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ORIGINAL ARTICLE

Adipocytokine and ghrelin levels in relation to bone mineral density in prepubertal rhythmic gymnasts entering puberty: a 3-year follow-up study

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Abstract

Purpose To investigate changes in bone mineral density (BMD) in rhythmic gymnasts (RG) entering puberty and their age-matched untrained controls (UC) over the 36-month period, and associations with leptin, adiponectin and ghrelin over this period.

Methods Whole body (WB), lumbar spine (LS) and femoral neck (FN) BMD, WB bone mineral content (BMC), and leptin, adiponectin and ghrelin were measured in 35 RG and 33 UC girls at baseline and at 12-month intervals over the next 3 years. The change over the 36 months was calculated (Δ score).

Results The pubertal development over the next 36 months was slower in RG compard to UC, while there was no difference in bone age development between the groups. BMD at all sites was higher in RG in comparison with UC at every measurement point. ΔLS BMD and ΔFN BMD, but not ΔWB BMD and ΔWB BMC, were higher in RG compared with UC. None of the measured hormones at baseline or their Δ scores correlated with ΔBMD and ΔBMC in RG. Baseline fat free mass correlated with ΔWB BMD and ΔWB BMC in RG, while baseline leptin was related to ΔWB BMC, ΔWB BMD and ΔLS BMD in UC.

Conclusions Measured baseline hormones and their Δ scores did not correlate with increases in bone mineral values in RG entering puberty. Although the pubertal development in RG was slower than in UC, high-intensity training appeared to increase BMD growth and counterbalance negative effects of slow pubertal develpment, lower fat mass and leptin in RG.

Keywords Rhythmic gymnasts · Puberty · Bone mineral density · Blood biomarkers

Abbreviations
BMC Bone mineral content
BMD Bone mineral density
BMI Body mass index
FFM Fat free mass
FM   Fat mass
FN   Femoral neck
LS   Lumbar spine
RG   Rhythmic gymnasts
UC   Untrained controls
WB   Whole body

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Introduction

Bone is a metabolically active tissue with a constant dynamic process of bone formation and resorption. Bone mineralization increases with age, height and body mass throughout childhood (Jürimäe 2010; Rizzoli et al. 2010). Pubertal development is related to an increased rate of bone mass accumulation over a relative brief period (Maimoun et al. 2013; Theintz et al. 1992). It is also well established

that regular high-impact weight-bearing physical activity plays an important role in maximizing bone mass gain during growth and maturation (Gruodyte-Raciene et al. 2013; Jürimäe 2010; Rizzoli et al. 2010). High impact mechanical loading is one of the most important and most effective factors determing bone mineral accrual (Jackowski et al. 2015; MacKelvie et al. 2002). Rhythmic gymnasts (RG) practice an intensive, weight-bearing sport, which is also known as a high-impact bone loading sport (Gruodyté et al. 2010; Maimoun et al. 2010). Many cross-sectional studies have demonstrated significantly higher bone mineral density (BMD) values at the load-bearing sites of the skeleton in prepubertal (Parm et al. 2011), early pubertal (Munoz et al. 2004), adolescent (Gruodyté et al. 2010) and adult (Helge and Kanstrup 2002) gymnasts in comparison with healthy untrained controls (UC). However, there are very limited longitudinal data on the effects of regular high-impact weight-bearing athletic activity on bone mass acquisition, and these studies in gymnasts to date have followed the development of BMD for only 1 year (Bass et al. 1998; Maimoun et al. 2010, 2013; Nickols-Richardson et al. 1999; Parm et al. 2012), and have been conducted only in prepubertal years (Bass et al. 1998; Nickols-Richardson et al. 1999; Parm et al. 2012) or in a very heterogeneous group of gymnasts at different age and sexual maturation (Maimoun et al. 2010, 2013).

