	0.490	16.6 ± 2.2	16.9±2.4	0.030
Fat mass (kg)	4.7±1.6	5.5±1.7	0.001	6.7 ± 2.90	8.0±3.7	0.001
Fat free mass (kg)	19.9±4.0	22.4±2.2	0.001	19.9 ± 2.5	22.0±3.1	0.001
Body fat (%)	18.4±4.5	19.4±4.5	0.001	24.1±7.1	25.5±7.9	0.001
LS BMD (g/cm²)	0.74±0.07	0.79±0.08	0.001	0.70 ± 0.09	0.74±0.09	0.001
FN BMD (g/cm²)	0.78±0.07	0.83±0.07	0.029	0.71 ± 0.08	0.75±0.08	0.083
WB BMD (g/cm²)	0.87±0.04	0.90±0.04	0.001	0.85 ± 0.05	0.87±0.06	0.001
Leptin (ng/ml)	2.0±1.3	2.3±1.5	0.125	4.2 ± 2.6	5.0±3.3	0.038
Adponectin (µg/ml)	9.8±4.0	11.3±4.2	0.002	10.4 ± 4.3	11.9±3.5	0.017
Ghrelin (pg/ml)	1404.0±494.6	1189.3±387.3	0.001	1172.0±341.0	829.0±385.8	0.001

Significantly different from the age ($p<0.05$); LS – lumbar spine; FN – femoral neck; WB – whole body; BMD – bone mineral density

Table 9. Pearson correlation coefficients of change (Δ scores) in bone mineral density (BMD) values during 12-month study period with baseline body composition and blood biochemical variables

	RG (n=33)			UC (n=35)		
	Δ WB BMD (g/cm²)	Δ LS BMD (g/cm²)	Δ FN BMD (g/cm²)	Δ WB BMD (g/cm²)	Δ LS BMD (g/cm²)	Δ FN BMD (g/cm²)
Age (y)	0.369*	0.402*	0.242	–0.006	0.131	0.315
Bone age (y)	0.126	–0.069	0.234	0.103	–0.060	0.234
Body height (cm)	0.138	0.118	0.434*	0.367*	0.036	0.340*
Body mass (kg)	0.080	0.000	0.361*	0.490*	–0.075	0.339*
Body mass index (kg/m²)	–0.005	–0.126	0.086	0.475*	–0.153	0.207
Fat mass (kg)	0.126	0.217	0.220	0.502*	–0.097	0.235
Fat free mass (kg)	0.058	–0.156	0.238	0.467*	–0.071	0.396*
Body fat (%)	0.153	0.275	0.130	0.483*	–0.108	0.111
Leptin (ng/ml)	–0.078	0.211	0.172	0.352*	–0.150	0.219
Adiponectin (μg/ml)	0.319	0.294	–0.008	0.029	–0.0084	–0.392*
Ghrelin (pg/ml)	–0.040	–0.033	–0.208	–0.394*	0.114	0.284

* Statistically significant correlation, $p<0.05$; RG – rhythmic gymnasts; UC – untrained controls; LS – lumbar spine; FN – femoral neck; WB – whole body

6. DISCUSSION

6.1. Relationships between anthropometric, body composition and bone mineral parameters in prepubertal rhythmic gymnasts compared with untrained controls

One of the main finding was that relatively young prepubertal rhythmic gymnasts have similar values with untrained controls in body height, body mass, BMI and FFM. However, body fat percentage and FM were significantly lower and BMD in LS, FN and WB were significantly higher in rhythmic gymnasts than in untrained controls. Skinfold thicknesses were thicker in untrained controls. On the girths, lengths and breadths there were very few significant differences between rhythmic gymnasts and untrained controls. Lower body anthropometrical parameters were sensitive to LS BMD in untrained controls. Similar relationships were absent in rhythmic gymnasts.

