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Relationships between bone parameters,  
jumping height and hormonal indices in  
adolescent female athletes



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## LIST OF ABBREVIATIONS

ANOVA	analysis of variance
APHV	age at peak height velocity
BMAD	volumetric BMD L2-L4
BMC	bone mineral content
BMD	bone mineral density
BMI	body mass index
CMJ	countermovement jump
CV	coefficient of variation
DXA	dual-energy X-ray absorptiometry
HOMA	homeostasis model assessment (insulin resistance index)
IGF-1	insulin-like growth factor-1
IGFBP-3	IGF binding protein-3
L2-L4	antero-posterior lumbar spine
RJ15s	rebound jump for 15 seconds
RJ30s	rebound jump for 30 seconds
SD	standard deviation

## LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original papers, which are referred to in the text by the Roman numerals (I–III):

### STUDY I

**Gruodytė R, Jürimäe J, Saar M, Maasalu K, Jürimäe T.** Relationships between areal bone mineral density and jumping height in pubertal girls with different physical activity patterns. *Journal of Sports Medicine and Physical Fitness*, 2009; 49(4):474–9

### STUDY II

**Gruodytė R, Jürimäe J, Saar M, Jürimäe T.** The relationships among bone health, insulin-like growth factor-1 and sex hormones in adolescent female athletes. *Journal of Bone and Mineral Metabolism*, 2010; 28(3):306–13

### STUDY III

**Gruodytė R, Jürimäe J, Cicchella A, Stefanelli C, Passariello C, Jürimäe T.** Adipocytokines and bone mineral density in adolescent female athletes. *Acta Paediatrica* (in press)

In all three studies, Rita Gruodytė had primary responsibility for protocol development, subjects' screening, performing measurements, preliminary and final data analyses, and writing of the manuscripts.

## INTRODUCTION

Bone is under a constant dynamic process of formation and resorption, imbalance of which leads to bone metabolic diseases, such as osteoporosis [Caetano-Lopes et al. 2009]. Although traditionally considered to be a disease affecting the elderly, osteoporosis is now recognized globally as a condition that has childhood antecedents [Faulkner and Bailey 2007]. Regular high-impact weight-bearing physical activity (ball games, racket sports, gymnastics, dance, running, or jumping exercises) during growth may play an important role in maximizing bone mineral mass gain, which may reduce the incidence of fractures in children and adolescents as well as in the elderly [Janz et al. 2010; Misra 2008; Rizzoli et al. 2010]. However, the relationships between jumping ability and bone parameters are yet unknown.

Bone mineralization increases with age, height, and body mass throughout childhood, with a significant gain during pubertal development, resulting in an increase of BMD of about 40% during this period [Gordon et al. 1991]. Early puberty is 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 increase of femoral neck and lumbar spine BMC between the ages of 7 and 17 years reaches up to 50–150% due to the increased bone size [Bass et al. 1999]. The main increase in BMC and mineralization at the femoral neck and lumbar spine in females occurs at the age of 11 to 15 years, whereas after 16 years of age the rate of gain drops dramatically [Bass et al. 1999; Theintz et al. 1992]. It is possible that the identification whether athletes did or did not accrue a normal amount of bone mineral may not have been recognizable until girls reach the age of 17–18 years [Barrack et al. 2010].

Several relationships of bone formation and resorption markers with physical activity and sport have been presented [Banfi et al. 2010]. However, few information is available concerning these characteristics in adolescent female athletes. Sex hormones, insulin-like growth factors and physical activity are among the main determinants of bone gain during puberty [Faulkner and Bailey 2007]. Physical activity *per se* is not always beneficial for bone health, thus BMD in adolescent athletes may be affected by the nature of the sport, a relative state of energy deficiency, and changes in body composition [Misra 2008]. Nevertheless, Courteix et al. [2007] suggested that physical activity has beneficial effects on bone and may counterbalance such negative factors of bone health such as low fat mass and insufficient blood leptin levels in adolescent females.

The use of bone metabolism biochemical markers in young athletes should be encouraged for studying bone turnover and general bone metabolism status. Strenuous efforts and physical stress, depending on sport event together with biological maturation in adolescent female athletes, can deeply affect bone cell activity; however, better biochemical markers are also needed. There is evidence that several adipocytokines may also have a positive influence on BMD of the growing skeleton [Garnett et al. 2004].

## **2. REVIEW OF THE LITERATURE**

### **2.1. Bone development and factors that influence it**

Bone is a unique, metabolically active tissue that undergoes a continuous remodeling throughout its life cycle. The skeleton grows as the body grows, in length, breadth, mass, and volumetric density [Javaid and Cooper 2002]. There are two functionally distinct phases of bone development: (1) the skeletal patterning during the embryonic period; and (2) the mineralization, the location of which is influenced by mechanical strain [Davies et al. 2005]. Bone mass increases substantially during the first two decades, reaching a plateau (i.e. peak bone mass) in the late teen or young adult years, remaining relatively stable throughout the early- to mid-adult years until the onset of the naturally progressive bone loss that accompanies ageing occurs [Faulkner and Bailey 2007]. Bone mineral is accrued at differing rates throughout the skeleton: before puberty, the limb growth is more rapid than axial growth; in early puberty, the limb growth remains constant and axial growth accelerates; in late puberty, growth of both regions decelerates [Bachrach 2001; Bass et al. 1999].

The accumulation of bone mineral is influenced by a number of factors: (1) endogenic, such as heredity, ethnicity, gender, or endocrine status; and (2) exogenic, such as nutrition or physical activity [Javaid and Cooper 2002; Rizzoli et al. 2010]. Chronological age, higher body mass, greater estrogen exposure (i.e. gynecological age), higher levels of lean tissue mass, and participation in a non lean-build variable-impact loading sport are among the positive predictors of BMD and BMC in adolescent female athletes [Barrack et al. 2010]. Lean body mass and fat mass contribute to bone mass by increasing compressive forces during skeletal loading [Ho and Kung 2005]. Late menarche, irregular menstrual cycles, insufficient leptin levels, hypoestrogenism, low body weight and/or fat mass are just a few risk factors for low BMD, which, in turn, is a risk factor for fractures later in life [Eastell 2005; Ho and Kung 2005; Meczekalski et al. 2010]. According to the model for mechanism underlying the association between early menarche and low risk of fracture [Eastell 2005], girls who enter menarche earlier have higher BMI and serum leptin, they soon establish regular menses, they tend to be shorter and heavier, and they have more years of exposure to estrogen.

### **2.2. Bone mineral density and weight-bearing physical activity in growth years**

It is well known that mechanical loading activity on bone is vitally important for skeletal strength and development [Eliakim et al. 2002; Khan et al. 2001]. The type, intensity and duration of the physical loading are affecting BMD. The influence of exercise and mechanical loading on skeleton peak bone mass has

been studied extensively in humans [Eliakim and Beyth 2003; Nichols et al. 2007 b]. Peak bone mass at skeletal maturity has been identified as a risk factor for osteoporosis in later life [Javaid and Cooper 2002]. Osteoporosis is a bone disease associated with low BMD that increases the risk of debilitating bone fractures [Khan et al. 2001]. Osteoporosis in females may be prevented or delayed by maximizing peak bone mass through modifications of lifestyle, such as in diet and physical activity, during childhood [Iuliano-Burns et al. 2005]. It is suggested that in healthy children with adequate dietary intakes, physical activity has a greater osteogenic effect than calcium or protein intake [Iuliano-Burns et al. 2005]. Of different exercise modalities, high-impact activity (for example, jumping) seems to be especially osteogenic [MacKelvie et al. 2002]. Sport activities, especially those involving high peak strain (team sports), are also important in determining peak bone mass in early adulthood [Neville et al. 2002]. Skeletal adaptations to mechanical loading appear to be site-specific [Duncan et al. 2002]. Accordingly, another important factor influencing peak bone mass might be anatomical distribution of mechanical load [Duncan et al. 2002]. In girls, with regard to physical activity pattern, greater attention must be paid to pre- and perimenarcheal period, when half of lumbar adult peak bone mass is acquired [Sabatier et al. 1999]. Although exercise is recommended to maximize bone health at all stages of life, a particularly opportune time to intervene with loading exercise may be the two years surrounding peak bone velocity which correspond to ages 11.5–13.5 for girls [Faulkner and Bailey 2007; MacKelvie et al. 2002]. Over 25% of final adult bone mass is accumulated during that period.

The effects of sport on bone health vary in relation to training modality, ranging from non-weight bearing (swimming) and low-impact activities (skiing) to moderate- and high-impact activities (racket sports, volleyball, gymnastics) [Nichols et al. 2007 a]. Characteristics, such as the type, direction, rate, frequency, and magnitude of strain of the exercise, play a role in the variation of the bone mineral accrual. For instance, distance runners experience more repetitive linear forces, concentrated on the lower limbs, while sport games activities may involve a great extent of turning and jumping with compression forces as well as locomotory landing impacts [Egan et al. 2006].

There are substantial data to support the notion that high impact loading is the most effective type of bone-building activity [MacKelvie et al. 2002; Witzke and Snow 2000]. Athletes whose skeletons are subjected to forces of high intensity, such as gymnasts, possess significantly greater bone mass at femoral neck and lumbar spine compared with athletes who participate in activities associated with lower skeletal forces [Sööt et al. 2005; Witzke and Snow 2000]. Accordingly, jumping ability seems to correlate well with different bone values. Different vertical jumps have been used to evaluate jumping ability in young athletes [Kellis et al. 1999]. Maximum vertical jump performed from the standing position with a countermovement (CMJ) as well as rebound jumps for 15 (RJ15s) and 30 (RJ30s) seconds adequately reflect the jumping abilities in young athletes [Kellis et al. 1999]. However, there is few information available

about the relationships between areal BMDs and jumping ability, using different jumping tests, in adolescent females with different training patterns.

### **2.3. Bone mineral parameters, IGF-1 and sex hormones in adolescent girls**

Bone mineral accrual is substantially altered between childhood and puberty through the concerted influences of growth and systemic hormones [MacKelvie et al. 2002]. Increase in BMC accrual during adolescence is associated with specific hormonal changes during this period [Misra 2008]. Manolagas et al. [2002] suggested that bone mass accrual during puberty is more dependable on sexual maturation than chronological age, and effects of sex hormones on the growing skeleton are associated with the osteoporosis in later life.