BMD is highly dependent on body mass that generates additional mechanical loading on the growing skeleton (Pacifico et al. 2009; Reid 2002) and body mass is therefore a strong predictor of BMD (Rizzoli et al. 2010). Body fat mass (FM) and fat free mass (FFM) are both positively associated with BMD during pubertal development in girls (Gruodyté et al. 2010; Maimoun et al. 2010). Furthermore, a positive influence of FM on bone mineral acquisition has been attributed to a combination of mechanical loading (Reid 2002) and the impact of several hormones linked to adipose tissue (Jürimäe 2014), such as leptin (Gruodyté et al. 2010; Remmel et al. 2015), adiponectin (Misra et al. 2007; Sayers et al. 2010) and ghrelin (Misra et al. 2005; Pacifico et al. 2009). Prolonged and intense training in childhood can lead to a relative state of energy deficiency, lower body FM and changes in these hormone levels (Jürimäe 2014). Thus, chronic physical exercise with high energy expenditure decreases basal leptin and increases basal adiponectin and ghrelin concentrations in young athletes (Jürimäe et al. 2011). However, in pubertal RG who had hypoleptinemia caused by intensive and stressful trainings in the presence of elevated energy expenditure, BMD was not affected (Courteix et al. 2007). Furthermore, basal leptin, adiponectin and ghrelin levels were not correlated with the increment of BMD before puberty in RG, whereas in healthy UC the increment of BMD was associated with the basal levels of these hormones (Parm et al. 2012).

Accordingly, regular physical activity may modify the possible relationships between these circulating adipose-modulated biochemical signals and bone mineral values in children during growth and pubertal maturation (Parm et al. 2011, 2012).

To date, the reports on the relationships of circulating leptin, adiponectin and ghrelin concentrations with bone mineralization during linear growth remain contradictory (Parm et al. 2012). It also has to be considered that leptin levels increase progressively during growth and pubertal maturation following a pattern that parallels increases in subcutaneous fat depots (Roemmich et al. 2003) as well as in body mass (Maimoun et al. 2010; Võsoberg et al. 2014). In addition, circulating adiponectin increases and ghrelin decreases in healthy UC girls entering puberty (Võsoberg et al. 2014). To our best of knowledge, no studies have been conducted to examine longitudinally the influence of leptin, adiponectin and ghrelin on bone mineralization in a specific group of prepubertal RG entering puberty. Therefore, the aim of this 3-year prospective study was to investigate the increases in BMD and bone mineral content (BMC) values during pubertal development in RG and their age-matched UC girls, and to evaluate the associations between leptin, adiponectin and ghrelin with BMD and BMC changes.

Materials and methods

Participants

Participants in this study were 35 RG and 33 UC Estonian prepubertal girls at the mean age of 8.0 ± 0.6 and 8.2 ± 0.6 years, respectively, at baseline, who were followed at 12-month intervals for the next 3-year period. All RG were recruited from the local training groups and had trained 6–14 h per week for the past 2 years before starting the study. All RG had very similar training lessons, including rhythmic gymnastics, ballet, and acrobatics 4–7 times a week. All RG were competing at the national level (Võsoberg et al. 2014). Control subjects were recruited from local secondary schools and had physical education classes twice a week. Participation only in compulsory school physical education classes (2–3 times of 45 min each) was inclusion criteria for UC subjects (Parm et al. 2011). None of the participants were receiving any medications or had a history of bone or renal diseases. Throughout the study period, no restrictions were placed on dietary intake and participants consumed their everyday diet (Jürimäe et al. 2007). The study protocol was reviewed and approved by the Medical Ethics Committee of the University of Tartu (Estonia), and was explained to the girls and their parents. All RG, UC and their parents gave written informed consent before entering the study.

Experimental design

Participants were studied once a year at 12-month intervals during a 3-year study period. Height, body mass, BMI, pubertal stage, bone age, body composition, BMC, BMD, and fasting blood samples were taken at baseline (T0), after 12 month (T1), 24 month (T2) and 36 month (T3) study period. Both absolute values as well as changes between T3 and T0 (Δ scores) were used in the analyses (Võsoberg et al. 2014). Body composition, sexual maturation and hormonal data of these subjects have been previously reported (Võsoberg et al. 2014), but not bone age, BMD and BMC characteristics.

Biological maturation

Pubertal development was assessed by self-report using an illustrated questionnaire of pubertal stages according to the criteria of Tanner (1962), which has been previously validated (Matsudo and Matsudo 1994) and used successfully in previous studies with girls (Gruodyté et al. 2010; Jürimäe et al. 2007, 2010). The girls 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. If a disagreement between the development of breast and pubic hair was found, the final decision was made according to the breast development (Matsudo and Matsudo 1994). Bone age was assessed with an X-ray of the left hand and wrist and determined according to the method of Greulich and Pyle (1959).

