Bone Mineralization in Rhythmic Gymnasts before Puberty: No Longitudinal Associations with Adipocytokine and Ghrelin Levels

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Key Words

Rhythmic gymnasts · Bone mineral density · Adipocytokines · Ghrelin

Abstract

Aim: Relationships between change of bone mineral density (BMD) and baseline adipocytokine and ghrelin levels over 12 months in prepubertal rhythmic gymnasts (RG) and age-matched untrained controls (UC) were studied. **Methods:** Adipocytokine, ghrelin, body composition, BMD and bone age values were measured in RG (8.0 ± 0.6 years; $n = 33$) and UC (8.2 ± 0.6 years; $n = 35$). **Results:** Increases in BMD and body composition parameters were seen in both groups as a result of the 12-month study period. Adiponectin increased and ghrelin decreased in both groups, leptin increased only in UC. Measured bone age, body composition and hormone values did not predict increases in BMD values in RG. The variables that were associated with increases in whole-body (Δ WB) and femoral neck (Δ FN) BMD values were fat mass and fat-free mass together in UC. Ghrelin and adiponectin were the most important hormonal predictors of Δ WB BMD and Δ FN BMD values in UC, respectively. In RG, adiponectin and ghrelin levels did not predict increases in measured BMD values. **Conclusion:** Body composition parameters and hor-

mone levels did not predict normal growth in measured BMD values as a result of the 12-month study period in RG. However, leptin together with specific body composition variables was associated with an increase in Δ WB BMD value in UC.

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Introduction

Prepubertal time period is a very sensitive period for bone mineral accumulation in children [1], which is influenced by genetic potential, endocrine status, nutritional factors, body composition and physical activity [2, 3]. Regular high-impact weight-bearing physical activity during growth and maturation plays an important role in maximizing bone mineral mass gain [3]. A number of cross-sectional studies have demonstrated significantly higher bone mineral density (BMD) values at the load-bearing sites of the skeleton in prepubertal [4], early pubertal [5] and adolescent [6] rhythmic gymnasts (RG) compared to untrained controls (UC). In addition, a recent cross-sectional study by Maimoun et al. [7] showed that BMD values are higher over the course of pubertal development in elite RG, where more mature girls dem-

onstrated significantly higher BMD values compared with less mature girls. However, there are fewer longitudinal data on specific bone mineral accrual during the prepubertal growth in RG who start to exercise with relatively high training loads already at a relatively early age [4].

Body mass has been considered as one of the strongest predictors of BMD [8]. Recent reports indicate that fat (FM) and fat-free (FFM) masses are positively related to BMD in prepubertal girls [2, 4]. Furthermore, a positive effect of FM on bone mineralization has been attributed to a combination of mechanical load exerted on the skeleton by FM [8] and by the adipose-modulated biochemical signals of appetite regulation and energy homeostasis [9, 10]. Among the numerous adipose-modulated biochemical signals that may participate in energy homeostasis and contribute to the relationship between FM and BMD in lean children and adolescents are leptin [6, 9], adiponectin [11, 12] and the gut hormone ghrelin [4, 10]. In general, in the presence of elevated energy expenditure, chronic physical activity decreases leptin, and increases adiponectin and ghrelin concentrations in athletes [13]. Leptin concentrations have been related to FM and BMD values in healthy lean prepubertal girls [2, 9], while the impact of lowered leptin concentrations on bone mass acquisition in the presence of elevated energy expenditure and reduced FM remains questionable in prepubertal and pubertal female RG [4, 5, 7, 14, 15]. Adiponectin has been related to BMD values in healthy untrained adolescent girls [11, 12], while adiponectin did not predict BMD among prepubertal [4] and adolescent [16] female athletes. Similarly, circulating ghrelin has been reported to influence bone mineralization in untrained prepubertal [4] and adolescent [17] girls. In contrast, our recent studies have demonstrated that ghrelin concentrations were not related to BMD values in female prepubertal RG [4] and pubertal swimmers [18]. Accordingly, to date, the reports on the possible interactions between adipocytokines and ghrelin concentrations with bone mineralization during linear growth remain contradictory. Therefore, leptin concentrations increase progressively during growth and maturation in girls following a pattern that parallels increases in subcutaneous fat depot [19] and also in body mass [7, 20]. In addition, it has been reported that circulating adiponectin increases [21] and ghrelin decreases [22] during linear growth in children.