Rhythmic gymnasts are usually taller and thinner than untrained controls (Cacciari et al., 2002; Claessens et al., 1992). In our study, there were no differences in body height and body mass values between prepubertal rhythmic gymnasts and untrained controls. However, our rhythmic gymnasts had significantly lower FM and body fat percentage (see Table 1). Similarly, Courteix et al. (2007) also found significantly lower FM among rhythmic gymnasts and there were no significant differences between FFM compared to untrained controls in their study. Thus, it appears that already prepubertal rhythmic gymnasts have lower values in body FM, while there are no differences in body mass compared to untrained controls.

There is little information available about the specific anthropometric parameters in relatively young prepubertal rhythmic gymnasts. Surprisingly, there were not many significant differences in measured anthropometric parameters except skinfold thicknesses between prepubertal rhythmic gymnasts and age-matched untrained controls. Therefore, all skinfold thicknesses were thinner in rhythmic gymnasts compared with untrained controls. This is understandable as rhythmic gymnasts had also lower FM and fat percentage compared to untrained controls.

Di Cagno et al. (2008) compared elite rhythmic gymnasts to sub-elite rhythmic gymnasts (age 14.7 ± 2.2 yrs) and found longer leg length and sitting height in elite rhythmic gymnasts. However, in our study rhythmic gymnasts had significantly lower values in sitting height compared to untrained controls and there were no differences in leg length between the studied groups. All these different results in this study compared to Di Cagno et al. (2008) might be caused by all the fact that girls in our study were very young and the differences in anthropometric parameters, for example in leg length, body height and body mass, might occur in later life.

Anthropometric parameters correlated significantly with LS and WB BMD (but not with FN BMD) mostly in untrained controls but not in rhythmic

gymnasts. Interestingly, some lower body girths, lengths and breadths were critical parameters influencing LS BMD in untrained controls. To our best of knowledge, these are the first results indicating that especially lower body anthropometrical parameters are sensitive to the LS BMD development in prepubertal untrained girls. In rhythmic gymnasts with high LS and FN BMD values, specific anthrpometric parameters mostly did not correlate with measured BMD values.

In our study, rhythmic gymnasts had significantly higher BMD values in LS, FN and WB compared to untrained controls (see Table 1). Courteix et al. (2007) also found higher BMD values in the WB and LS in rhythmic gymnasts. Our results confirm the finding that rhythmic gymnastics as a high-impact activity has a strong impact on BMD development (Dowthwaite et al., 2006; Laing et al., 2005; Ward et al., 2005) and rhythmic gymnastics has therefore similar effect on bone development like artistic gymnastics (Dowthwaite et al. 2006). On the other hand, there is one study reporting that artistic gymnastics had higher osteogenic stimulus than rhythmic gymnastics (Vicente-Rodriguez et al., 2007). To our best of knowledge, no study has yet reported morphological differences in 7–9-year-old rhythmic gymnasts compared with untrained controls.

Impact-loading sport can increase BMD in the stressed sites of the skeleton (Courteix et al., 1999) and the correlation between body composition and BMD values seems to be also site-specific (Taaffe et al., 2000; Van Langendonck et al., 2004). Our results confirmed these findings. Besides the site-specific effect, body composition parameters seem to be more related to BMD values in untrained controls than rhythmic gymnasts. Total body mass appears to be a good predictor for bone mineral accrual in children (Lima et al., 2001).

Higher FM promotes higher BMD through the weight bearing activity (Hsu et al., 2006). In rhythmic gymnasts, changes in body fat are related to changes in BMD (Courteix et al., 1999). In our study, rhythmic gymnasts had significantly lower values for FM and body fat percentage compared with untrained controls, while rhythmic gymnasts had significantly higher values for BMD in all measured sides compared to the results in untrained controls. Untrained control subjects had higher values in FM and they also had more significant correlations with measured BMD values. These results show significant relationships between FM and BMD values in untrained controls. However, it can be suggested that rhythmic gymnasts have higher values in BMD because of the influence of the specific gymnastics training.