Bone mineral density and body composition assessment

Bone mineral density (BMD, g/cm^2) of the whole body (WB), lumbar spine (L2–L4) (LS), femoral neck (FN), and the WB BMC (g), FM (kg) and FFM (kg) 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 the back, with arms at their sides. The fast scan mode and standard subject positioning were used for total body measurements, and were analyzed using the extended analysis option. DXA measurements and results were evaluated by the same examiner. Coefficients of variations (CVs) for bone mineral and body composition measurements were less than 2 % in our laboratory (Jürimäe et al. 2005).

Blood analysis

Venous blood samples were drawn between 7:30 and 8:30 a.m. after an overnight fast from an antecubital vein with the participant sitting in the upright position. The plasma

was separated and frozen at −20 °C for later analysis. Samples from one individual were run in the same assay, and all hormones were determined in duplicate. Leptin concentration was determined by radioimmunoassay (RIA) (Mediagnost GmbH, Reutlingen, Germany). This assay has intra- and interassay CVs less than 5 %, and the least detection limit was 0.01 ng/mL. Adiponectin was determined with a commercially available RIA kit (Linco Research, St. Charles, MO, USA). The intra- and interassay CVs were less than 7 %, and the least detection limit was 1 μg/mL. Ghrelin was determined using a commercially available RIA kit (Linco Research, St. Charles, MO, USA). The sensitivity of this kit was 93 pg/mL, and intra- and interassay CVs were less than 10 and 14.7 %, respectively.

Statistical analysis

All statistical analyses were performed using SPSS version 20.0 package for Windows (Chicago, IL, USA). Mean and standard deviation (±SD) were calculated using standard statistical methods. Comparison of baseline measurements and the changes over time between RG and UC were made by ANOVA and paired t tests. Spearman correlation coefficient was used to examine the bivariate relationships between baseline blood biochemical and body composition values and the increases in bone mineral parameters as well as between the changes in blood biochemical and body composition variables, and the changes in BMD and BMC values over the 36-month study period (Võsoberg et al. 2014). Stepwise multiple regression analysis was performed to determine the possible independent associations of an increase in measured BMD and BMC values over the 36-month study period with baseline age, bone age, BMI, FM, FFM, leptin, adiponectin and ghrelin values (Parm et al. 2012). Significance was set at $p < 0.05$.

Results

Mean age, bone age and FFM were not different at baseline and increased similarly in both groups over the 36-month study period (Table 1), whereas pubertal progression was slower in RG compared to UC. All girls were prepubertal at the beginning of study. After the first 12-month period, all girls in RG remained at pubertal stage 1, while seven girls in UC were at pubertal stage 2. After 36-month period, only four out of 35 girls in RG reached at least pubertal stage 3, while there were 14 girls in the UC who reached at least pubertal stage 3 (Võsoberg et al. 2014). RG had lower mean FM compared to UC and this difference remained throughout the study period (Table 1). Similarly, RG had significantly ($p < 0.05$) lower BMI in comparison with UC at all measurement points, while BMI

Table 1 Mean (±SD) clinical characteristics of subjects and their changes over the 36-month period (Δ score)