Bone mineral development in girls may be especially promoted by the increase of estrogen and free, biologically active IGF-1 levels which occur between pubertal stages 2 and 4, corresponding to the timing of menarche and peak bone mineral accrual velocity [MacKelvie et al. 2002]. Estrogens are a group of steroid hormones that function as the primary female sex hormone and are known to have an important role not only in regulating the menstrual cycle, but also in controlling bone formation [Ter Horst 2010]. The most potent and abundant estrogen during puberty and throughout the fertile life of females is estradiol [Wang et al. 2004]. In early puberty, low levels of circulating sex hormones in combination with increasing levels of growth hormone and IGF-1 stimulate chondrocytes in the epiphyseal growth plate thus stimulating linear growth; in turn, growth hormone and IGF-1 stimulate the secretion of sex hormones by the gonads and potentiate their effects on bone [Van Coeverden et al. 2002]. As circulating levels of sex hormones increase, the concentrations of locally available estradiol increase at the growth plate [Van Coeverden et al. 2002]. At the end of puberty, the high levels of estradiol inhibit chondrocyte proliferation despite the high levels of growth hormone and IGF-1, which results in inhibition of growth velocity and bone mineralization [Van Coeverden et al. 2002]. In addition, IGF-1 has a marked effect on body composition: it stimulates increase in muscle mass and bone mineral density, and reduces fat distribution [Eliakim et al. 2005].

The positive association between estradiol and bone turnover markers in early puberty (pubertal stages 2 and 3) disappears in late puberty (pubertal stages 4 and 5), as has also been observed for IGF-1 [Van Coeverden et al. 2002]. Increasing sex hormones with pubertal development also increase the biologically active IGF-1 levels that affect bone growth [Kanbur-Öksüz et al. 2004; Pomerants et al. 2007]. Estrogens are needed for calcium absorption into bone [Baxter-Jones and Mundt 2007] and may influence linear bone growth indirectly via modulation of the IGF-axis [Jull 2001]. It appears that sex hormone levels in female athletes are related to the amount and type of exercise performed, i.e. in endurance-trained athletes sex hormone levels may be

abnormally low while strength-trained athletes may have higher levels, although even their sex hormone levels may be lower than those of sedentary control subjects [Sööt et al. 2006; Voss et al. 1998]. Bone health is extremely compromised if IGF-1 and estrogen levels are low, particularly in those young female athletes who participate in competitive sports where leanness may be necessary or aesthetically pleasing (gymnasts, runners, swimmers) [Baxter-Jones and Mundt 2007; Zanker and Hind 2007].

Many studies have investigated IGF-axis and sex hormone levels in relation with age, pubertal development, and physical activity [Van Coeverden et al. 2002; Kanbur-Öksüz et al. 2004; Pomerants et al. 2007; Yilmaz et al. 2005]. To our knowledge there are no studies that have investigated the effect of exercising systematically in different sport events on the relationships between these fasting blood hormones and bone mineral variables in pubertal female athletes.

## **2.4. Adipocytokines and bone mineral parameters**

Visfatin, adiponectin and leptin are cytokine-like hormones, secreted by adipocytes, and associated with adiposity, i.e. the levels of adiponectin are lower while visfatin and leptin concentrations are higher in obese adolescents compared to their healthy counterparts [Haider et al. 2006; Jin et al. 2008]. Furthermore, it is suggested that these adipocytokines carry signals from adipose tissue to bone and contribute to the relationship between fat mass and BMD [Huang et al. 2004 a; Jürimäe and Jürimäe 2007; Xie et al. 2007].

Evidence of the effects of visfatin on bone is scant. Xie et al. [2007] hypothesized that visfatin may carry signals from fat to bone. Visfatin has been found to increase bone mineralization, promote glucose uptake, and down-regulate osteocalcin secretion in human osteoblasts [Xie et al. 2007].

Leptin is involved in the accumulation, maintenance, and loss of BMD throughout life and plays a major role in mediating the fat-bone axis [Hamrick and Ferrari 2008]. In girls, the leptin concentrations steadily increase with pubertal stages [Garcia-Mayor et al. 1997], decrease with increased physical activity levels [Courteix et al. 2007; Ischander et al. 2007; Romon et al. 2004], are strongly associated with body fat [Matkovic et al. 1997; Misra et al. 2007], and are inversely related to menarche [Matkovic et al. 1997]. Leptin has been designated to have a number of physiological roles, the most important of which may be its influence on energy balance: leptin decreases when energy intake is restricted and increases when body fat is increased [Jürimäe et al. 2010]. It is possible that leptin regulates bone mass concurrently with changes in body mass [Zanker and Hind 2007].

Our previous laboratory study has demonstrated an independent relationship between adiponectin and BMD in healthy women [Jürimäe and Jürimäe 2007]. It has also been reported that adiponectin and leptin were related to total body BMD and BMC in healthy female adolescents [Huang et al. 2004 a]. Misra et

al. [2007] also suggested that adiponectin is inversely and independently associated with BMD in adolescent girls.

Since different adipocytokines are produced by adipose tissue, and fat mass being a strong determinant of BMD in healthy 14-16-year-old girls [El Hage et al. 2009], we hypothesized that levels of visfatin, adiponectin, and leptin concentration might be related to bone mineral parameters in pubertal girls with different training patterns.

## **2.5. Summary**

Maximizing BMD and BMC during growth years by adopting weight-bearing physical activity in childhood and adolescence may be one of the most effective osteoporosis prevention strategies [French et al. 2000]. The activity-induced bone gain is greater if exercise begins before or during puberty, and is especially accentuated in the premenarcheal period [Heinonen et al. 2000]. High-impact activities (gymnastics, volleyball, soccer, running, weight lifting) promote bone mineral accrual to a greater extent than low-impact activities (swimming, cycling) [Bellew and Gehrig 2006; Duncan et al. 2002]. The possible synergism between advancing pubertal status and loading induced bone gain suggests that a “window of opportunity” for bone response may exist in early puberty [MacKelvie et al. 2002]. However, whether there are significant relationships between jumping ability and BMD and BMC, is unknown. Secondly, fasting hormone relationships with bone parameters have not been well studied, especially in connection with different adipocytokines.

### **3. AIM AND PURPOSES OF THE STUDY**

The general aim of this study was to investigate the relationships between bone parameters and jumping height, fasting serum IGF-1, IGFBP-3, sex hormones, and concentrations of selected adipocytokines in pubertal girls with different training patterns.

The specific purposes of this study were:

- to determine the relationships between single and 15 and 30 seconds vertical jumping series height and BMD at femoral neck and lumbar spine in pubertal girls with different training patterns;
- to investigate the relationships of BMD and BMC with IGF-1, IGFBP-3, and estradiol concentrations in pubertal girls with different training patterns;
- to evaluate the relationships of adipocytokines (visfatin, adiponectin, and leptin) concentrations with BMD and BMC in adolescent female athletes with different training patterns.

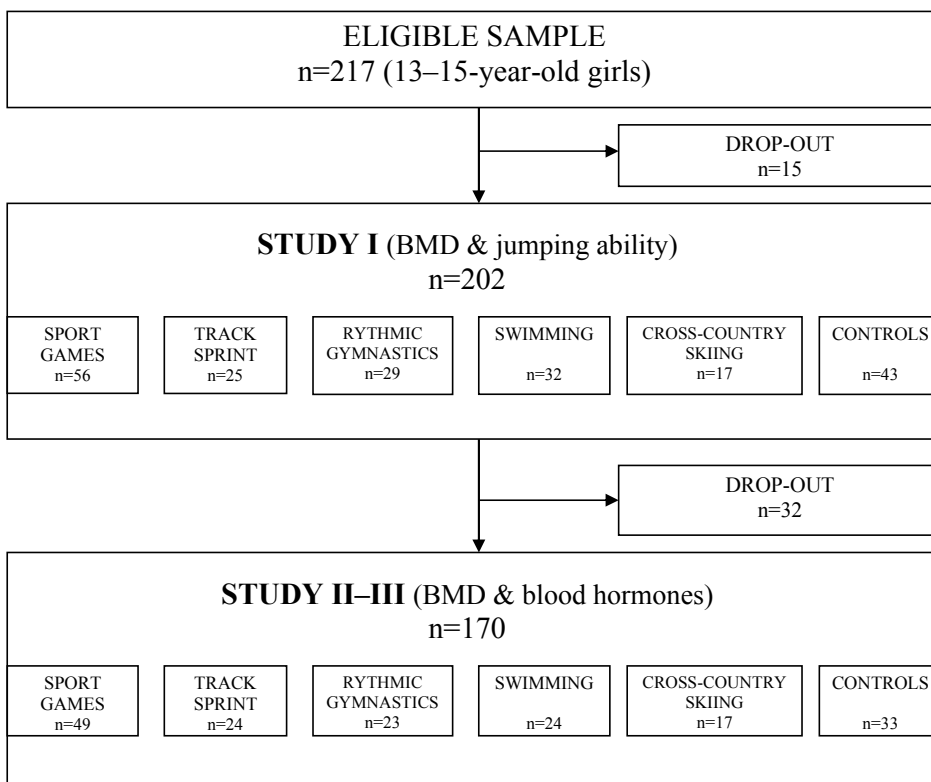
We hypothesized that:

- the relationships between areal BMDs and different jumping tests should be well pronounced in adolescent female athletes, especially in those girls who perform many and various jumping exercises during their training sessions (in sport games or rhythmic gymnastics);
- systematic and regular exercising in different sport events may have a different effect on the relationships between fasting blood hormones (i.e. IGF-1, IGFBP-3 and estradiol) and bone mineral variables in pubertal female athletes;
- visfatin, adiponectin, and leptin concentrations might be related to bone mineral parameters in pubertal girls with different training patterns.

## **4. MATERIAL AND METHODS**

### **4.1. Subjects**

In total 202 healthy adolescent (13–15-year-old) girls from different schools and sport clubs in Estonia (Tallinn, Tartu, Pärnu) took part in this cross-sectional study. Before entering the study, volunteers completed medical and physical activity questionnaires. All participants were free from past or present diseases known to affect skeletal metabolism. None of the girls used birth control or medications known to affect bone. Girls were also asked not to change their eating habits. According to their physical activity pattern (determined by answers to questions on the onset of the training and weekly hours of sporting), participants were divided into six groups. Girls who participated in competitive extramural athletic programmes comprised the groups of sport games (basketball, volleyball, badminton) (n=56), track sprint (n=25), rhythmic gymnastics (n=29), swimming (n=32), and cross-country skiing (n=17) (Figure 1). To be included in the study, the girls had to have participated in their selected sports for at least the recent two years. Control group (n=43) consisted of the girls who only took part in compulsory physical education classes at school (45 min twice per week). In the Studies II and III the number of participants was cut to 170 due to the lacking data on blood biochemical analysis since some of the girls refused to undergo the blood drawing procedure. As a result, the group sizes in the Studies II and III were the following: sport games (n=49), track sprint (n=24), rhythmic gymnastics (n=23), swimming (n=24), cross-country skiing (n=17), and controls (n=33) (Figure 1).