There were no significant differences in FFM between rhythmic gymnasts and untrained controls (see Table 1). However, differences emerged in the relationships between FFM and BMD between the two studied groups. In untrained control group, FFM correlated significantly with WB BMD. Unlike WB BMD value, WB BMC was significantly correlated with FFM in rhythmic gymnasts (see Table 6). There was also a significant relationship between FFM and WB BMC in untrained controls. In previous studies, change in FFM has been strongly correlated with the change in WB BMC during linear growth

(Lima et al., 2001; Vicente-Rodriguez et al., 2007; Young et al., 2001) and FFM appears to be best predictor for WB BMC development (Vicente-Rodriguez et al., 2007). The muscle-bone relationship during linear growth is explained by the indirect osteogenetic effect theory – bigger muscles exert higher tensile forces on the growing bones they attach (Rauch et al., 2002), and by direct osteogenetic effect theory – exercise stimulate both muscle and bone development (Vicente-Rodriguez, 2006).

It was surprising that at least two years of serious training with high energy expenditure did not cause any delay in bone age maturation in relatively young rhythmic gymnasts (mean chronological age 8.0 years). This finding differs from the study by Maimoun et al. (2010a) in which prepubertal rhythmic gymnasts at the mean age of 11.5 years had a significant delay in bone age (mean 9.9 years). In a longitudinal study, Theintz et al. (1992) reported that bone development in sedentary girls was particularly substantial after chronological age of 11 years at the skeletal sites of LS and FN, whereas the increment fell dramatically after age 16 or two years after menarche. In addition, bone age has been found to be delayed for 2 years in adolescent rhythmic gymnasts at the average age of 16.2 years (Munoz et al., 2004). A 1-year follow-up study in peripubertal female rhythmic gymnasts who received intensive training showed a significant increase in BMD at all bone sites throughout puberty, but the maximal gain was around Tanner stage III (Maimoun et al., 2010a). Therefore, it is very likely that our girls were too young (Tanner stage I) and in too narrow age range to see any relationships between BMD and chronological age or bone age in the first study-year. It is interesting to note that chronological age and bone age were both significantly related to WB and LS BMD values only in untrained controls but not in rhythmic gymnasts (see Table 6). These results together suggest that bone age is not delayed in 8-year-old girls despite increased physical activity levels for at least two years. Therefore, the possible delay in bone age in rhythmic gymnasts may occur later, just before puberty. However, we also agree that the narrow age range of the participants and the probably homogeneous group of prepubertal rhythmic gymnasts may affect our results. In addition, other factors that were not studied might also affect the rhythmic gymnasts results, namely, intensity of physical activity, amount of nutrition, and intake of vitamin D and minerals such as calcium and phosphate.

In summary, the results of our study show that the relationships between anthropometry, body composition and bone mineral parameters are mostly absent in prepubertal rhythmic gymnasts.

6.2. Adipocytokine and ghrelin levels in relation to bone mineral density in prepubertal rhythmic gymnasts compared with untrained controls

In this study, possible differences in the relationships between adipocytokines, ghrelin, and BMD values in prepubertal rhythmic gymnasts and untrained control subjects were assessed. It was found that adipocytokine and ghrelin levels were not significantly correlated with measured WB and areal BMD parameters in female prepubertal rhythmic gymnasts. In contrast, leptin and ghrelin were related to measured BMD values in untrained control subjects. It appears that despite lower leptin and FM values, prepubertal rhythmic gymnasts have higher BMD parameters.