Variable	RG (n = 35)	UC (n = 33)	ΔRG (n = 35)	ΔUC (n = 33)
Age (years)				
T0	8.0 ± 0.6	8.2 ± 0.6	3.0 ± 0.1	3.0 ± 0.2
T1	9.0 ± 0.6	9.2 ± 0.6		
T2	10.0 ± 0.6	10.2 ± 0.6		
T3	10.9 ± 0.6[#]	11.2 ± 0.5[#]		
Bone age (years)				
T0	7.9 ± 1.3	7.7 ± 1.0	3.1 ± 1.0	3.4 ± 0.8
T1	8.7 ± 1.4	9.0 ± 0.8		
T2	9.8 ± 1.2	10.3 ± 1.1		
T3	10.9 ± 0.9[#]	11.1 ± 1.0[#]		
Fat mass (kg)				
T0	4.8 ± 1.8	6.8 ± 3.0*	2.2 ± 1.4	5.1 ± 2.9[‡]
T1	5.6 ± 1.9	8.1 ± 3.6*		
T2	6.2 ± 1.9	9.6 ± 4.5*		
T3	7.0 ± 2.3#	11.7 ± 5.3*[#]		
Fat free mass (kg)				
T0	20.3 ± 1.8	19.7 ± 2.5	6.7 ± 1.8	7.4 ± 2.5
T1	22.2 ± 2.0	21.8 ± 2.9		
T2	24.4 ± 2.4	23.8 ± 3.6		
T3	27.0 ± 3.0[#]	27.0 ± 4.4[#]		
WB BMC (g)				
T0	1002.5 ± 117.3	933.8 ± 157.6*	474.6 ± 104.4	507.4 ± 171.1
T1	1145.5 ± 132.1	1081.4 ± 172.8		
T2	1304.0 ± 149.9	1218.0 ± 229.1		
T3	1477.1 ± 188.8[#]	1441.2 ± 308.9[#]		
WB BMD (g/cm^2)				
T0	0.87 ± 0.04	0.84 ± 0.05*	0.08 ± 0.02	0.09 ± 0.03
T1	0.90 ± 0.04	0.87 ± 0.05*		
T2	0.93 ± 0.04	0.89 ± 0.06*		
T3	0.96 ± 0.04[#]	0.93 ± 0.06*[#]		
LS BMD (g/cm^2)				
T0	0.75 ± 0.08	0.69 ± 0.08*	0.16 ± 0.05	0.13 ± 0.06‡
T1	0.89 ± 0.08	0.73 ± 0.09*		
T2	0.86 ± 0.08	0.76 ± 0.10*		
T3	0.91 ± 0.09[#]	0.82 ± 0.13*[#]		
FN BMD (g/cm^2)				
T0	0.78 ± 0.07	0.71 ± 0.08*	0.14 ± 0.06	0.11 ± 0.07‡
T1	0.83 ± 0.07	0.74 ± 0.09*		
T2	0.87 ± 0.09	0.76 ± 0.09*		
T3	0.93 ± 0.09#	0.82 ± 0.1*[#]		

TO Baseline, *T1* after 12 months, *T2* after 24 months, *T3* after 36 months, *WB* whole body, *BMC* bone mineral content, *BMD* bone mineral density, *LS* lumbar spine, *FN* femoral neck

* Statistically significant differences between RG and UC; $p < 0.05$

[#] Statistically significant changes between T3 and T0; $p < 0.05$

[‡] Statistically significant changes between Δ RG and Δ UC; $p < 0.05$

increased significantly in both groups over the 36-month study period (RG, T0: 15.7 ± 1.2; T3: 16.7 ± 1.5 kg/m^2; UC, T0: 16.7 ± 2.3; T3: 18.6 ± 3.1 kg/m^2). However, the increase in BMI was significantly ($p < 0.05$) lower in RG in comparison with UC (0.9 ± 0.8 vs 2.0 ± 1.6 kg/m^2, respectively). Over the 36-month period, both groups

Table 2 Spearman correlation coefficients between changes (Δ scores) in bone mineral values during the 36-month study period with baseline body composition and blood biochemical variables

Variable	Rhythmic gymnasts ($n = 35$)				Untrained controls ($n = 33$)			
	ΔWB BMC (g)	ΔWB BMD (g/cm^2)	ΔLS BMD (g/cm^2)	ΔFN BMD (g/cm^2)	ΔWB BMC (g)	ΔWB BMD (g/cm^2)	ΔLS BMD (g/cm^2)	ΔFN BMD (g/cm^2)
Age (years)	0.33	0.23	0.23	−0.02	0.50*	0.36*	0.22	0.24
Bone age (years)	0.16	0.10	0.16	−0.06	0.49*	0.47*	0.28	0.17
BMI (kg/m^2)	0.20	0.20	0.04	0.19	0.62*	0.49*	0.22	0.24
Fat mass (kg)	0.26	0.22	0.20	0.16	0.76*	0.64*	0.44*	0.40*
Fat free mass (kg)	0.44*	0.38*	0.22	0.27	0.73*	0.65*	0.42*	0.38*
Leptin (ng/mL)	0.03	0.11	0.09	0.21	0.66*	0.73*	0.45*	0.29
Adiponectin (μg/mL)	0.01	−0.08	−0.04	−0.06	−0.33	−0.25	−0.47*	−0.20
Ghrelin (pg/mL)	−0.16	−0.21	−0.09	0.06	−0.17	−0.36*	−0.10	0.04