**Figure 1.** Tracking the study population (13–15-year-old girls from different schools and sport clubs in Estonia).

Each girl and her parent (or legal guardian) received a full written description of the nature of the study and signed an informed consent form before participating. The study was approved by the Medical Ethics Committee of the University of Tartu (Estonia).

## 4.2. Anthropometric measurements

Body height and sitting height were 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 girls were dressed in light clothing and were wearing no shoes. Body height and body mass data were used to calculate BMI ( $\text{kg/m}^2$ ).

### **4.3. Sexual maturity assessment**

Pubertal development of the participants was assessed by self-report using an illustrated questionnaire of pubertal stages according to the criteria of Tanner [Tanner 1962], which has been previously validated [Matsudo and Matsudo 1994] and used in our previous laboratory studies [Jürimäe et al. 2007]. The girls were given photographs, figures and descriptions of breast and pubic hair development stages, and asked to choose the one which most accurately reflected their appearance. If a disagreement between the development of breast and pubic hair was found, the final decision was made according to the breast [Jürimäe et al. 2007; Matsudo and Matsudo 1994]. Predicted age at peak height velocity (APHV) and biological maturity age (years from APHV) of the participants were estimated using chronological age, body height, sitting height, and body mass data [Baxter-Jones and Sherar 2007]. The girls were also asked if they had experienced menarche. Using a simple questionnaire, the data on the age at menarche and the duration of a single menstrual cycle of the girls was obtained. None of the subjects were amenorrhoeic or had irregular menses.

### **4.4. Bone mineral parameters**

The whole-body BMD, and femoral neck and lumbar spine (L2-L4) BMD ( $\text{g}/\text{cm}^2$ ) and BMC (g) were measured using 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 the back, with arms at the sides. The fast 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 (CVs) for BMD and BMC measurements in female adolescents were less than 2%. The formula by Kröger et al. [1995] was used to calculate the apparent volumetric mineral bone density (BMAD) of the lumbar spine to reduce the effect of body size on BMD values:  $\text{BMAD} = \text{BMD} \times [4/(\pi \times \text{width of L2 to L4})]$ .

### **4.5. Jumping performance**

The maximal height of two-footed hands-on-the-hips vertical jumps was measured using the contact mat (Newtest OY, Oulu, Finland), connected to a digital recorder that calculated the jumping height. The girls performed three jumping tests: (1) a CMJ from a standing position with a preliminary countermovement; (2) the rebound jump, with continuous countermovement jumps for 15 seconds (RJ15s); and (3) the rebound jump for 30 seconds (RJ30s) [Kellis et al. 1999]. In CMJ, the best result out of three attempts was recorded.

The girls were first instructed and then verbally encouraged to jump as high and as rapidly as they could. The hands remained on the waist throughout all jumping tests to avoid upper extremities contribution to the jump height [Kellis et al. 1999].

#### 4.6. Blood analysis

Venous blood samples to determine the concentration of estradiol, IGF-1, IGFBP-3, visfatin, adiponectin, leptin, insulin and glucose were drawn between 07:30 and 08:30 a.m. after an overnight fasting. For those girls who had regular menstruation, the fasting blood samples were drawn in the early follicular phase of the menstrual cycle, i.e. on days 5-7 after menstrual bleeding started [Jürimäe et al. 2007; Sööt et al. 2006]. The blood serum was separated and stored at -20 °C for later analysis. IGF-1, IGFBP-3, estradiol and insulin concentrations were analysed in duplicate on Immulite 2000 radioimmunoassay (DPC, Los Angeles, CA, USA). The intra- and inter-assay CVs for IGF-1, IGFBP-3, and estradiol were <7%. The intra- and inter-assay CVs for insulin were <5% and <12%, respectively, at an insulin concentration of 6.6  $\mu$ IU/mL. The levels of visfatin and adiponectin concentration were analyzed using ELISA kits (AdipoGen and Mediagnost, Aspenhastr, Germany). The intra- and inter-assay CVs for visfatin were <10% and <8%, and for adiponectin <5% and <6%, respectively. For leptin concentration analysis an ELISA sandwich (DRG Instruments GmbH, Marburg, Germany) was used. The intra- and inter-assay CVs were <7% and <12%, respectively. Glucose concentration was measured with a commercial kit (Boehringer, Mannheim, Germany) that employed the hexokinase/glucose-6-phosphate dehydrogenase method.

In addition, the IGF-1/IGFBP-3 molar ratio was calculated as it is suggested to be an indirect indicator of serum-free IGF-1 [Pomerants et al. 2007]. The molar ratio was obtained as follows: IGF-1 (ng/mL) x 0.130/IGFBP-3 (ng/mL) x 0.036 [Morimoto et al. 2005]. The insulin resistance index was calculated using homeostasis model assessment (HOMA): fasting plasma insulin ( $\mu$ IU/mL) x fasting plasma glucose (mmol/L)/22.5 [Matthews et al. 1985]. The greater HOMA values indicate the greater level of insulin resistance.

#### 4.7. Statistical analysis

All statistical analyses were performed using SPSS 15.0 package for Windows (Chicago, IL, USA). Standard statistical methods were used to calculate means and standard deviations ( $\pm$ SD). Normality of parameters was controlled by one-sample Kolmogorov-Smirnov test ( $p > 0.05$ ). Statistical comparisons between the groups were made using analysis of variance (ANOVA) and Tukey *post hoc* tests. Adjustments for the major confounders (i.e. age, body height, and body mass) in the analysis of the statistical differences among the athletes' groups and the controls in BMD, BMC, and blood biochemical parameters were

performed using general linear modelling. Pearson product moment correlation coefficients were computed to evaluate the relationships between bone mass parameters, jumping performance, and measured blood hormones. Partial correlation analysis was performed to assess these relationships while controlling for age, body height, and body mass [Wang et al. 2004]. In addition, partial correlation analysis was performed to assess the relationships between selected variables while controlling for insulin resistance. The effect of IGF-1, IGFBP-3 and estradiol on the BMD and BMC was analysed by stepwise multiple regression analysis. The level of significance was set at  $p < 0.05$ .

## **5. RESULTS**

### **5.1. Study I**

#### **5.1.1. Anthropometric, maturational, bone mineral and performance characteristics of the subjects**

The physical and performance characteristics of pubertal girls with different training patterns are presented in Table 1. While there were no significant differences in biological maturity age (years from APHV) and pubertal status between athletes and controls, the rhythmic gymnasts were significantly older than track sprinters. The girls practising sport games and track sprinters were significantly taller than controls, swimmers, and cross-country skiers. The sport games group had greater body mass than controls ( $p < 0.05$ ). The predicted APHV of the girls practising sport games, track sprinters and swimmers was lower than in controls; the rhythmic gymnasts' predicted APHV was higher than that of the girls practising sport games, track sprinters and swimmers ( $p < 0.05$ ). Rhythmic gymnasts had been training for the longest period in years of all athletes ( $p < 0.05$ ), and swimmers had trained longer than the girls in sport games group ( $p < 0.05$ ). Both rhythmic gymnasts' and swimmers' training duration in weekly hours was greater than in other athletes ( $p < 0.05$ ).

Rhythmic gymnasts had significantly higher BMD values at femoral neck of all groups and sport games group girls' femoral neck BMD was greater than in controls and swimmers (Table 1). There were no differences between track sprinters, swimmers, cross-country skiers and controls in BMD values at femoral neck ( $p > 0.05$ ). Girls practising sport games, track sprinters and rhythmic gymnasts had significantly greater lumbar spine BMD than controls and cross-country skiers.

Track sprinters, rhythmic gymnasts, swimmers and cross-country skiers had significantly higher results in CMJ than controls; track sprinters jumped significantly higher in CMJ than the girls from sport games group (Table 1). Track sprinters jumped higher than the girls from control, sport games, and swimming groups in both rebound jumps (RJ15s and RJ30s) ( $p < 0.05$ ). Rhythmic gymnasts jumped higher than controls in RJ15s ( $p < 0.05$ ) and also higher than the girls from sport games and swimming groups in RJ30s ( $p < 0.05$ ). There were no differences between the girls of control, sport games and cross-country skiing groups in both rebound jumps (RJ15s and RJ30s).

**Table 1.** Physical and performance characteristics (mean  $\pm$  SD) of the subjects and differences between the groups.