Leptin, adiponectin and ghrelin seem to play an important role in the regulation of body composition during growth and maturation in children. Furthermore, regular physical activity may modify the possible relation-

ships between these circulating adipocytokine and ghrelin levels with specific body composition parameters in children [6, 7]. However, to our best knowledge, no studies have been conducted to longitudinally examine the possible role of different adipocytokines and ghrelin at the same time on the development of BMD in a specific group of physically active prepubertal girls. Therefore, a recent 12-month follow-up study found that baseline leptin was not associated with BMD gain in peripubertal RG (10.7–16.1 years old) [15]. Accordingly, we conducted a 12-month prospective study to investigate the relationship between baseline leptin, adiponectin and ghrelin concentrations with the normal increase in BMD values in a specific group of prepubertal RG and UC. In addition, we evaluated different body composition values that are known to affect bone metabolism.

Subjects and Methods

Subjects

The study protocol was reviewed and approved by the Medical Ethics Committee of the University of Tartu (Estonia). All RG, UC and their parents gave written informed consent before entering the study. Participants of this study were 68 7- to 9-year-old girls from different schools in Estonia. The participants were divided into RG ($n = 33$) and UC ($n = 35$). All RG were recruited from local training groups and had usually trained 10–12 h/week (5–6 training sessions per week) for the past 2 years before starting the study. However, there were some easy periods in their training when gymnasts trained a mean 6 h/week (3 training sessions per week). All these RG were training in the same club and had very similar training lessons (ballet, acrobatics and rhythmic gymnastics training). Most training sessions lasted 2 h and consisted of a warm-up, routine training, and strength and stretching exercises. All RG were competing at the national level. Controls had compulsory physical education classes twice a week at school. Participation only in school's physical education classes was inclusion criteria for UC subjects [4]. All participants were free from present or past diseases known to affect skeletal metabolism, and none of the girls were receiving medications known to affect bone. Throughout the study period, no restrictions were placed on dietary intake and participants consumed their usual everyday diet [4, 23].

Methods

Body height was measured using a Martin metal anthropometer to the nearest 0.1 cm according to the standard technique, body mass was measured with minimal clothing to the nearest 0.05 kg with a medical electronic scale (A&D Instruments, Ltd, Abingdon, UK) and body mass index (BMI) was calculated as body mass divided by square of body height. Pubertal development was assessed by self-report using an illustrated questionnaire of pubertal stages according to the criteria of Tanner [24]. The participants 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.

Pubertal development according to the method of Tanner, which uses self-assessment of breast and pubic hair stages in girls, has been validated previously [25, 26] and was used in our previous studies with girls [4, 6, 18, 23]. All girls in this study were in Tanner stage 1. Bone age was assessed with an X-ray of the left hand and wrist, and determined according to the method of Greulich and Pyle [27].

Body Composition and Bone Mineral Measurements

Body composition (body fat %, FM, and FFM), and BMD (g/cm²) from whole body (WB), and BMD from lumbar spine (LS) and femoral neck (FN) were measured by dual energy X-ray absorptiometry (DXA) using the DPX-IQ densitometer (Lunar Corp., Madison, Wisc., USA) equipped with proprietary software, version 3.6. Participants were scanned in light clothing while lying flat on their backs with arms on their sides. The fast scan mode and standard subject positioning were used for total body measurements, which were analyzed with use of the extended analysis option. DXA measurements were evaluated by the same examiner. Coefficients of variation (CVs) for the DXA measurements were less than 2%. DXA is widely available, precise and safe and has become the preferred clinical instrument to assess bone development in children [28].

Blood Sampling and Analysis

Venous blood samples were drawn in both years between 07:30 and 08:30 h after an overnight fasting from an antecubital vein with the participant sitting in the upright position. The plasma was separated and frozen at -20°C for later analysis. Adiponectin was determined in duplicate via commercially available radioimmunoassay (RIA) kit (cat. No. HADP-61HK; Linco Research, Inc. St. Charles, Mo., USA), the intra- and interassay CVs were <7%, and the least detection limit was 1 µg/ml. Leptin concentration was determined in duplicate by RIA (Mediagnost GmbH, Reutlingen, Germany) and this assay has the intra- and interassay CV values less than 5%, and the least detection limit was 0.01 ng/ml. Ghrelin was also determined in duplicate using a commercially available RIA kit (Linco Research, Inc.). The sensitivity of this kit was 93 pg/ml, and the intra- and interassay CVs were <10 and 14.7%, respectively.