WB Whole body, *BMC* bone mineral content, *BMD* bone mineral density, *LS* lumbar spine, *FN* femoral neck

* Statistically significant; $p < 0.05$

showed a significant increase in body height (RG, T0: 1.30 ± 0.05; T3: 1.47 ± 0.06 m; UC: T0: 1.29 ± 0.06; T3: 1.48 ± 0.07 m), which was not different ($p > 0.05$) between RG and UC at any measurement point (Võsoberg et al. 2014). RG had higher ($p < 0.05$) mean WB, LS and FN BMD compared to the UC and these differences remained at each time points over the 36-month study period. The increments in LS BMD and FN BMD were higher in RG than in UC (Table 1). While mean WB BMC was higher ($p < 0.05$) in RG in comparison with UC at the beginning of the study, no difference in WB BMC was observed in other measurement points between groups (Table 1). However, WB BMC increased significantly over the 36-month period in both groups.

RG had significantly ($p < 0.05$) lower leptin level and leptin remained relatively unchanged over the 36-month study period (T0: 2.1 ± 1.4; T3: 2.4 ± 1.1 ng/mL) in RG in comparison with UC. Leptin concentration in UC significantly increased ($p < 0.05$) over the 36-month study period (T0: 4.1 ± 2.7; T3: 7.5 ± 4.4 ng/mL). Adiponectin concentrations were not different between the groups during the first 24-month of the study, while RG (T0: 10.0 ± 3.9; T3: 12.9 ± 5.8 μg/mL) had significantly higher ($p < 0.05$) adiponectin levels than UC (TO: 10.2 ± 4.5 μg/mL; T3: 9.8 ± 3.6 μg/mL) after the 36-month study period. Ghrelin levels were significantly ($p < 0.05$) higher at the beginning and after the first 12-month period but not after the 24- or 36-month study period in RG (T0: 1429.4 ± 495.2; T3: 1077.7 ± 424.4 pg/mL) compared with UC (T0: 1200.6 ± 401.9; T3: 1054.9 ± 599.9 pg/mL). Therefore, the changes (Δ scores) over the 36-month study period

were significantly ($p < 0.05$) different in leptin and ghrelin values between studied groups (Võsoberg et al. 2014).

In the RG, the only baseline body composition and hormonal characteristics correlated significantly to increases in bone mineral values was baseline FFM, which was positively related to Δ WB BMD and Δ WB BMC (Table 2). The changes in BMI, FM and FFM were positively correlated to changes in WB BMD and WB BMC in the RG (Table 3). However, if we looked the girls in the RG who remained prepubertal (pubertal stage 1) at the end of the study ($n = 8$), the increase in adiponectin level was significantly correlated to Δ WB BMD ($r = 0.82$; $p < 0.05$). Stepwise multiple regression analysis revealed that baseline FFM was a significant predictor of Δ WB BMD and Δ WB BMC in RG, explaining 21.8 and 13.3 % of their variabilites, respectively. Baseline leptin, adiponectin and ghrelin levels did not predict ($p > 0.05$) increases in measured BMD and BMC values over the 36-month study period in RG.

In the UC, baseline age, BMI, FM, FFM and leptin (Fig. 1) were all positively, and ghrelin negatively correlated with Δ WB BMD, whereas baseline adiponectin was negatively correlated to Δ LS BMD (Table 2). Similarly, baseline age, bone age, BMI, FM, FFM and leptin were positively related to Δ WB BMC. Δ leptin was correlated to Δ WB BMC, Δ LS BMD and Δ FN BMD, but not to Δ WB BMD (Table 3). Stepwise multiple regression analysis demonstrated that baseline FFM was the most significant predictor of Δ WB BMC, Δ WB BMD and Δ LS BMD values, explaining 52.3, 28.8 and 10.5 % of their variabilities, respectively. The most important predictor of Δ FN

Table 3 Spearman correlation coefficients between changes (Δ scores) in bone mineral values during the 36-month study period with changes (Δ scores) in body composition and blood biochemical variables