GROUP	CONT (n=43)	SG (n=56)	SPR (n=25)	GYMN (n=29)	SW (n=32)	CCS (n=17)
Age (years)	14.2 $\pm$ 1.2	14.2 $\pm$ 1.0	14.2 $\pm$ 1.1	14.4 $\pm$ 0.9 <sup>c</sup>	13.8 $\pm$ 1.3	13.9 $\pm$ 0.9
Body height (cm)	162.4 $\pm$ 6.9	167.2 $\pm$ 7.8 <sup>a</sup>	168.1 $\pm$ 5.9 <sup>a</sup>	165.0 $\pm$ 7.0	164.1 $\pm$ 7.1 <sup>b,c</sup>	162.8 $\pm$ 6.4 <sup>b,c</sup>
Body mass (kg)	53.7 $\pm$ 8.8	57.6 $\pm$ 9.3 <sup>a</sup>	54.2 $\pm$ 6.5	53.6 $\pm$ 10.5	54.2 $\pm$ 9.1	52.7 $\pm$ 9.5
BMI (kg/m <sup>2</sup> )	20.3 $\pm$ 2.6	20.6 $\pm$ 2.8	19.2 $\pm$ 2.0 <sup>b</sup>	19.6 $\pm$ 2.6	20.1 $\pm$ 2.9	19.7 $\pm$ 2.6
Years of training	–	3.8 $\pm$ 1.5	4.3 $\pm$ 2.0	6.1 $\pm$ 2.2 <sup>b,c</sup>	4.9 $\pm$ 1.9 <sup>b,d</sup>	4.3 $\pm$ 2.1 <sup>d</sup>
Training duration (h/week)	–	5.1 $\pm$ 1.5	4.7 $\pm$ 2.1	8.6 $\pm$ 5.0 <sup>b,c</sup>	8.9 $\pm$ 3.2 <sup>b,c</sup>	6.3 $\pm$ 1.1 <sup>d,e</sup>
Predicted APHV (years)	12.4 $\pm$ 0.4	12.1 $\pm$ 0.6 <sup>a</sup>	12.1 $\pm$ 0.5 <sup>a</sup>	12.4 $\pm$ 0.5 <sup>b,c</sup>	12.1 $\pm$ 0.4 <sup>a,d</sup>	12.2 $\pm$ 0.5
Biological age	1.8 $\pm$ 1.0	2.1 $\pm$ 0.8	2.2 $\pm$ 0.8	2.0 $\pm$ 0.8	1.7 $\pm$ 1.1	1.7 $\pm$ 0.9
Tanner stage (1/2/3/4/5)	1/2/7/28/5	0/2/3/42/9	0/2/5/14/4	0/1/7/16/5	0/2/6/21/3	1/1/2/11/2
Girls having menses (n (%))	36 (83.7)	42 (75)	20 (80)	19 (65.5)	19 (59.4)	10 (58.8)
BMD femoral neck (g/cm <sup>2</sup> )	0.996 $\pm$ 0.114	1.066 $\pm$ 0.109 <sup>a</sup>	1.051 $\pm$ 0.087	1.124 $\pm$ 0.136 <sup>a,b,c</sup>	1.005 $\pm$ 0.124 <sup>b,d</sup>	1.005 $\pm$ 0.130 <sup>d</sup>
BMD lumbar spine (g/cm <sup>2</sup> )	1.058 $\pm$ 0.136	1.132 $\pm$ 0.124 <sup>a</sup>	1.135 $\pm$ 0.128 <sup>a</sup>	1.125 $\pm$ 0.111 <sup>a</sup>	1.085 $\pm$ 0.144	1.037 $\pm$ 0.148 <sup>b,c,d</sup>
CMJ (cm)	24.4 $\pm$ 3.7	25.6 $\pm$ 3.4	27.7 $\pm$ 4.3 <sup>a,b</sup>	27.1 $\pm$ 4.3 <sup>a</sup>	26.8 $\pm$ 4.2 <sup>a</sup>	27.7 $\pm$ 4.6 <sup>a</sup>
RJ15s (cm)	21.3 $\pm$ 3.5	21.8 $\pm$ 3.2	24.3 $\pm$ 3.1 <sup>a,b</sup>	23.0 $\pm$ 3.8 <sup>a</sup>	22.2 $\pm$ 3.1 <sup>c</sup>	23.0 $\pm$ 4.0
RJ30s (cm)	21.0 $\pm$ 3.3	20.5 $\pm$ 3.3	23.8 $\pm$ 2.8 <sup>a,b</sup>	22.3 $\pm$ 3.5 <sup>b</sup>	20.4 $\pm$ 3.5 <sup>c,d</sup>	22.0 $\pm$ 3.7

CONT: control group; SG: sport games group; SPR: track sprinters; GYMN: rhythmic gymnasts; SW: swimmers; CCS: cross-country skiers; BMI: body mass index; BMD: bone mineral density; CMJ: countermovement jump; RJ15s: the rebound jump for 15 seconds; RJ30s: the rebound jump for 30 seconds; APHV: age at peak height velocity

<sup>a</sup> difference from control group

<sup>b</sup> difference from sport games group

<sup>c</sup> difference from track sprint group

<sup>d</sup> difference from rhythmic gymnasts' group

<sup>e</sup> difference from swimmers' group

In all cases –  $p < 0.05$

### 5.1.2. The relationships between bone mineral parameters and jumping height

BMD at femoral neck was significantly correlated only with jumping height in RJ15s for track sprint group (Table 2). Concerning BMD of the spine, a negative correlation ( $r=-0.508$ ;  $p<0.01$ ) emerged with jumping height in CMJ for rhythmic gymnasts, and a positive correlation with jumping height in both rebound jumps for sport games group ( $r=0.265-0.269$ ;  $p<0.05$ ). There were no significant relationships between BMD variables and jumping height of different jumps in control, swimming and cross-country skiing groups. There were no significant correlations between BMD and jumping ability in control, swimming and cross-country skiing groups.

**Table 2.** Pearson correlation for BMD variables and jumping height in athlete and control groups. Partial correlation analysis (controlled for age, body height, and body mass) is in parentheses.

GROUP	CONT (n=43)	SG (n=56)	SPR (n=25)	GYMN (n=29)	SW (n=32)	CCS (n=17)
<i>BMD femoral neck (g/cm<sup>2</sup>)</i>						
CMJ	0.144 (0.206)	0.141 (0.274)*	0.210 (-0.033)	-0.173 (0.080)	-0.211 (-0.074)	0.164 (0.261)
RJ15s	0.087 (0.166)	0.170 (0.282)*	0.473* (0.300)	0.214 (0.510)**	-0.009 (0.040)	0.216 (0.359)
RJ30s	0.200 (0.162)	0.087 (0.204)	0.375 (0.198)	0.217 (0.489)*	-0.018 (-0.036)	0.320 (0.344)
<i>BMD lumbar spine (g/cm<sup>2</sup>)</i>						
CMJ	0.179 (0.164)	0.228 (0.401)**	0.020 (-0.402)	-0.508** (0.106)	-0.251 (-0.087)	0.173 (0.188)
RJ15s	0.168 (0.189)	0.269* (0.369)**	0.140 (-0.358)	-0.218 (0.386)	-0.121 (-0.093)	0.285 (0.333)
RJ30s	0.300 (0.220)	0.265* (0.386)**	0.170 (-0.188)	-0.142 (0.437)*	0.010 (-0.038)	0.372 (0.309)

CONT: control group; SG: sport games group; SPR: track sprinters; GYMN: rhythmic gymnasts; SW: swimmers; CCS: cross-country skiers; BMD: bone mineral density; CMJ: countermovement jump; RJ15s: rebound jump for 15 seconds; RJ30s: rebound jump for 30 seconds

\* $p<0.05$

\*\* $p<0.01$

When the correlation between BMD variables and different jumps was controlled for age, body height, and body mass, the significant relationships were revealed between BMD of femoral neck and both CMJ and RJ15s ( $r=0.274-0.282$ ;  $p<0.05$ ) in sport games group; BMD of lumbar spine and all jumps (CMJ, RJ15s, and RJ30s) in sport games group ( $r=0.369-0.401$ ;  $p<0.01$ ); BMD of femoral neck and both rebound jumps (RJ15s and RJ30s) in rhythmic gymnasts' group ( $r=0.489-0.510$ ;  $p<0.05$ ); and BMD of lumbar spine and RJ30s in rhythmic gymnasts' group ( $r=0.437$ ;  $p<0.05$ ) (Table 2). There were no significant relationships between BMD variables and jumping height of various jumps when controlled for age, body height, and body mass in control, track sprint, swimming, and cross-country skiing groups.

## **5.2. Study II**

### **5.2.1. Anthropometry, pubertal status, and training history of the subjects**

The characteristics of the Study II population are summarised in Table 3 using ANOVA with Tukey *post hoc* adjustments. The girls in the swimmers' group were significantly younger than those in the control, track sprinters' and rhythmic gymnasts' groups. Girls practising sport games and track sprinters were taller than control group girls, rhythmic gymnasts and cross-country skiers ( $p<0.05$ ). Body mass was higher in girls practising sport games compared with rhythmic gymnasts and cross-country skiers ( $p<0.05$ ). BMI was lower in track sprinters compared with controls and girls practising sport games ( $p<0.05$ ). When comparing all the sport groups, the rhythmic gymnasts had the highest mean training time ( $p<0.05$ ). Training duration in weekly hours was greater in both rhythmic gymnasts and swimmers compared to the other athletes ( $p<0.05$ ).

**Table 3.** Mean ( $\pm$  SD) anthropometric, biological maturation and training history in pubertal girls with different training patterns.

GROUP	CONT (n=33)	SG (n=49)	SPR (n=24)	GYMN (n=23)	SW (n=24)	CCS (n=17)
Age (years)	14.2 $\pm$ 1.1	14.0 $\pm$ 0.9	14.3 $\pm$ 1.1	14.3 $\pm$ 1.0	13.7 $\pm$ 1.2 <sup>a,c,d</sup>	13.9 $\pm$ 0.9
Body height (cm)	163.3 $\pm$ 6.5	167.6 $\pm$ 7.9 <sup>a</sup>	168.1 $\pm$ 6.1 <sup>a</sup>	163.8 $\pm$ 6.7 <sup>b,c</sup>	164.2 $\pm$ 6.8	162.8 $\pm$ 6.4 <sup>b,c</sup>
Body mass (kg)	55.2 $\pm$ 8.1	57.9 $\pm$ 9.6	54.0 $\pm$ 6.6	52.4 $\pm$ 8.9 <sup>b</sup>	55.4 $\pm$ 9.2	52.7 $\pm$ 9.5 <sup>b</sup>
BMI (kg/m <sup>2</sup> )	20.6 $\pm$ 2.4	20.6 $\pm$ 2.8	19.1 $\pm$ 2.0 <sup>a,b</sup>	19.4 $\pm$ 2.4	20.5 $\pm$ 2.9	19.7 $\pm$ 2.6
Tanner stage (1/2/3/4/5)	0/1/5/23/4	0/2/2/39/6	0/2/5/13/4	0/1/7/11/4	0/1/3/18/2	1/1/2/11/2
Girls having menses (n (%))	29 (87.9)	35 (71.4)	19 (79.2)	13 (56.5)	13 (54.2)	10 (58.8)
Menarcheal age (years) <sup>f</sup>	12.5 $\pm$ 0.8	12.2 $\pm$ 0.7	12.8 $\pm$ 1.0 <sup>b</sup>	13.0 $\pm$ 0.7 <sup>b</sup>	12.5 $\pm$ 0.8	12.5 $\pm$ 1.1
Years of training	–	3.8 $\pm$ 1.6	4.4 $\pm$ 2.1	6.5 $\pm$ 1.8 <sup>b,c</sup>	4.8 $\pm$ 1.5 <sup>b,d</sup>	4.3 $\pm$ 2.1 <sup>d</sup>
Training duration (h/week)	–	4.8 $\pm$ 1.3	4.8 $\pm$ 2.2	9.6 $\pm$ 4.9 <sup>b,c</sup>	9.4 $\pm$ 3.2 <sup>b,c</sup>	6.3 $\pm$ 1.1 <sup>d,e</sup>

CONT: control group; SG: sport games group; SPR: track sprinters; GYMN: rhythmic gymnasts; SW: swimmers; CCS: cross-country skiers;

BMI: body mass index

<sup>a</sup> difference from control group

<sup>b</sup> difference from sport games group

<sup>c</sup> difference from track sprint group

<sup>d</sup> difference from rhythmic gymnasts' group

<sup>e</sup> difference from swimmers group

<sup>f</sup> of the girls that have experienced menarche

In all cases –  $p < 0.05$

### **5.2.2. Bone mineral and blood biochemical characteristics of the subjects**

Table 4 shows bone mineral and blood biochemical values in Study II population according to the training patterns. While sport games group girls' femoral neck BMD was greater than in control group ( $p < 0.05$ ), rhythmic gymnasts had significantly greater femoral neck BMD than all the other groups. Girls of cross-country skiing group had significantly lower BMD at lumbar spine than girls practising sport games, track sprint, and rhythmic gymnastics. Girls practising sport games had significantly greater femoral neck BMC than controls, swimmers and cross-country skiers. The BMC at lumbar spine was higher in track sprinters than in cross-country skiers and controls ( $p < 0.05$ ). In sport games group the BMC at lumbar spine was higher than in cross-country skiers ( $p < 0.05$ ). There were no significant differences in the whole-body and volumetric BMD of lumbar spine between the groups.