Leptin concentrations in our 8-year-old rhythmic gymnasts were similar to the results reported in previous studies with older prepubertal rhythmic gymnasts (Maimoun et al., 2010b). However, leptin values in prepubertal rhythmic gymnasts were lower in comparison with rhythmic gymnasts who have already reached puberty (Courteix et al., 2007; Gruodyté et al., 2010; Munoz et al., 2004). In addition, leptin levels in these prepubertal rhythmic gymnasts were significantly lower compared to the results obtained in age-matched untrained control subjects (see Table 1). The mean lepin concentration in our untrained controls was similar to a recent study in normal weight girls at the same age (Rhie et al., 2010). The independent relationship between leptin concentration and WB and LS BMD values was observed only in untrained prepubertal girls. Other studies have also reported an independent effect of leptin on LS (Garnett et al., 2004) and FN (Rhie et al., 2010) BMD values in untrained prepubertal children. In contrast, Roemmich et al. (2003) did not find significant relationships between leptin concentrations and WB BMD and areal BMD values in children and adolescents. A recent study by Gruodyté et al. (2010) found significant relationships between leptin levels and FN BMD and also LS BMD in a group of 13- to 15-year-old adolescent rhythmic gymnasts, whereas Maimoun et al. (2010b) did not find leptin as an independent predictor of BMD in elite female rhythmic gymnasts at different pubertal stages (age range 10.6–17.2 years). Although some studies have provided evidence of leptin and bone interaction in humans (Kaufman et al., 2002; Thomas et al., 1999), the impact of leptin on growing human bone remains controversial (Maimoun et al., 2010b). Accordingly, no relationship between leptin concentration and measured WB and areal BMD values was observed in prepubertal rhythmic gymnasts. In a current study, the high training volume in a rhythmic gymnasts group that caused higher energy expenditure may have influenced the relationships between measured adipocytokines and bone mineral values. Courteix et al. (2007) found that leptin concentrations in adolescent rhythmic gymnasts were as low as those observed in anorectic subjects, while the measured BMD values were greater in rhythmic gymnasts than in untrained controls, concluding that physical activity counterbalanced the negative effect

that low FM and lepin deficiency has on bone. Physical training has been shown to have a direct effect on bone macro- and microarchitecture (Courteix et al., 2007). The other reason might be that our rhythmic gymnasts were too young and in a very narrow age range to show the variability in leptin levels that may influence the relationship between the hormone and BMD.

It has been reported that adiponectin promotes osteogenesis, increasing osteogenic markers and augmenting osteoblast differentiation (Lee et al., 2009), suggesting that adiponectin may link bone and fat metabolism (Blüher et al., 2007). In accordance, our previous study revealed adiponectin to be an independent predictor of BMD in healthy females (Jürimäe & Jürimäe, 2007). Misra et al. (2007) demonstrated that adiponectin was a significant and independent contributor of WB and areal BMD values in healthy adolescent girls compared to girls with anorexia nervosa. Similarly to the recent study by Rhie et al. (2010) in prepubertal children, we did not find any relationship between adiponectin and measured BMD values in the two studied groups. In addition, adiponectin concentrations were not different among prepubertal rhythmic gymnasts and untrained control groups (see Table 1). It has been suggested that chronic exercise causes increases in adiponectin concentration in adults (Jürimäe et al., 2011). Taken together, the results of the present study suggest that in this young age group of girls adiponectin level is not directly related to bone mineral parameters and is not influenced by the physical activity pattern.

Ghrelin directly promotes bone formation and increases BMD in rats (Fukushima et al., 2005), but in humans these relationships are contradictory. In this study, ghrelin was not related to measured BMD values in prepubertal rhythmic gymnasts, whereas an independent relationship was observed between ghrelin concentration and FN BMD in untrained control subjects. These results are similar to those of Makovey et al. (2007), who found also an inverse relationship between ghrelin and BMD, which was statistically significant only for total hip after adjustment for FM, body size, and lifestyle factors. Accordingly, the authors suggested that there is no evidence that ghrelin plays a role in human bone metabolism. It was suggested, however, that the weight-bearing nature of the hip site might partly explain the relationship between FN BMD and ghrelin (Makovey et al., 2007). In contrast, no relationships between circulating ghrelin and BMD values have been observed in adolescent athletes with high energy expenditure values (Jürimäe et al., 2010). It has been shown that ghrelin has proliferative effects on osteoblasts in cell culture (Maccarinelli et al., 2005). However, of interest, Maccarinelli et al. (2005) reported that the ghrelin effect on osteoblast proliferation was dose dependent: osteoblast proliferation was seen only with lower doses of ghrelin, whereas no stimulating effect was seen in higher ghrelin doses. Furthermore, Misra et al. (2005b) speculated that very high ghrelin levels may even indirectly decrease osteoblast proliferation. In accordance with other studies (Jürimäe et al., 2007), our prepubertal rhythmic gymnasts presented significantly higher ghrelin concentrations in comparison with untrained controls (see Table 1). The relatively