Statistical Analysis

Statistical analyses were performed with SPSS 16.0 for Windows (SPSS, Chicago, Ill., USA), and the means and standard deviations ($\pm$ SD) were determined. Paired *t* tests were performed to determine the changes in measured variables over the 12-month study period. The comparisons between chronological age and bone age were also performed using the paired *t* test. Independent *t* tests were used to compare differences between groups. The least significant change (LSC) for measured BMD variables was also calculated [29] resulting in an LSC of 3.1% at the measured sites. Pearson correlation coefficients were computed to explore the relationship between changes in BMD values during a 12-month study period with baseline body composition and blood biochemical variables. Stepwise multiple regression analysis was performed to determine the possible independent associations of an increase in measured BMD values over a 12-month study period with baseline bone age, BMI, FM, FFM, leptin, adiponectin and ghrelin values. The level of significance was set at $p < 0.05$.

Results

Mean age, bone age, height (+4.8%), body mass (+8.5%), body fat % (+5.4%), FM (+5.8%), FFM (+9.8%), WB BMD (+3.4%), LS BMD (+6.7%), FN BMD (+6.4%) and adiponectin (+14.1%) were significantly increased ($p < 0.05$), while ghrelin (-17.2%) was significantly decreased ($p < 0.05$) after a 12-month study period in RG (table 1). No changes ($p > 0.05$) in BMI and leptin were observed over the 12-month study period in RG. In UC, significant increases ($p < 0.05$) in mean age, bone age, height (+4.9%), body mass (+12.9%), BMI (+2.4%), FM (+19.6%), FFM (+11.1%), WB BMD (+3.6%), LS BMD (+7.2%), FN BMD (+5.6%), leptin (+22.0%) and adiponectin (+17.8%) were observed. In addition, significant decreases ($p < 0.05$) in ghrelin (-30.3%) occurred over the 12-month study period in controls (table 1). Therefore, the increases in bone age over the 12-month study period were significantly lower ($p = 0.013$) in RG when compared with UC, while the increases in LS BMD over the 12-month study period were significantly higher ($p = 0.044$) in RG when compared with UC. Changes in all other measured variables over the 12-month study period were not significantly different ($p > 0.05$) between the groups studied. In addition, the increases in measured BMD variables in both groups were higher with respect to the calculated LSC of 3.1%.

Changes in WB and LS BMD values were significantly related to baseline age, while changes in FN BMD were significantly related to baseline height and body mass values in RG (table 2). All other relationships between changes in BMD values with measured body composition and blood biochemical variables were not significant ($r < 0.242$; $p > 0.05$) in RG. In addition, stepwise multiple regression analysis revealed that measured baseline-independent variables did not predict ($p > 0.05$) increases in BMD values over the 12-month study period in RG.

Increases in WB BMD were significantly correlated with baseline height, body mass, BMI, body fat %, FM, FFM, leptin and ghrelin values in UC (table 2). No significant correlations were observed between increases in LS BMD and measured baseline body composition and blood biochemical variables, while increases in FN BMD were related to baseline height, body mass, FFM and adiponectin values in UC. In UC, stepwise multiple regression analysis demonstrated that baseline FM and FFM values together were the most significant predictors of Δ WB BMD and Δ FN BMD values, explaining 25.2 and 15.7% of the variability in Δ WB BMD and Δ FN BMD values, respectively. When the influence of baseline bone

Table 1. Mean ($\pm$ SD) of subject characteristics before and after a 12-month study period