Serum levels of IGF-1 were significantly higher in cross-country skiers compared to track sprinters and controls (Table 4). In swimmers the levels of IGF-1 were higher than in track sprinters ( $p < 0.05$ ). The IGFBP-3 was significantly lower in track sprinters compared to rhythmic gymnasts and cross-country skiers. The levels of estradiol in track sprinters were significantly higher compared to all the other groups. No differences were found among the groups in IGF-1/IGFBP-3 molar ratio ( $p > 0.05$ ).

**Table 4.** Mean ( $\pm$  SD) bone mineral parameters and blood hormones in pubertal girls with different training patterns.

GROUP	CONT (n=33)	SG (n=49)	SPR (n=24)	GYMN (n=23)	SW (n=24)	CCS (n=17)
Whole-body BMD (g/cm <sup>2</sup> )	1.06 $\pm$ 0.09	1.09 $\pm$ 0.08	1.10 $\pm$ 0.08	1.08 $\pm$ 0.10	1.07 $\pm$ 0.07	1.06 $\pm$ 0.08
BMD femoral neck (g/cm <sup>2</sup> )	1.01 $\pm$ 0.11	1.06 $\pm$ 0.11 <sup>a</sup>	1.05 $\pm$ 0.09	1.13 $\pm$ 0.15 <sup>a,b,c</sup>	1.01 $\pm$ 0.11 <sup>d</sup>	1.01 $\pm$ 0.13 <sup>d</sup>
BMD lumbar spine (g/cm <sup>2</sup> )	1.08 $\pm$ 0.13	1.12 $\pm$ 0.12	1.14 $\pm$ 0.13	1.12 $\pm$ 0.11	1.08 $\pm$ 0.13	1.04 $\pm$ 0.15 <sup>b,c,d</sup>
BMAD lumbar spine (g/cm <sup>3</sup> )	0.36 $\pm$ 0.04	0.36 $\pm$ 0.03	0.36 $\pm$ 0.04	0.35 $\pm$ 0.04	0.36 $\pm$ 0.03	0.36 $\pm$ 0.04
BMC femoral neck (g)	4.7 $\pm$ 0.7	5.1 $\pm$ 0.8 <sup>a</sup>	4.9 $\pm$ 0.5	4.9 $\pm$ 0.7	4.6 $\pm$ 0.7 <sup>b</sup>	4.7 $\pm$ 0.8 <sup>b</sup>
BMC lumbar spine (g)	41.4 $\pm$ 7.9	45.4 $\pm$ 9.8	46.6 $\pm$ 8.6 <sup>a</sup>	44.5 $\pm$ 8.2	41.6 $\pm$ 9.9	38.9 $\pm$ 10.5 <sup>b,c</sup>
IGF-1 ( $\mu$ g/L)	419.2 $\pm$ 144.9	444.4 $\pm$ 84.2	388.3 $\pm$ 92.2	443.2 $\pm$ 104.9	463.7 $\pm$ 138.6 <sup>c</sup>	509.5 $\pm$ 137.0 <sup>a,c</sup>
IGFBP-3 (mg/L)	5.8 $\pm$ 0.9	5.9 $\pm$ 0.8	5.5 $\pm$ 0.7 <sup>b</sup>	6.0 $\pm$ 0.7 <sup>c</sup>	5.9 $\pm$ 0.7	6.3 $\pm$ 1.1 <sup>c</sup>
IGF-1/IGFBP-3 molar ratio	0.26 $\pm$ 0.07	0.27 $\pm$ 0.04	0.26 $\pm$ 0.05	0.27 $\pm$ 0.06	0.28 $\pm$ 0.07	0.30 $\pm$ 0.06
Estradiol (pmol/L)	103.1 $\pm$ 134.5	77.3 $\pm$ 68.0	199.1 $\pm$ 165.4 <sup>a,b</sup>	67.7 $\pm$ 35.2 <sup>c</sup>	108.0 $\pm$ 91.4 <sup>c</sup>	89.7 $\pm$ 63.9 <sup>c</sup>

CONT: control group; SG: sport games group; SPR: track sprinters; GYMN: rhythmic gymnasts; SW: swimmers; CCS: cross-country skiers;

BMD: bone mineral density; BMAD: volumetric BMD of lumbar spine; BMC: bone mineral content; IGF-1: insulin-like growth factor-1;

IGFBP-3: IGF binding protein-3

<sup>a</sup>difference from control group

<sup>b</sup>difference from sport games group

<sup>c</sup>difference from track sprint group

<sup>d</sup>difference from rhythmic gymnasts' group

In all cases –  $p < 0.05$

### 5.2.3. The relationships between bone mineral and blood biochemical parameters

The significant correlations were found between IGF-1, IGF-1/IGFBP-3 molar ratio and BMD at both femoral neck and lumbar spine ( $r=0.39-0.59$ ) in rhythmic gymnasts and controls (Table 5). After adjusting for age, body height, and body mass, the relationships remained significant ( $r=0.46-0.52$ ) only in rhythmic gymnasts' group. Similar patterns were found for BMAD, but no relationships were found between whole-body BMD and blood hormones (data not shown). There were significant correlations between BMD values and estradiol levels ( $r=0.45-0.60$ ) in rhythmic gymnasts' group only. After adjusting for age, body height, and body mass, the associations remained significant ( $r=0.53-0.60$ ). No significant relationships were found between fasting blood hormones and BMD variables in other athlete groups, i.e. sport games, track sprint, swimming and cross-country skiing. In addition, estradiol concentration was related ( $p<0.05$ ) with serum IGF-1 ( $r=0.52$ ) and IGFBP-3 ( $r=0.42$ ) levels only in rhythmic gymnasts' group.

Significant correlations were found between BMC at femoral neck and (a) IGF-1 in controls, sport games group, rhythmic gymnasts, and cross-country skiers; and (b) IGF-1/IGFBP-3 molar ratio in controls and rhythmic gymnasts (Table 6). The correlations were significant between lumbar spine BMC and (a) IGF-1 in controls, sport games group, and cross-country skiers; (b) IGF-1/IGFBP-3 molar ratio in controls; and (c) estradiol in cross-country skiers. After adjusting for age, body height, and body mass, the significant correlations were revealed between femoral neck BMC and IGF-1 and IGF-1/IGFBP-3 molar ratio in rhythmic gymnasts' group only.

**Table 5.** Pearson correlation for BMD and blood hormones in athlete and control groups. Partial correlation analysis (controlled for age, body height, and body mass) is in parentheses.

GROUP	CONT (n=33)	SG (n=49)	SPR (n=24)	GYMN (n=23)	SW (n=24)	CCS (n=17)
<i>BMD femoral neck (g/cm<sup>2</sup>)</i>						
IGF-1 (µg/L)	0.54** (0.31)	0.19 (-0.06)	-0.27 (-0.17)	0.59** (0.52*)	-0.22 (-0.01)	0.60 (-0.84)
IGFBP-3 (mg/L)	0.31 (0.25)	0.03 (-0.10)	-0.18 (-0.36)	0.32 (0.24)	-0.11 (0.22)	0.63 (-0.46)
IGF-1/IGFBP-3 molar ratio	0.57** (0.25)	0.19 (-0.01)	-0.22 (0.02)	0.52* (0.46*)	-0.17 (-0.09)	0.27 (-0.70)
Estradiol (pmol/L)	0.20 (0.21)	0.03 (0.10)	-0.05 (-0.08)	0.60** (0.60**)	0.24 (0.38)	0.43 (-0.12)
<i>BMD lumbar spine (g/cm<sup>2</sup>)</i>						
IGF-1 (µg/L)	0.39* (0.04)	0.18 (-0.04)	-0.00 (-0.06)	0.56** (0.49*)	-0.20 (0.01)	0.52 (-0.38)
IGFBP-3 (mg/L)	0.22 (0.05)	0.03 (-0.07)	-0.14 (-0.31)	0.44* (0.43)	-0.05 (0.20)	0.60 (0.24)
IGF-1/IGFBP-3 molar ratio	0.44* (0.04)	0.19 (0.02)	0.10 (0.11)	0.44* (0.35)	-0.19 (-0.06)	0.19 (-0.72)
Estradiol (pmol/L)	0.10 (0.08)	-0.08 (-0.08)	0.22 (0.40)	0.45* (0.53*)	0.05 (0.16)	0.44 (-0.20)

CONT: control group; SG: sport sprinters; SPR: track sprinters; GYMN: rhythmic gymnasts; SW: swimmers; CCS: cross-country skiers; BMD: bone mineral density; IGF-1: insulin-like growth factor-1; IGFBP-3: IGF binding protein-3

\*p<0.05

\*\*p<0.01

**Table 6.** Pearson correlation for BMC and blood hormones in athlete and control groups. Partial correlation analysis (controlled for age, body height, and body mass) is in parentheses.