high ghrelin levels caused by elevated energy expenditure observed in our prepubertal rhythmic gymnasts may explain why we did not find a relationship between ghrelin and measured BMD values. In accordance with these results, high ghrelin concentrations in girls with anorexia nervosa and adolescent athletes with amenorrhea had no direct influence on bone tissue (Misra, 2008). Taken together, further longitudinal studies are necessary to clarify the role of ghrelin in bone metabolism in girls with different physical activity patterns.

In conclusion, no significant relationships were found between leptin, adiponectin, and ghrelin concentrations and BMD parameters in prepubertal rhythmic gymnasts. In untrained control subjects, leptin correlated with WB and LS BMD values, whereas ghrelin was related to FN BMD. Prepubertal rhythmic gymnasts had greater measured bone parameters despite of lower FM values in comparison with age-matched untrained controls.

6.3. Changes in bone mineral density values after 12-month period in relation to baseline body composition, and adipocytokine and ghrelin concentrations in rhythmic gymnasts compared with untrained controls

The relationships between increased BMD values with baseline leptin, adiponectin and ghrelin concentrations were studied in two different groups of prepubertal girls. 12-month prospective study demonstrated significant changes in measured adipocytokine, ghrelin and body composition variables in untrained controls, while no significant increases in leptin concentrations were found in rhythmic gymnasts over 12-month study period. Therefore, the increases in measured BMD variables were higher with respect to the calculated LSC, indicating normal growth of BMD values in both groups of prepubertal girls over 12-month study period. In addition, while increases in adiponectin and body composition variables, and decreases in ghrelin were not significantly different ($p>0.05$) between studied groups, the increases in bone age over 12-month study period were significantly lower ($p<0.05$) in rhythmic gymnasts when compared with untrained controls, the increases in LS BMD over 12-month study period were significantly higher ($p<0.05$) in rhythmic gymnasts when compared with untrained controls. It appeared that initial body composition variables together with measured adipocytokine and ghrelin levels did not predict a normal growth in BMD values in prepubertal rhythmic gymnasts. In contrast, it appeared that initial leptin and ghrelin concentrations together with specific body composition variables were associated with an increase in WB BMD value as a result of the 12-month observation period in untrained prepubertal girls. However, initial adiponectin concentration together with FFM was a significant predictor of an increase in FN BMD in control subjects. These results suggest that due to mechanical loading, prepubertal rhythmic gymnasts may have a beneficial effect on bone mineralization and

may have counterbalanced the negative factors on bone development such as low FM and leptin concentrations.