Variable	Rhythmic gymnasts ($n = 35$)				Untrained controls ($n = 33$)			
	ΔWB BMC (g)	ΔWB BMD (g/cm^2)	ΔLS BMD (g/cm^2)	ΔFN BMD (g/cm^2)	ΔWB BMC (g)	ΔWB BMD (g/cm^2)	ΔLS BMD (g/cm^2)	ΔFN BMD (g/cm^2)
ΔAge (years)	−0.16	−0.11	0.05	−0.17	−0.13	−0.11	−0.03	0.06
ΔBone age (years)	0.23	0.07	0.26	−0.14	0.20	0.11	−0.04	0.16
ΔBMI (kg/m^2)	0.43*	0.36*	0.31	0.12	0.59*	0.44*	0.44*	0.55*
ΔFat mass (kg)	0.50*	0.42*	0.18	0.13	0.66*	0.47*	0.42*	0.45*
ΔFat free mass (kg)	0.74*	0.42*	0.63*	0.24	0.82*	0.83*	0.60*	0.61*
ΔLeptin (ng/mL)	0.33	0.22	0.15	-0.20	0.52*	0.32	0.38*	0.45*
ΔAdiponectin (μg/mL)	−0.19	−0.05	−0.27	0.22	0.22	0.10	0.27	0.19
ΔGhrelin (pg/mL)	−0.01	0.11	0.08	0.23	−0.24	−0.06	−0.06	−0.11

WB Whole body, *BMD* bone mineral density, *LS* lumbar spine, *FN* femoral neck

* Statistically significant; $p < 0.05$

BMD was baseline FM, explaining 13.6 % of the variability in UC.

Discussion

Different body composition and hormonal factors may predict an increase in BMD values in girls with different physical activity levels entering from prepubertry to puberty. Our 3-year longitudinal study was conducted to describe the changes in BMD and BMC in RG and age-matched UC girls entering puberty, and to study the possible role of leptin, adiponectin and ghrelin as predictors of these increases. As already reported, later onset of puberty and a slower progression was found in RG (Võsoberg et al. 2014), similar to the study of Georgopoulos et al. (2010). The 3-year prospective study demonstrated significant changes in measured adipocytokine, ghrelin and body composition variables in RG and UC, while no significant increases in leptin levels were seen in RG. In addition, all measured BMD and BMC values were higher in RG compared to UC, and while these values increased in both groups over the 3-year period, the increases in LS and FN BMD were significantly higher in RG when compared with UC. It appeared that baseline hormone concentrations together with specific body composition indices were associated with an increase in measured BMD and BMC values in UC, but not in RG. These results suggest that although the pubertal development in RG may be slower due to the chronic intense physical exercise (Donoso et al. 2010;

Võsoberg et al. 2014), mechanical loading of high-intensity rhythmic gymnastic training has beneficial effect on bone mineralization and may have counterbalanced such negative factors on bone development as low FM and leptin levels in prepubertal RG entering puberty.

Mean BMD values of all measured sites were significantly higher at all measurement times in RG in comparison with UC, indicating that regular high-impact weight-bearing athletic activity may promote significant annual gains in BMD in prepubertal RG entering puberty. Furthermore, RG presented significantly higher annual gains in LS and FN BMD values compared with UC. These results suggest that mechanical loading of high-impact physical exercises appears to be a more important factor for bone mineralization than inadequate energy intake due to the chronic high intense physical exercise as indicated by relatively low FM and leptin values in RG. Our results are similar to the study by Maimoun et al. (2010) where 1-year follow-up in a heterogenous group of peripubertal female RG with intensive gymnastics training demonstrated a significant increase of BMD in all measured bone sites throughout puberty. The annual gain in BMD values in our RG was very close to the results of Slemenda et al. (1994) where the mean annual gains in LS and FN BMD were 0.077 and 0.047 g/cm^2 per year, very similar to the respective values of 0.053 and 0.047 g/cm^2 in the present study. The smaller annual gain in LS BMD in the present study can be due to the gender effect as the former study investigated both boys and girls.