GROUP	CONT (n=33)	SG (n=49)	SPR (n=24)	GYMN (n=23)	SW (n=24)	CCS (n=17)
<i>BMC femoral neck (g)</i>						
IGF-1 ( $\mu\text{g/L}$ )	0.47** (0.09)	0.29* (-0.05)	-0.32 (-0.43)	0.58* (0.64*)	-0.30 (-0.14)	0.66* (-0.31)
IGFBP-3 (mg/L)	0.25 (0.04)	0.09 (-0.11)	-0.14 (-0.46)	-0.04 (0.20)	-0.14 (0.14)	0.48 (0.01)
IGF-1/IGFBP-3 molar ratio	0.52** (0.09)	0.27 (0.03)	-0.31 (-0.25)	0.67* (0.77*)	-0.27 (-0.25)	0.47 (-0.54)
Estradiol (pmol/L)	0.21 (0.22)	-0.09 (-0.02)	-0.27 (-0.07)	0.14 (0.21)	0.14 (0.30)	0.51 (-0.50)
<i>BMC lumbar spine (g)</i>						
IGF-1 ( $\mu\text{g/L}$ )	0.38* (-0.14)	0.29* (0.05)	0.01 (-0.13)	0.42 (0.11)	-0.31 (-0.13)	0.69* (-0.17)
IGFBP-3 (mg/L)	0.21 (-0.07)	0.12 (0.08)	0.05 (-0.36)	0.14 (0.08)	-0.12 (0.13)	0.46 (0.20)
IGF-1/IGFBP-3 molar ratio	0.44* (-0.11)	0.25 (-0.02)	-0.03 (0.06)	0.45 (0.17)	-0.30 (-0.22)	0.52 (-0.35)
Estradiol (pmol/L)	0.17 (0.16)	-0.10 (-0.27)	-0.00 (0.37)	0.10 (0.04)	0.08 (0.20)	0.71* (0.63)

CONT: control group; SG: sport sprinters; SPR: track sprinters; GYMN: rhythmic gymnasts; SW: swimmers; CCS: cross-country skiers; BMC: bone mineral content; IGF-1: insulin-like growth factor-1; IGFBP-3: IGF binding protein-3

\*p<0.05

\*\*p<0.01

Stepwise multiple regression analysis indicated that IGF-1 and estradiol together explained 42.6% ( $R^2 \times 100$ ) of the total variance at the femoral neck BMD, and IGF-1 alone 35.4% ( $R^2 \times 100$ ) of the total variance at BMC at femoral neck in rhythmic gymnasts group only (Table 7).

**Table 7.** Results of stepwise multiple regression analysis with femoral neck BMD and BMC as dependent variables and IGF-1, IGFBP-3, and estradiol as independent variables ( $R^2 \times 100$ ) in rhythmic gymnasts' group.

Dependent variable	Independent variable	$R^2 \times 100$	F	SEE	p
BMD femoral neck	IGF-1 and estradiol	42.6	11.6	0.12	0.003
BMC femoral neck	IGF-1	35.4	5.2	0.57	0.046

BMD: bone mineral density; BMC: bone mineral content; IGF-1: insulin-like growth factor-1

### 5.3. Study III

#### 5.3.1. Adipocytokines and bone mineral parameters

Basic characteristics of the groups are presented in Table 3. The mean femoral neck BMD was significantly higher in the rhythmic gymnasts' group than in all other five groups after adjusting for age, body height, and body mass (Table 8). The adjusted lumbar spine BMD was also significantly greater in rhythmic gymnasts' group but only when compared to the control group. Swimmers had significantly lower femoral neck BMC than girls in all other groups except for cross-country skiers. There were no significant differences among the groups with respect to the adjusted lumbar spine BMC.

**Table 8.** Mean ( $\pm$  SD) bone mineral and blood biochemical parameters in pubertal girls with different training patterns.

GROUP	CONT (n=33)	SG (n=49)	SPR (n=24)	GYMN (n=23)	SW (n=24)	CCS (n=17)
<i>Bone variables</i>						
BMD femoral neck (g/cm <sup>2</sup> )	1.01 $\pm$ 0.11	1.06 $\pm$ 0.11	1.05 $\pm$ 0.09	1.13 $\pm$ 0.15 <sup>a,b,c</sup>	1.01 $\pm$ 0.11 <sup>d</sup>	1.01 $\pm$ 0.13 <sup>d</sup>
BMD lumbar spine (g/cm <sup>2</sup> )	1.08 $\pm$ 0.13	1.12 $\pm$ 0.12	1.14 $\pm$ 0.13	1.12 $\pm$ 0.11 <sup>a</sup>	1.08 $\pm$ 0.13	1.04 $\pm$ 0.15
BMC femoral neck (g)	4.7 $\pm$ 0.7	5.1 $\pm$ 0.8	4.9 $\pm$ 0.5	4.9 $\pm$ 0.7	4.6 $\pm$ 0.7 <sup>a,b,c,d</sup>	4.7 $\pm$ 0.8
BMC lumbar spine (g)	41.4 $\pm$ 7.9	45.4 $\pm$ 9.8	46.6 $\pm$ 8.6	44.5 $\pm$ 8.2	41.6 $\pm$ 9.9	38.9 $\pm$ 10.5
<i>Blood biochemical parameters</i>						
Visfatin (ng/mL)	0.66 $\pm$ 0.79	0.95 $\pm$ 0.78	1.01 $\pm$ 0.88	0.92 $\pm$ 0.94	0.77 $\pm$ 0.76	1.02 $\pm$ 0.82
Adiponectin ( $\mu$ g/mL)	16.0 $\pm$ 7.0	14.9 $\pm$ 6.4	17.0 $\pm$ 6.1	14.1 $\pm$ 6.0	13.1 $\pm$ 6.2	10.9 $\pm$ 7.7 <sup>a,c</sup>
Leptin (ng/mL)	8.6 $\pm$ 8.0	11.8 $\pm$ 9.1	7.5 $\pm$ 6.1	7.8 $\pm$ 5.5	11.4 $\pm$ 9.5	8.8 $\pm$ 5.4
Insulin ( $\mu$ IU/mL)	6.0 $\pm$ 3.5	6.2 $\pm$ 4.6	8.0 $\pm$ 3.7 <sup>a,b</sup>	5.6 $\pm$ 3.2 <sup>c</sup>	5.0 $\pm$ 3.5 <sup>c</sup>	7.7 $\pm$ 5.3
Glucose (mmol/L)	4.8 $\pm$ 0.3	4.7 $\pm$ 0.4	5.1 $\pm$ 0.5 <sup>a,b</sup>	4.9 $\pm$ 0.3 <sup>c</sup>	4.9 $\pm$ 0.4 <sup>c</sup>	4.9 $\pm$ 0.3
HOMA	1.31 $\pm$ 0.79	1.30 $\pm$ 0.98	1.87 $\pm$ 0.96 <sup>a,b</sup>	1.22 $\pm$ 0.73 <sup>c</sup>	1.09 $\pm$ 0.73 <sup>c</sup>	1.68 $\pm$ 1.15

CONT: control group; SG: sport games group; SPR: track sprinters; GYMN: rhythmic gymnasts; SW: swimmers; CCS: cross-country skiers; BMD: bone mineral density; BMC: bone mineral content; HOMA: homeostasis model assessment

<sup>a</sup>difference from control group after adjusting for age, height, and body mass

<sup>b</sup>difference from sport games group after adjusting for age, height, and body mass

<sup>c</sup>difference from track sprint group after adjusting for age, height, and body mass

<sup>d</sup>difference from rhythmic gymnasts' group after adjusting for age, height, and body mass

In all cases –  $p < 0.05$

No significant differences in visfatin and leptin concentration levels were found among the studied groups (adjusted data are reported in Table 8). Adiponectin levels were significantly lower in cross-country skiers than in track sprinters and controls. In track sprinters the insulin levels, glucose, and HOMA were significantly higher than in all the other groups except for cross-country skiers.

### **5.3.2. The relationships between adipocytokines and bone mineral parameters**

Visfatin concentrations were not correlated with femoral neck or lumbar spine BMD and BMC in any group ( $p>0.05$ ). Adiponectin was found to be inversely related ( $p<0.05$ ) to BMD at femoral neck ( $r=-0.48$ ) and lumbar spine ( $r=-0.60$ ) as well as to BMC of femoral neck ( $r=-0.47$ ) and lumbar spine ( $r=-0.62$ ) only in the swimmers' group. In addition, the levels of adiponectin were positively related with lumbar spine BMC, but only in sport games group ( $r=0.39$ ). These relationships disappeared after controlling for age, body height, and body mass (data not shown). Leptin concentrations correlated positively with femoral neck and lumbar spine BMD ( $r=0.43-0.52$ ) and femoral neck BMC ( $r=0.56$ ) in the rhythmic gymnasts' group only, even after adjustment for age, body height, and body mass ( $r=0.41-0.63$ ). No relationships between the measured adipocytokines and bone mineral parameters were found in track sprinters, cross-country skiers and controls.

Finally, no relationships were found between visfatin, adiponectin, and leptin in any of the groups ( $p>0.05$ ). Leptin correlated only with HOMA ( $r=0.43$ ) in the rhythmic gymnasts' group. A partial correlation test revealed that correlation between leptin and BMD and BMC in rhythmic gymnasts (reported above) were present even after adjusted for HOMA ( $r=0.72-0.89$ ).

## 6. DISCUSSION

### 6.1. The relationships between bone parameters and jumping height

The participants in Study I were lean, healthy 13–15-year-old pubertal girls representing different sport events with different impact characteristics to the bones. There were no differences between the groups in pubertal status and biological age. The results of the present cross-sectional investigation demonstrated that girls participating in high-impact activities (rhythmic gymnastics and sports games) had higher BMD values at the femoral neck skeletal site compared with moderate-impact (track sprint), low-impact (swimming and cross-country skiing) and physically inactive groups of pubertal girls. In addition, rhythmic gymnasts had higher femoral neck BMD values compared with girls in sport games group. However, BMD at the lumbar spine region of the skeleton did not differ between girls in high-impact sports (rhythmic gymnastics and sport games), medium-impact sports (track sprint) and low-impact sport (swimming) groups (Table 1). This indicates that BMD at the femoral neck is more sensitive to the specific physical activity pattern in pubertal girls compared to BMD at the lumbar spine. Similarly to our results, in the study of specific types of mechanical loading and menstrual status influence on bone tissue in female high school athletes, Nichols et al. [2007 b] found higher BMD at the proximal femur in eumenorrhic athletes participating in high-impact sports (soccer, softball, volleyball, track sprinters and throwers, tennis, or lacrosse) compared to low-impact sports (swimming and long-distance running). Accordingly, it can be suggested that BMD at femoral neck experiences greater mechanical loading during high-impact activities than BMD at lumbar spine.

Different vertical jump tests had no significant correlation with measured areal BMD values in physically inactive controls, low-impact sports (swimming and cross-country skiing) and moderate-impact sport (track sprint) groups. The RJ15s test characterized best BMD at lumbar spine and the RJ30s test characterized best BMD at femoral neck in high-impact sports (rhythmic gymnasts and sport games) groups. Furthermore, the CMJ test was significantly related to BMD at the lumbar spine skeletal region only in rhythmic gymnasts (Table 2). These results suggest that continuous high-impact activities are more important in bone health compared to single jumping activities and the effect of different exercises on areal BMD values is more pronounced at femoral neck compared to lumbar spine region in the skeleton in pubertal girls with different training patterns.