	Gymnasts (n = 33)			Controls (n = 35)		
	before	after	p value	before	after	p value
Age, years	8.0 $\pm$ 0.6	9.0 $\pm$ 0.6	0.001	8.2 $\pm$ 0.6	9.3 $\pm$ 0.5	0.001
Bone age, years	7.9 $\pm$ 1.4	8.6 $\pm$ 1.5*	0.001	8.0 $\pm$ 1.1	9.3 $\pm$ 0.8	0.001
Height, cm	130.2 $\pm$ 5.1	136.4 $\pm$ 6.5	0.001	129.9 $\pm$ 5.8	135.9 $\pm$ 6.4	0.001
Body mass, kg	27.2 $\pm$ 3.3	29.5 $\pm$ 3.3	0.001	28.1 $\pm$ 5.3	31.5 $\pm$ 6.3	0.001
BMI	15.7 $\pm$ 1.1	15.9 $\pm$ 1.3	0.490	16.6 $\pm$ 2.2	16.9 $\pm$ 2.4	0.030
Fat mass, kg	4.7 $\pm$ 1.6	5.5 $\pm$ 1.7	0.001	6.7 $\pm$ 2.9	8.0 $\pm$ 3.7	0.001
Fat-free mass, kg	19.9 $\pm$ 4.0	22.4 $\pm$ 2.2	0.001	19.9 $\pm$ 2.5	22.0 $\pm$ 3.1	0.001
Body fat, %	18.4 $\pm$ 4.5	19.4 $\pm$ 4.5	0.001	24.1 $\pm$ 7.1	25.5 $\pm$ 7.9	0.001
WB BMD, g/cm ²	0.87 $\pm$ 0.04	0.90 $\pm$ 0.04	0.001	0.85 $\pm$ 0.05	0.87 $\pm$ 0.06	0.001
LS BMD, g/cm ²	0.74 $\pm$ 0.07	0.79 $\pm$ 0.08	0.001	0.70 $\pm$ 0.09	0.74 $\pm$ 0.09	0.001
FN BMD, g/cm ²	0.78 $\pm$ 0.07	0.83 $\pm$ 0.07	0.029	0.71 $\pm$ 0.08	0.75 $\pm$ 0.08	0.083
Leptin, ng/ml	2.0 $\pm$ 1.3	2.3 $\pm$ 1.5	0.125	4.2 $\pm$ 2.6	5.0 $\pm$ 3.3	0.038
Adiponectin, μ g/ml	9.8 $\pm$ 4.0	11.3 $\pm$ 4.2	0.002	10.4 $\pm$ 4.3	11.9 $\pm$ 3.5	0.017
Ghrelin, pg/ml	1,404.0 $\pm$ 494.6	1,189.3 $\pm$ 387.3	0.001	1,172.0 $\pm$ 341.0	829.0 $\pm$ 385.8	0.001

WB = Whole body; LS = lumbar spine; FN = femoral neck; BMD = bone mineral density; BMI = body mass index.

p shows the significance before and after a study period in both groups. * Significantly different from the age: p < 0.05.

Table 2. Pearson correlation coefficients of change (Δ scores) in BMD values during the 12-month study period with baseline body composition and blood biochemical variables

	Gymnasts (n = 33)			Controls (n = 35)		
	Δ WB BMD g/cm ²	Δ LS BMD g/cm ²	Δ FN BMD g/cm ²	Δ WB BMD g/cm ²	Δ LS BMD g/cm ²	Δ FN BMD g/cm ²
Age, years	0.369*	0.402*	0.242	-0.006	0.131	0.315
Bone age, years	0.126	-0.069	0.234	0.103	-0.060	0.234
Body height, cm	0.138	0.118	0.434*	0.367*	0.036	0.340*
Body mass, kg	0.080	0.000	0.361*	0.490*	-0.075	0.339*
BMI	-0.005	-0.126	0.086	0.475*	-0.153	0.207
Fat mass, kg	0.126	0.217	0.220	0.502*	-0.097	0.235
Fat free mass, kg	0.058	-0.156	0.238	0.467*	-0.071	0.396*
Body fat, %	0.153	0.275	0.130	0.483*	-0.108	0.111
Leptin, ng/ml	-0.078	0.211	0.172	0.352*	-0.150	0.219
Adiponectin, μ g/ml	0.319	0.294	-0.008	0.029	-0.0084	-0.392*
Ghrelin, pg/ml	-0.040	-0.033	-0.208	-0.394*	0.114	0.284

Δ WB = Whole body; Δ LS = lumbar spine; Δ FN = femoral neck; BMD = bone mineral density; BMI = body mass index.

* Statistically significant: p < 0.05.

age, BMI, FM and FFM was excluded, baseline ghrelin and adiponectin concentrations were the most important hormonal predictors of Δ WB BMD and Δ FN BMD values in the UC group, explaining 15.5% in Δ WB BMD and

15.3% in Δ FN BMD values, respectively. In addition, stepwise regression analysis demonstrated no association between Δ LS BMD and measured baseline-independent variables in UC.

Discussion

The relationships between increased BMD values with baseline leptin, adiponectin and ghrelin were studied in two different groups of prepubertal girls. The 12-month prospective study demonstrated significant changes in measured adipocytokine, ghrelin and body composition variables in UC, while no significant increases in leptin concentrations were found in RG. Therefore, the increases in measured BMD variables were higher with respect to the calculated LSC, indicating normal growth of BMD values in both groups of prepubertal girls over the 12-month study period. While increases in adiponectin and body composition variables, and decreases in ghrelin were not significantly different between the groups studied, the increases in bone age over the 12-month study period were lower ($p < 0.05$) in RG compared with UC, and the increases in LS BMD were higher ($p < 0.05$) in RG compared with UC. It appeared that initial hormone concentrations together with specific body composition variables were associated with an increase in BMD in prepubertal UC but not in RG groups. These results suggest that due to mechanical loading, prepubertal RG may have a beneficial effect on bone mineralization and may have counterbalanced the negative factors on bone development such as low FM and leptin concentrations.