One of the main findings of the present investigation was that regular high-impact weight-bearing athletic activity promoted significant annual gains in bone mineral parameters in relatively young prepubertal rhythmic gymnasts (see Table 8). The annual gain in BMD values in rhythmic gymnasts was close to the results of Courteix et al. (1999), who found annual change rates of 0.04, 0.03 and 0.03 g/cm^2/y at the WB, LS and FN BMD values, respectively, in prepubertal rhythmic gymnasts compared to 0.03, 0.05 and 0.05 g/cm^2/y for our subjects at the same skeletal sites. However, prepubertal girls in Courteix et al. (1999) study were two years older (mean chronological age 10 yrs) compared to our rhythmic gymnasts (mean chronological age 8 yrs) and had trained for 12–15 hrs per week three years before starting the study. Similarly to previous study (Courteix et al., 1999), the measured BMD values were greater at all skeletal sites in our rhythmic gymnasts at the start of the study and one year later, and the annual gain in LS BMD area was significantly higher ($p<0.05$) in rhythmic gymnasts compared to the untrained controls. Interestingly, the results of present study demonstrated that bone age (mean bone age 8.6 yrs) was significantly lower ($p<0.05$) from chronological age after a 12-month study period in our 9-year-old rhythmic gymnasts (see Table 8). In comparison, a recent cross-sectional study by Maimoun et al. (2010a) suggested that pre-pubertal rhythmic gymnasts at the mean chronological age of 11.5 years had a significant delay in bone age (mean bone age 9.9 yrs), while Courteix et al. (1999) found only a trend towards a lower bone age after 12-month study period in highly trained prepubertal rhythmic gymnasts at the mean chronological age of 11.6 years (mean bone age 11.0 yrs). Georgopoulos et al. (2010) indicated that prepubertal stage is prolonged and pubertal development is shifted to a later age following the bone maturation rather than the chronological age in elite rhythmic gymnasts. Bone age has been reported to be delayed for two years in rhythmic gymnasts at the average chronological age of 16.2 years (Munoz et al., 2004). These results together suggest that since prepubertal rhythmic gymnasts present significant annual gains in BMD and higher BMD values in comparison with same chronological age untrained controls, they may also maintain higher bone mineral values although bone age and biological maturation have been delayed.

Another main finding of the present investigation was that leptin concentration was not significantly increased in prepubertal rhythmic gymnasts, while leptin was significantly increased in untrained control subjects over 12-month study period (see Table 8). The mean leptin values in our untrained control subjects were similar to previous results in normal weight girls at the similar age (Rhie et al., 2010). It has been reported that circulating leptin concentrations start to rise in the prepubertal period from five years and continue to progressively rise throughout puberty in healthy physically inactive girls (Blum et al., 1997; Garcia-Mayor et al., 1997; Mann et al., 2003). While

no significant increases in leptin concentrations were found in our rhythmic gymnasts over 12-month study period from eight to nine years of age, Maimoun et al. (2010b) reported that leptin concentrations rise in parallel with the increase in FM in highly trained rhythmic gymnasts even with a reduced amount of adipose tissue progressing from prepuberty to puberty. These results together suggest that the specific physical activity pattern seen in our rhythmic gymnasts during earlier prepubertal years may have counterbalanced the age-dependent increase in circulating leptin concentrations and the increase in leptin levels could be seen at the onset of puberty in a specific group of young rhythmic gymnasts.

Baseline leptin concentrations did not predict increases in measured BMD values over 12-month study period in rhythmic gymnasts, while leptin was significantly correlated with ΔWB BMD in untrained controls. In accordance with these results, our previous cross-sectional study found positive correlations between leptin levels and WB and LS BMD only in prepubertal untrained controls but not in rhythmic gymnasts (see Table 6). While other investigations have reported independent relationships betweeen leptin and measured BMD variables in untrained prepuburtal children (Garnett et al., 2004; Rhie et al., 2010), recent studies did not find circulating leptin as an independent predictor of BMD in a group of elite female rhythmic gymnasts at different pubertal stages (Maimoun et al., 2010a,b). However, there are studies to report significant relationships between leptin and BMD values in adolescent rhythmic gymnasts (Gruodyté et al., 2010; Munoz et al., 2004). Although leptin has been reported to be involved in the accumulation, maintenance and loss of BMD throughout life (Hamrick & Ferrari, 2008), the impact of leptin on growing human bone remains controversial (Maimoun et al., 2010b). Accordingly, the results of present study suggest that leptin is not longitudinally involved in the development of BMD in a specific group of rhythmic gymnasts before puberty. It appears that specific physical activity pattern seen in rhythmic gymnasts may have counterbalanced the possible negative effect that leptin deficiency may have on growing bone.