The results of our study demonstrated that girls participating in rhythmic gymnastics benefit the most from mechanical loading of the skeleton to the development of areal BMD, followed by girls practising sport games, while the benefits of track sprint running on areal BMD were less apparent similarly to the previous results [Egan et al. 2006]. However, the difference between

athletes and sedentary controls may be attributable to genetic factors and selection bias. For example, individuals with higher BMD may excel in certain sports (for example, gymnastics), whereas individuals with lower BMD may excel in other sports (for example, swimming) [Williams et al. 2005].

It is interesting to note that single vertical jumping height (CMJ test) did not appear to be a significant predictor of areal BMD values in studied pubertal girls with different training patterns (Table 2). The results of our study demonstrated that repeated jumps (RJ15s and RJ30s) characterize better the areal BMD in pubertal girls. This suggests that repeated high-impact activity is necessary for the development of areal BMD in pubertal girls. Rhythmic gymnasts had higher performance in different jumping tests and significant correlations in areal BMD values and jumping ability. Surprisingly, the sport games group had lower performance in jumping tests than other groups, but revealed a significant relationship with regard to areal BMD and jumping ability. Although track sprinters in our study had high performance in all jumping tests, the relationship between BMD and jumping ability was revealed only in BMD at femoral neck and mean jumping height in RJ15s. The results of our study suggest that repeated jumping test (RJ15s and/or RJ30s) should be applied instead of a single jump test (CMJ) to assess the relationship between muscle performance and BMD in pubertal girls with different training patterns.

In conclusion, the continuous high-impact physical activity has beneficial effects on the development of areal BMD in pubertal girls with different training patterns. BMD at femoral neck appears to be more sensitive to the mechanical loading compared to the BMD at lumbar spine. Finally, repeated jumping tests (RJ15s and RJ30s) characterize bone development better than a single maximal jump test (CMJ) in pubertal girls with different training patterns.

## **6.2. Bone parameters and their relationships with IGF-1 and estradiol concentration**

The results of Study II indicate the specificity of the rhythmic gymnasts' (a very high-impact sport event) group among other athletes. In rhythmic gymnasts, the correlations of femoral neck and lumbar spine BMD with IGF-1 and estradiol, as well as femoral neck BMD and BMC with IGF-1/IGFBP-3 molar ratio, remained significant even after adjusting for age, body height, and body mass (Tables 5 and 6). Additional stepwise multiple regression analysis emphasised the importance of IGF-1 and estradiol on femoral neck BMD and the importance of IGF-1 alone on femoral neck BMC. In contrast, the whole-body BMD did not correlate with the measured blood hormones. Snow et al. [2000] reported IGF-1/IGFBP-3 molar ratio to be the most robust predictor of femoral neck BMD and IGF-1 as a potential mediator of the muscle-bone relationship in young women with different exercise patterns. Recently, in a one-year follow-up study of high-training peripubertal female rhythmic gymnasts, the plasma

IGF-1/IGFBP-3 molar ratio was found to be a strong and independent factor influencing BMD gain at weight-bearing (proximal femur) and at less mechanically solicited (lumbar spine and mid-radius) bone sites as well as at the whole body [Maimoun et al. 2010].

Estradiol is not only a vital determinant for the development of the reproductive system, but also plays a significant role in bone formation. Wang et al. [2006] suggests that estradiol inhibits bone resorption during rapid growth and acts at higher concentrations to promote bone formation after menarche is reached. In our study, serum estradiol levels had a significant correlation ( $p < 0.05$ ) with both femoral neck ( $r = 0.60$ ) and lumbar spine ( $r = 0.45$ ) BMD only in rhythmic gymnasts' group. The same associations were revealed after adjusting for age, body height, and body mass (Table 5). In the cross-sectional study of healthy pubertal girls from Turkey emerged a relationship between the levels of serum estradiol and BMD at lumbar spine [Yilmaz et al. 2005]. Although estradiol is positively associated with total volumetric BMD ( $\text{g}/\text{cm}^3$ ) in pubertal girls, it was found to account for only 5.2% of the variance in total volumetric BMD [Wang et al. 2004]. It appears that estradiol levels differ between pubertal stages and increase with proceeding stages [Kanbur-Öksüz et al. 2004]. The lowest estradiol levels are associated with the lowest BMDs [Voss et al. 1998]. Rhythmic gymnasts, who had the highest femoral neck BMD among all athlete groups and controls, had the lowest estradiol levels (Table 4). Probably their hypoestrogenism was partly compensated by engaging in frequent high-impact loading [Fehling et al. 1995]. On the other hand, estradiol levels in track sprinters were the highest among other athletes and controls, although their BMD was found to be significantly greater only at lumbar spine, and only compared with cross-country skiers (Table 4).

IGF-1 increases dramatically during puberty, augmented by the increasing levels of sex hormones, and positively affects bone turnover by stimulating osteoblasts proliferation and differentiation [Davies et al. 2005]. The relationships of estradiol with serum IGF-1 levels and IGF-1/IGFBP-3 ratios were previously revealed in Kanbur-Öksüz et al. [2004] study. In the present study, estradiol was found to correlate with IGF-1 and IGFBP-3 significantly only in rhythmic gymnasts' group. Correlations between estradiol and IGF-axis indicate that increase in IGF-1 and IGFBP-3 may have affected the measured BMDs in rhythmic gymnasts due to the increases in sex hormones.

The rhythmic gymnasts' femoral neck BMD was the greatest among all the groups in our study and this was to be expected since the previous studies have shown gymnastics training to be beneficial to bone health in children and adolescents [Courteix et al. 2001; Nurmi-Lawton et al. 2004]. In a cross-sectional study of prepubescent girls, Scerpella et al. [2003] observed a dose-dependent relationship between BMD and hours per week of gymnastics activity. Although longer duration of gymnastics participation was associated with greater total and regional BMD, even moderate doses of impact activity were related to increase BMD [Scerpella et al. 2003]. Furthermore, Zanker et al. [2004] reported that an elevated bone mass in female former gymnasts is

retained during early adulthood, in spite of a cessation of training for up to 12 years. In a cross-sectional study of pubertal gymnasts, runners, and nonathletic controls, Lehtonen-Veromaa et al. [2000] found that unadjusted mean value of BMD at femoral neck was 15.2% higher in the pubertal gymnasts than in the controls. Others imply that it is the strain *per se* that generates an adaptive response of bone to loading [Rosen 2005]. The ground-reaction forces generated in rhythmic gymnasts' group was possibly related to this difference in femoral neck BMD among other athletes and controls in the present study.

Running activities are also associated with higher BMD values [Drysdale et al. 2007]. A cross-sectional study of elite adolescent female athletes specializing in endurance events (cyclists, runners, swimmers, triathletes) found runners to have higher BMD at femoral neck than swimmers and controls, and higher BMD at lumbar spine than controls [Duncan et al. 2002]. Nevertheless, gymnasts subject their skeletons to impact forces that are 3-4 times higher than runners [Snow et al. 2000]. Our findings are consistent with those of a previous study of collegiate runners, gymnasts and noncompetitive women, in which gymnasts had significantly higher femoral neck BMD than runners [Snow et al. 2000].

Although swimming as a non-weight-bearing sport is considered to be associated with lower BMD in athletes [Bellew and Gehrig 2006], the results of another cross-sectional study contradicts this, suggesting that swimming activities may be beneficial to bone properties as well [Falk et al. 2004]. The quantitative ultrasound method was used to find that tibial speed of sound was higher in non-elite female swimmers than in controls, indicating a possibility of mechanical loads on the lower extremities (i.e. repeated pushing and pressing against the wall at each turn during swimming training) to affect bone properties other than density [Falk et al. 2004]. However, in the present study, using the DXA method, no significant differences emerged in femoral neck and lumbar spine BMD between swimmers, track sprinters and controls.

Although there were explicit differences among the groups concerning BMD at femoral neck in Study II, these tendencies were not revealed when comparing groups in regard to BMD at lumbar spine (the only exception being cross-country skiers having significantly lower values than the girls of high-impact activity groups – sport games, track sprint, rhythmic gymnastics) (Table 4). The whole-body BMD and BMAD were also similar among the groups. This could be explained by the fact that skeletal response to the weight-bearing exercise is site-specific. Numerous physical activity intervention trials, aiming to increase BMD, have reported the most significant effects at the femoral neck. The reason for this has been seen in the inclusion of different jumping exercises that generate impact forces through the lower limbs and further create a greater loading at the hip than at the spine [MacKelvie et al. 2002]. The greatest increase in BMD among active exercisers occurs at the point of impact (i.e. the hip) and is most pronounced in gymnasts rather than runners or walkers [Rosen 2005]. On the other hand, BMD is being accrued at different rates throughout the skeleton, i.e. limb growth being completed before the growth of the axial

skeleton [Bachrach 2001]. Before puberty, the same amount of mineral mass in the legs is being accrued up to 40%, while in the spine – only 24% [Bass et al. 1999]. In our study, the rhythmic gymnasts were less mature (as indicated by the distribution of Tanner stages and menarcheal age) than the other groups (Table 3). It seems that the differences in BMD are related to the higher impact associated with the rhythmic gymnastics training.

Some limitations of the current study (Study II) include a lack of dietary information, the use of two-dimensional DXA technology, and the differences in training history of the sports-practising girls. Due to the nature of the cross-sectional data analysis, the cause and effect conclusions in the present study could not be drawn. However, this is one of the largest cohorts of adolescent female athletes with information regarding femoral neck and lumbar spine BMD, BMC, IGF-1, IGF-1/IGFBP-3 and estradiol published to date.

In summary, this study demonstrated that femoral neck and lumbar spine BMD correlated with IGF-1, IGF-1/IGFBP-3 molar ratio, and estradiol in pubertal rhythmic gymnasts. No relationships were found between femoral neck and lumbar spine BMD, IGF axis, and sex hormones in other athletes' groups (sport games, track sprint, swimming, and cross-country skiing). Only BMC at femoral neck remained associated with IGF-1/IGFBP-3 molar ratio in rhythmic gymnasts' group after adjusting for age, body height, and body mass.