One of the main findings of the present investigation was that regular high-impact weight-bearing athletic activity promoted significant annual gains in BMD in relatively young prepubertal RG (table 1). The annual gain in BMD values in RG was close to the results of Courteix et al. [30]. Our previous cross-sectional study with 8-year-old RG found that at least 2 years of athletic training with high energy expenditure did not cause any delay in bone age maturation [4], which is a good marker of biological maturation [31, 32]. Interestingly, the results of the present study demonstrated that bone age was lower ($p < 0.05$) than chronological age after a 12-month study period in RG. A recent cross-sectional study by Maimoun et al. [7] also suggested that prepubertal RG had a significant delay in bone age, while Courteix et al. [30] found only a trend towards a lower bone age after a 12-month study period in highly trained prepubertal RG. Georgopoulos et al. [32] indicated that prepubertal stage is prolonged and pubertal development is shifted to a later age following the bone maturation rather than the chronological age in elite RG. These results together suggest that since prepubertal RG present significant annual gains in BMD and higher BMD values in comparison with same chronological age UC, they may also maintain higher bone

mineral values although bone age and biological maturation have been delayed.

Another main finding was that leptin was significantly increased in UC but not in RG over the 12-month study period (table 1). The mean leptin values in UC were similar to previous results in normal weight girls at a similar age [2]. It has been reported that circulating leptin starts to rise in the prepubertal period from 5 years and continues to progressively rise throughout puberty in healthy physically inactive girls [20, 33, 34]. Maimoun et al. [7] reported that leptin concentrations rise in parallel with the increase in FM in highly trained RG even with a reduced amount of adipose tissue progressing from prepuberty to puberty. These results together suggest that the specific physical activity pattern seen in our RG during earlier prepubertal years may have counterbalanced the age-dependent increase in circulating leptin concentrations and the increase in leptin levels could be seen at the onset of puberty in a specific group of young RG.

Baseline leptin concentrations did not predict increases in measured BMD values over the 12-month study period in RG. However, leptin was significantly correlated with Δ WB BMD in UC. While other investigations have reported independent relationships between leptin and measured BMD variables in untrained prepubertal children [2, 4, 9], recent studies did not find leptin as an independent predictor of BMD in a group of elite female RG at different pubertal stages [4–7, 15]. Although leptin has been reported to be involved in the accumulation, maintenance and loss of BMD throughout life [35], the impact of leptin on growing human bone remains controversial [4, 7]. Accordingly, the results of present study suggest that leptin is not longitudinally involved in the development of BMD in a specific group of RG before puberty.

Similar to previous studies, adiponectin increased [21] and ghrelin decreased [22] over the 12-month study period in both groups studied (table 1). Baseline adiponectin and ghrelin levels did not predict significant increases in BMD values in prepubertal RG, while baseline adiponectin was inversely correlated with Δ FN BMD in UC. However, the independent variables that were associated with increases in BMD values were baseline FM and FFM values together in UC and only after excluding the influence of body composition parameters, ghrelin and adiponectin concentrations predicted increases in WB BMD and FN BMD values in UC, respectively. These results demonstrate that baseline adiponectin and ghrelin may not have a direct role in the development of BMD variables in prepubertal girls. In accordance with our results, previous studies have also found no relationship between

adiponectin [4, 16] and ghrelin [4, 18] with BMD variables in physically active prepubertal children. Although some studies have reported significant correlations of BMD variables with adiponectin [11, 12] and ghrelin [17, 36], the results of current study suggest that during prepubertal development, adiponectin and ghrelin are not independent predictors of increases in BMD levels in contrast to FM and FFM values in prepubertal girls with different physical activity patterns. Taken together, further longitudinal studies from prepuberty to pubertal maturation are necessary to clarify the possible roles of adiponectin and ghrelin in the bone development in girls with different physical activity parameters.

In conclusion, body composition variables together with measured adipocytokine and ghrelin levels did not predict a normal growth in BMD values in RG. However, initial leptin together with specific body composition variables were associated with an increase in WB BMD value as a result of the 12-month study period in UC.

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