Similarly to previous studies, adiponectin increased (Cambuli et al., 2008) and ghrelin decreased (Whatmore et al., 2003) over 12-month study period in both groups of prepubertal girls (see Table 8). Baseline adiponectin and ghrelin levels did not predict significant increases in BMD values in prepubertal rhythmic gymnasts, while baseline adiponectin was inversely correlated with ΔFN BMD in untrained controls. However, the independent variables that were associated with increases in BMD values were baseline FM and FFM values together in untrained controls and only after excluding the influence of body composition parameters, ghrelin and adiponectin concentrations predicted increases in WB BMD and FN BMD values in untrained controls, respectively. These results demonstrate that baseline adiponectin and ghrelin concentrations may not have a direct role in the development of BMD variables prepubertal girls. In accordance with these results, previous studies have also find no

relationship between adiponectin (Russell et al., 2009) and ghrelin (Jürimäe et al., 2010) with BMD variables in physically active prepubertal children. Although some studies have reported significant correlations of BMD variables with adiponectin (Huang et al., 2004; Misra et al., 2007) and ghrelin (Makovey et al., 2007; Misra et al., 2005b), the results of current study suggest that during prepubertal development, adiponectin and ghrelin are not independent predictors of increases in BMD levels in contrast to FM and FFM values in prepubertal girls with different physical activity patterns. Taken together, further longitudinal studies from prepuberty to pubertal maturation are necessary to clarify the possible roles of adiponectin and ghrelin in the bone development in girls with different physical activity parameters.

In conclusion, body composition variables together with measured adipocytokine and ghrelin levels did not predict a normal growth in BMD values in rhythmic gymnasts but in untrained controls it appeared that initial leptin together with specific body composition variables were associated with an increase in WB BMD value as a result of the 12-month study period.These results suggest that specific physical activity pattern seen in rhythmic prepubertal gymnasts may have a beneficial effect on bone mineralization and may have counterbalanced the negative factors on bone development such as low FM and decreased leptin concentrations.

7. CONCLUSIONS

1. Body FM was the most important parameter to predict whole-body BMD in prepubertal rhythmic gymnasts. Relationships of lumbar spine and femoral neck BMD values with measured body composition, and skinfold, girth, length and breadth indices were mostly absent in prepubertal rhythmic gymasts. In untrained controls, FFM was the most important parameter to predict whole-body and lumbar spine BMD values. Lumbar spine BMD was also related to measured skinfold, girth, length and breadth indices, while no relationship was seen between femoral neck BMD with measured body composition and anthropometric chracteristics in untrained controls.
2. Leptin, adiponectin and ghrelin concentrations were not related to measured BMD parameters in prepubertal rhythmic gymnasts. In untrained controls, leptin was correlated with whole-body and lumbar spine BMD values, whereas ghrelin was associated with femoral neck BMD value.
3. Baseline adipocytokine and ghrelin levels together with baseline body composition variables did not predict a normal growth in BMD values as a result of 12-month study period in prepubertal rhythmic gymnasts. In untrained controls, initial leptin together with specific body composition variables were associated with an increase in whole-body BMD value after 12-month prospective study period.

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SUMMARY IN ESTONIAN

Prepuberteediealiste iluvõimlejate luutihedus: seosed antropomeetriliste, keha koostise ja hormonaalsete parameetritega