### **6.3. The relationships between bone parameters and measured adipocytokines**

The main finding of the current study (Study III) was that out of all adipocytokines measured, leptin concentration was found to be the only one related to bone mineral parameters and only in rhythmic gymnasts' group. To our knowledge, this is the first study investigating the relationships of visfatin with bone mineral parameters in pubertal girls with different training patterns. To date, the only study aiming to investigate the relationships of visfatin and bone mineral found visfatin to be related with hip BMD but neither with lumbar spine nor total body BMD in a population of Chinese men aged 20-80 [Peng et al. 2008]. The aforementioned association disappeared after adjusting for age and fat mass [Peng et al. 2008].

On the other hand, the relationships of adiponectin and leptin with bone metabolism have been studied more extensively. In a study of healthy female adolescents, both adiponectin and leptin were related with total body BMD and BMC: levels of adiponectin correlated negatively to total body BMD and BMC, while leptin had a positive relationship [Huang et al. 2004 a]. Similarly, in the current study, femoral neck and lumbar spine BMD and BMC were found to be inversely related to adiponectin levels in the swimmers' group, while femoral neck and lumbar spine BMD and BMC were positively related to leptin concentration levels in the rhythmic gymnasts' group. After age, body height, and body mass adjustments were performed, the association between adiponectin

and bone mineral in swimmers ceased to exist. Interestingly, girls from the swimmers' group had lower BMD and BMC; their adiponectin and insulin concentration levels as well as insulin resistance were also lower (Table 8). While decreased BMD and BMC values in swimmers may be sport-specific [Misra 2008], it is difficult to explain the low levels of adiponectin in this particular group. Ischander et al. [2007] has reported higher adiponectin levels in physically active pubertal girls when comparing them with non-athletic controls. It seems that adiponectin is a significant and independent contributor of lumbar spine, femoral neck, and total body BMD in healthy adolescent girls compared to the girls with anorexia nervosa [Misra et al. 2007]. Similarly, our previous laboratory study revealed adiponectin to be an independent predictor of BMD in healthy females [Jürimäe and Jürimäe 2007].

The relationship of leptin and bone mass is complex: leptin exerts opposite effects on the skeleton, linking the changes in body composition with bone formation and bone resorption [Hamrick and Ferrari 2008]. Correlation study by Roemmich et al. [2003] failed to detect an independent relationship between serum leptin concentrations and total body and regional BMC and BMD. A 4-year follow-up of adolescent girls revealed that leptin deficiency is a primary reason for delayed puberty and menarche in populations accustomed to a negative energy balance, which can be caused by fasting and/or exercise [Matkovic et al. 1997]. In a current study, higher training volume in the swimmers' and rhythmic gymnasts' groups that caused higher energy expenditure may have influenced the relationships between adipocytokines and bone mineral parameters. Track sprinters and rhythmic gymnasts in this study had the lowest leptin levels, and their onset of menarche also occurred later (Tables 3 and 8). Rhythmic gymnasts' femoral neck BMD was greater than in all other groups even after adjusting for age, body height, and body mass; track sprinters' lumbar spine BMD and BMC were greater than in the other athletes and controls, but the difference was not significant ( $p > 0.05$ ) after adjusting for age, body height, and body mass (Table 8). The results presented correspond with the study by Munoz et al. [2004] in which leptin concentrations were found to be lower and femoral neck BMD higher in adolescent rhythmic gymnasts when compared to controls. Courteix et al. [2007] reported leptin concentrations of rhythmic gymnasts to be as low as those observed in anorectic subjects; nevertheless, the BMD and BMC values in gymnasts were greater than in controls, concluding that physical activity counterbalanced the negative effect that low fat mass and leptin deficiency has on bone.

We established no differences in visfatin levels between the girls specializing in different sports and non-athletic controls (Table 8). Higher adiponectin and lower leptin and visfatin levels emerge in lean subjects [Jin et al. 2008]. In a study of healthy adolescent girls, lower leptin and higher adiponectin levels were found in a physically active group when compared to sedentary controls [Ischander et al. 2007]. In our study, the levels of adiponectin were found to be significantly lower in cross-country skiers when compared to track sprinters and controls (after adjusting for age, body height, and body

mass); leptin concentration was lower in track sprinters and rhythmic gymnasts when compared to the other groups, but the difference after adjustment was not significant ( $p>0.05$ ) (Table 8). Furthermore, track sprinters had significantly higher adjusted insulin and glucose levels as well as greater insulin resistance when compared to all other groups, except for cross-country skiers, suggesting that insulin plays a primary role in bone formation, acting as an anabolic agent in bone, preserving and increasing BMD and bone strength [Thraill et al. 2005]. We speculate that greater insulin resistance may partly explain the absence of the relationships between measured bone mineral parameters and blood hormones (including adipokines, insulin and HOMA) in track sprinters.

In our study, leptin correlated with bone mineral parameters and insulin resistance in the rhythmic gymnasts' group. Our finding supports the previously published studies reporting that leptin levels are associated with plasma insulin, independent of body fat in healthy children [Romon et al. 2004] and with insulin resistance in non-diabetic adolescents [Huang et al. 2004 b]. Moreover, insulin also plays an important role in maintaining normal bone mass [Misra et al. 2007], suggesting that the leptin relationship found with bone mineral parameters could be caused by the insulin effect. However, the correlations between leptin and bone mineral parameters in this group were significant after controlling for insulin resistance values, allowing us to conclude that leptin has an independent effect on bone formation.

In summary, our study demonstrates that selected adipocytokines were differently associated with bone mineral parameters in adolescent female athletes. Visfatin was not associated with femoral neck and lumbar spine BMD and BMC in healthy pubertal girls with different training patterns. Adiponectin was inversely related with BMD and BMC in adolescent swimmers, but the relationship did not prove to be significant after controlling for age, body height, and body mass. Leptin was significantly correlated with BMD and BMC of femoral neck and lumbar spine in rhythmic gymnasts even after adjusting for major confounders. We conclude that from all adipocytokines measured, only leptin positively correlated with femoral neck and lumbar spine BMD and femoral neck BMC in rhythmic gymnasts' group.

## 7. CONCLUSIONS

1. Repeated jumping tests (RJ15s and RJ30s) characterize bone development better than a single maximal jump test (CMJ) in representatives of high-impact sport events (sport games and rhythmic gymnastics). Significant relationships were absent in representatives of low-impact sport events (swimming, cross-country skiing) and controls.
2. Femoral neck and lumbar spine BMD correlated with IGF-1, IGF-1/IGFBP-3 molar ratio, and estradiol only in rhythmic gymnasts. No significant relationships were found between bone parameters and studied hormones in other athletes' groups.
3. Leptin is the only adipocytokine from those measured which correlates to femoral neck and lumbar spine BMD and femoral neck BMC in rhythmic gymnasts' group.

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## SUMMARY IN ESTONIAN

### Luuparameetrite seos üleshüppe kõrguse ning hormonaalsete näitajatega puberteedialistel tütarlastel-sportlastel

Viimastel aastatel on osteoporoosi profülaktikaks järjest rohkem hakatud soovitava kehalist aktiivsust, eriti hüppelise iseloomuga koormuste rakendamist. On täheldatud, et nende spordialade esindajatel, kus kasutatakse palju hüppeid (võimlemine, sportmängud) on luude tihedus suurem. Luude kaalu suurenemise ning luukoe mineraliseerumise protsess toimub väga kiiresti puberteediperioodil, mil luukude on eriti tundlik hüppelise suunitlusega treeningutele, samuti toimuvad sel perioodil suured hormonaalsed muutused. Uurimistöös püstitati järgmised hüpoteesid:

1. Luude tihedus on usutavalt seotud sportlase hüppevõimega spordialadel, kus kasutatakse palju hüppeid (näiteks sportmängud ja võimlemine).
2. Erinevate spordialade treeningute spetsiifika avaldab erinevat mõju puhkeoleku hormoonide kontsentratsioonile (eriti IGF-1, IGFBP-3 ja estradiool) ning mitmetele luuparameetritele.
3. Vere visfatiini, adiponektiini ja leptiini sisaldus on usutavalt seotud luuparameetritega erinevate spordialade puberteedialistel tütarlastel.

Uurimistöö hüpoteeside lahendamiseks püstitati järgmised ülesanded:

1. Leida seoseid üleshüppe kõrguse ja luutiheduse vahel selja nimmelülides (L2-L4) ja reieluu kaelal erinevaid spordialasid harrastavatel puberteedialistel tütarlastel.
2. Uurida seoseid luumarkerite ja IGF-1, IGFBP-3 ning estradiooli kontsentratsioonide vahel noorsportlastel.
3. Adipotsütokiinide visfatiini, adiponektiini ja leptiini seoste leidmine luuparameetritega erinevate spordialade esindajatel.

Uuritavateks olid 13-15-aastased erineva kehalise aktiivsuse tasemega puberteedialised tütarlapsed, kes jagunesid järgmistesse gruppidesse: sportmängijad (korvpall, võrkpall, sulgpall) (n=56 esimeses uuringus ning n=49 teises ja kolmandas uuringus), sprinterid (n=25 ja n=24), võimlejad (n=29 ja n=23), ujujad (n=32 ja n=24), murdmaasuusatajad (n=17 ja n=17). Kontrollgrupi (n=43 ja n=33) moodustasid samaealised tütarlapsed, kes osalesid ainult kooli kehalise kasvatuses tundides kaks korda nädalas.

Uuritavatel mõõdeti keha pikkus ja kaal ning arvutati kehamassiindeks. Bioloogilist vanust hinnati Tanneri skaala alusel (Tanner 1962) ning neid küsitleti menstruatsiooni olemasolu kohta. Kõik luuparameetrid (luude tihedus ja kaal L2-L4 selgroolülidel ja reieluu kaelal) määrati DXA meetodil. Paigalt üleshüppe kõrgust määrati spetsiaalse hüppemati abil. Kasutati ühekordse üleshüppe kõrgust ja keskmist üleshüpete kõrgust 15- ja 30-sekundilise testi kestel.

Vereseerumis määrati estradioli, IGF-1, IGFBP-3, visfatiini, adiponektiini ja leptiini kontsentratsioon.

Uuringust tehti järgmised järeldused:

1. Reieluu kaela luutihedus on mehaanilistele koormustele tundlikum kui L2-L4 selgrootülilide tihedus. Pikemaajaliste hüppetestide tulemused on paremini seotud luu parameetritega kui ühekordse üleshüppe kõrgus.
2. Usutav seos nii reieluu kaela kui ka selgrootülilide L2-L4 luutiheduse ja estradioli ning IGF-1/IGFBP-3 molaarse suhte vahel saadi ainult võimlejate grupis.
3. Uuritud adipotsütokiinidest korreleerub ainult leptiin usutavalt luutiheduse ja luu kaaluga reieluu kaelal ja L2-L4 selgrootülilides võimlejate grupis.

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