In addition to the importance of regular high-impact weight-bearing athletic activity in bone mineral acquisition,
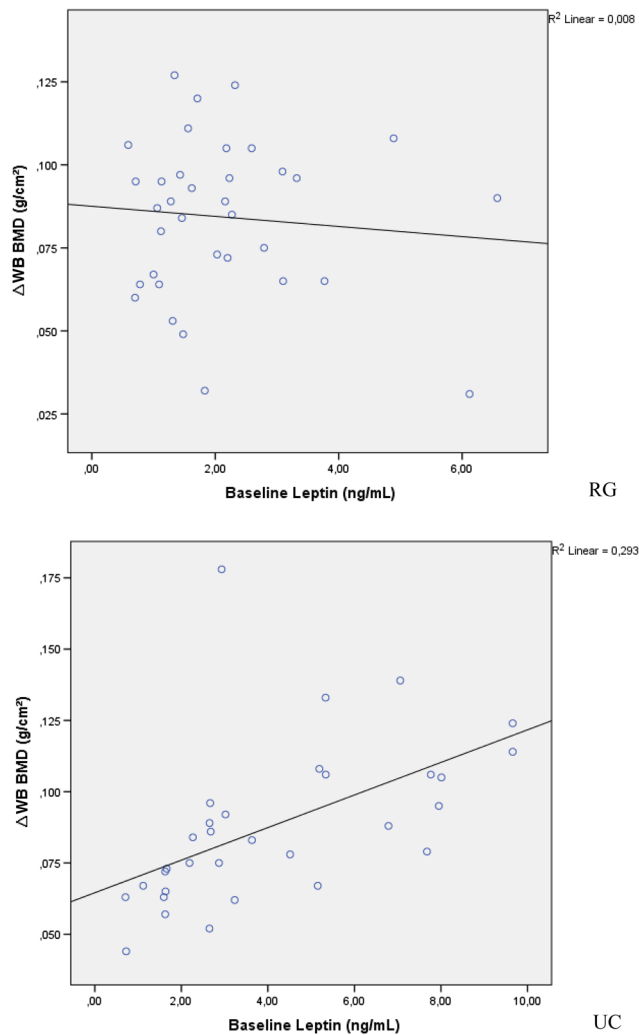
Fig. 1 Baseline leptin concentration association with **Δ** whole body BMD in rhythmic gymnasts (RG) and untrained controls (UC)

baseline FFM and Δ FFM were the most significant predictors of Δ WB BMD and BMC values in RG. The results demonstrated that FFM values were better determinants of bone mineralization than FM or measured blood hormonal values in both groups of girls with different physical activity levels entering from prepuberty to puberty. To date, there is a disagreement in the literature regarding the relative contributions of fat and fat free body compartments to bone mineral values in growing children (Ivuskans et al. 2013). It has been suggested that the association between FFM and FM values with measures of BMD and/or BMC could be dependent on the weight status of the studied population (Ivuskans et al. 2013). It appears that FFM is a better determinant of bone mineral acquisition in normal weight children (Ivuskans et al. 2013), which is in accordance with our results. In contrast, FM characterizes better BMD in overweight children (Ivuskans et al. 2013). In

agreement with our results, an independent association of FFM with increased BMD and BMC values has been demonstrated in different study populations (Cure-Cure et al. 2005; Moon 2014).

Interestingly, the 36-month study period did not cause any delay in bone age maturation in our prepubertal RG entering puberty. This is similar to other studies with prepubertal RG (Courteix et al. 1999; Parm et al. 2011) whereas studies in pubertal RG found delayed bone age (Maimoun et al. 2013; Munoz et al. 2004). In elite RG, the prepubertal stage is prolonged and pubertal maturation is shifted to a later age following the bone maturation rather than the chronological age (Georgopoulos et al. 2010). Thus, due to the delayed sexual maturation bone mass acquisition lasts longer in RG (Maimoun et al. 2013) and they have high likelyhood to achieve high peak bone mass. Our results also suggest that in spite of later onset of puberty and its slower progression in RG, their mean BMD at all measured sites was higer at all time points and the mean gain in BMD over the study period was similar (WB) or higher (LS and FN) compared to age-matched UC.

There was no change in leptin over the 36-month study period in prepubertal RG entering puberty. In accordance, low leptin concentrations have been reported in young female athletes in relation to their reduced body mass (Jürimäe 2014). Therefore, leptin levels rise in parallel with the increase in FM in highly trained RG even with a reduced amount of adipose tissue during puberty (Maimoun et al. 2010). Our findings are similar to the results of previous longitudinal studies with prepubertal (Parm et al. 2012) and peripubertal (Maimoun et al. 2010) RG. In the RG, neither baseline leptin nor the Δ leptin correlated to Δ BMD and Δ BMC values. However, in the UC, baseline leptin was significantly correlated to Δ WB and Δ LS BMD, and Δ WB BMC values, whereas Δ leptin correlated also to Δ WB BMC, and Δ LS and Δ FN BMD values, similar to many other studies in untrained children (Parm et al. 2011; Remmel et al. 2015; Rhie et al. 2010). Although leptin has been reported to be independent predictor of BMD values in adults (Lorentzon et al. 2006), its impact on bone mineralization in growing human bone remains controversial and may be altered by the specific chronic athletic activity (Maimoun et al. 2010; Parm et al. 2011). The results of the present investigation suggest that leptin is not longitudinally involved in bone mineral acquisition in prepubertal RG entering puberty, while leptin may play a biological role in regulating bone metabolism in UC.