Sissejuhatus

Osteoporoos on tõsine ülemaailmne terviseprobleem. On leitud, et parem luutihedus lapsena võib kanduda täiskasvanuikka ja nii on tulevikus suurem võimalus ennetada osteoporoosi. Lisaks geneetilistele faktorite ja toitumisele mõjutavad laste luutihedust kehamass, kehaline koormus, endokriinsüsteem ja puberteedi algusaeg. Kehamass mõjutab luutihedust mehaanilise koormuse ja hormoonide (sh leptiin, adiponektiin, greliin) vahendusel. Mainitud hormoonid mõjutavad luud aga läbi kehakoostise ja samuti kesknärvisüsteemi vahendusel. Viimastel andmetel peetakse lastel rasvavabamassi oluliseks luu mineraaltiheduse tõstjaks, samas on leitud, et rasvamass võib luutihedusele mõjuda nii positiivselt kui ka negatiivselt. Selgelt mõjub luu mineraaltihedusele positiivselt aga regulaarne ning intensiivne hüppeline ehk löögiline kehaline koormus. Iluvõimlemine on kindlasti löögilise spordiala esindajaks, kuna ühel treeningul võib iluvõimleja sooritada isegi kuni 400 maksimaalse pingutusega hüpet. Iluvõimlemisega alustatakse juba 3–4 aastaselt, treeningud on suhteliselt varakult intensiivsed ja mahukad langetades oluliselt võimlejate keha- ning rasvamassi. Kuigi puberteediealiste sportlaste luutiheduse seoseid mainitud faktoritega on korduvalt uuritud, on siiani vähem informatsiooni, kuidas mõjutab intensiivne kehaline koormus prepuberteediealiste noorsportlaste luutihedust, kelle kehamass on vähemaktiivsete eakaaslastega võrreldes tunduvalt madalam.

Uurimustöö eesmärk ja ülesanded:

Antud töö eesmärgiks oli hinnata prepuberteediealiste iluvõimlejate luutiheduse seoseid kehakoostise ja hormoonidega (leptiin, adiponektiin, greliin). Lähtuvalt eesmärgist olid ülesanneteks:

1. hinnata luutiheduse seoseid antropomeetriliste ja kehakoostise näitajatega;
2. hinnata luutiheduse seoseid adipotsütokiinide ja greliini kontsentratsiooniga;
3. hinnata, kuidas on aastane luutiheduse kasv seotud kehakoostise ja hormonaalsete parameetritega.

Uuritavad ja metoodika

Uuringus osales algselt 89 7–9 aastast tüdrukut, kellest 46 olid iluvõimlejad ja 53 kontrollgrupi tüdrukud. Võimlejad olid enne uuringu alustamist regulaarselt

tegelenud iluvõimlemisega vähemalt 2 aastat ning treenisid keskmiselt 10–12 tundi nädalas. Kontrollgrupi tüdrukud osalesid vaid 2–3 korda nädalas kooli kehalise kasvatuse tundides. Ühelgi tüdukul ei esinenud skeletilihassüsteemi haigusi, mis võinuks mõjutada luutihedust. Teisel uuringu-aastal osales uuringus 33 iluvõimlejat ja 35 kontrollgrupi tüdrukut. Uuringust väljalangemise sagedasemaiks põhjuseks iluvõimlejate seas oli iluvõimlemistreeningute katkestamine ja kontrollgrupi tüdrukute seas regulaarsete treeningutega alustamine.

Kõigil uuritavatel määrati kehamass ja pikkus, kehamassiindeks arvutati vastava valemiga. Bioloogiline vanus määrati Tanneri metoodikaga, luuline vanus röntgeniga ning määrati luustumise aste. DXA aparaadiga määrati kehakoostise parameetrid, nii rasva- ja rasvavaba mass kui ka luutihedus (kogu keha luutihedus, lumbaarosa (L2-L4) ja reieluukaela luutihedus).

Järeldused:

1. Prepuberteediealistel iluvõimlejatel olid luutihedusel olulised seosed rasvamassiga, samaealistel regulaarselt spordiga mittetegevatel tüdrukutel aga praktiliselt kõikide määratud antropomeetriliste ja kehakoostise näitajatega.
2. Iluvõimlejatel ei olnud luutihedusel seoseid adipotsütokiinide ega greliini tasemega, kontrollgrupi tüdrukutel leiti aga olulised seosed leptiini ja kogu keha ja lumbaarosa luutiheduse ning greliini kontsentratsiooni ja reieluukaela luutiheduse vahel.
3. Iluvõimlejate aastase luutiheduse kasvu, kehakoostise parameetrite ning adipotsütokiinide ja greliini taseme vahel olulisi seoseid ei ilmnenud. Kontrollgrupi tüdrukutel olid luutihedusel olulised seosed leptiini taseme ja kehakoostise näitajatega.

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