Baseline adiponectin levels were not correlated with increases in measured BMD and BMC values in prepubertal RG entering puberty, neither were changes in adiponectin levels related to changes in measured BMD and BMC values in the whole group of RG over the 36-month study period. These results demonstrate that adiponectin is not

involved in bone mineralization in RG entering from prepuberty to puberty. In the UC, we found a negative correlation between baseline adiponectin and Δ LS BMD. Adiponectin has been found to be a negative independent predictor of LS BMD in healthy untrained adolescent girls (Misra et al. 2007) and healthy females (Jürimäe et al. 2005), and has been suggested as a link between bone and fat metabolism (Donoso et al. 2010). However, if we split the RG group into two, then in the group who remained prepubertal over the 36-month study period ($n = 8$), the increase in adiponectin was positively correlated to increase in WB BMD. Similar positive correlation has been found in early pubertal ballet dancers (Donoso et al. 2010). These positive associations are possibly due to the intense exercise that can increase circulating adiponectin level (Donoso et al. 2010; Jürimäe 2014). Taken together, our longitudinal results show that adiponectin may be involved in bone mineral development in prepuberty in RG.

In our study, baseline ghrelin was not associated with significant increases in bone mineral values, and Δ ghrelin was not correlated with Δ BMD and Δ BMC in RG during the 36-month study period. This is in accordance with the results of adolescent female swimmers with high energy expenditure values (Jürimäe et al. 2010). Although some studies have found significant associations of ghrelin levels with BMD variables in girls (Makovey et al. 2007; Misra et al. 2005), the relatively high ghrelin levels at the beginning of the study and throughout the study period caused by elevated energy expenditure could explain why there was no relationship between ghrelin and BMD values in RG. In contrast, baseline ghrelin was associated with Δ WB BMD in UC, confirming that physical activity may modify the association between ghrelin and bone mineralization in girls during growth and pubertal maturation. These results together indicate that circulating ghrelin is not involved in bone mineralization in prepubertal RG entering puberty, and chronic physical activity may modify the relationship between ghrelin and bone.

The strength of the current study is the 36-month longitudinal observation period, which allowed us to have more reliable data on the bone mineral acquisition and actual changes in blood hormonal and body composition parameters in a specific group of prepubertal RG entering puberty, compared to cross-sectional design or 12-month observation period. The used longitudinal design indicated positive effects of regular high-impact weight-bearing athletic activity on bone mineralization in a specific group of growing athletes despite their low body fat values. The limitations of the study include the DXA measurement of BMD as DXA-derived BMD is an areal rather than a true volumetric BMD measurement, and may not precisely reflect bone mineral acquisition (Binkovitz and Henwood 2007). However, similarly to our study, previous longitudinal studies

have also used DXA and measured areal BMD values in RG (Maimoun et al. 2010, 2013; Parm et al. 2012). In addition, BMC was also measured in our RG, which is determined with great precision by DXA in growing children (Binkovitz and Henwood 2007) and used in other longitudinal studies with young recreational gymnasts (Gruodyte-Raciene et al. 2013; Jackowski et al. 2015). Another limitation was that we were not able to track the nutrition of the subjects (including calcium and vitamin D intake) and the possible interaction with bone mineral acquisition. In the future, young RG should be evaluated at a shorter intervals to more precisely monitor growth and maturation during a sensitive period of pubertal development.

In conclusion, baseline leptin, adiponectin and ghrelin, and their Δ scores did not correlate with Δ BMD in RG entering puberty. Although the pubertal development in RG was slower than in UC, mechanical loading of high-intensity training may have beneficial effect on BMD rise and have counterbalanced the negative effects of slow pubertal development, lower FM and leptin levels in RG.

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