

Research article

## A Longitudinal Study of Bone Mineral Accrual during Growth in Competitive Premenarcheal Rhythmic Gymnasts

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### Abstract

The purpose of this investigation was to study whether prolonged competitive rhythmic gymnastics training influenced bone mineral accrual in premenarcheal girls. Eighty-nine girls (45 rhythmic gymnasts [RG] and 44 untrained controls [UC]) between 7 and 9 years of age were recruited and measured annually for four years (not all participants were measured at every occasion). Dual energy x-ray absorptiometry was used to assess the development of whole body (WB), femoral neck (FN) and lumbar spine (LS) bone mineral content (BMC). In addition, body composition, blood adipokine and jumping performance characteristics were obtained. For longitudinal analyses, hierarchical mixed-effects models were constructed to predict differences in the development of WB, FN and LS BMC between RG and UC groups, while accounting for differences in body composition, blood adipokine and jumping performance values. It appeared that from 8 years of age, RG had lower ( $p < 0.05$ ) fat mass and leptin values, and higher ( $p < 0.05$ ) jumping performance measures in comparison with UC girls. Hierarchical mixed-effects models demonstrated that RG had  $71.9 \pm 12.0$ ,  $0.23 \pm 0.11$  and  $1.39 \pm 0.42$  g more ( $p < 0.05$ ) WB, FN and LS BMC, respectively, in comparison with UC girls. In addition, WB, FN and LS BMC increased more ( $p < 0.05$ ) between 7 to 12 years of age in RG girls in comparison with UC. In conclusion, these findings suggest that the prolonged exposure to competitive rhythmic gymnastics trainings in premenarcheal girls is associated with greater bone mineral accrual despite lower body fat mass and leptin values.

**Key words:** Rhythmic gymnastics, premenarcheal girls, bone mineral content, longitudinal development, adipokines, fat mass.

### Introduction

The amount of bone mineral gained during childhood is greatly associated with the adult peak bone mass (Gruodyte-Racienė et al., 2013; Scerpella et al., 2011). Currently, osteoporosis is a major public health problem and the increase in peak bone mass by 10% during childhood growth may reduce risk of fractures by 50% in later life (Hasselstrøm et al., 2008). Bone mineral content (BMC) increases with age, height and body mass throughout childhood (Jürimäe, 2010), while maximal increases in bone mineral accrual occur over a relatively brief period in the years surrounding peak height velocity (PHV) (Bailey et al., 1999; Baxter-Jones et al., 2011). It appears that up to 40% of total BMC is acquired in the five-year window surrounding the attainment of PHV, which is approximately 12 years of age for females (Bailey et al., 1999; Baxter-Jones et al., 2011). Many factors influence the accumulation of bone mineral content during childhood including

genetics (Hasselstrøm et al., 2008), nutritional status (Maimoun et al., 2014), body composition (Gruodyte et al., 2010), endocrine factors (Jürimäe, 2014) and physical activity (Vaitkeviciute et al., 2014). It has been suggested that physical activity may have the greatest potential to influence bone mineral accrual during the growing years (Jackowski et al., 2015) as the level of physical activity during the years surrounding PHV greatly influences bone development (Vaitkeviciute et al., 2014). It is also known that the exposure to high-impact weight-bearing exercises produces a higher osteogenic effect on bone compared to activities characterized by low-impact and weight-supported loads in growing athletes (Gruodyte et al., 2010; Jürimäe et al., 2021).

Gymnastics training is an athletic activity that exposes the growing skeleton to high-impact weight-bearing mechanical loads and has been shown to be highly osteogenic (Jürimäe et al., 2018). Previous cross-sectional studies have found that girls involved in prolonged rhythmic gymnastics training demonstrate enhanced areal bone mineral density (aBMD) and BMC values in prepubertal (Parm et al., 2011), pubertal (Gruodyte et al., 2009) and adolescent (Jürimäe et al., 2021) years. It has also been demonstrated that the exposure to early childhood recreational artistic gymnastics activity in 4- to 6-year old children over a four year period is associated with greater bone mineral accrual in comparison with children participating in other recreational sports (Erlandson et al., 2011; Jackowski et al., 2015). In another study, BMC increment was significantly higher in well-trained artistic gymnasts progressing from prepuberty (Tanner stage I) to early puberty (Tanner stage III) during a three year period when compared with age- and maturity-matched untrained controls (Laing et al., 2002). Furthermore, skeletal advantages of childhood artistic gymnastics training have been reported to persist into young adulthood even up to 10 years after retiring from the sport and potentially prevent the risk of osteoporosis and related fracture in later life (Erlandson et al., 2012). These results suggest that the structured athletic activity of artistic gymnastics training during childhood before menarche is an effective tool to increase bone mineral accrual in growing females. However, there is a paucity of research examining the longitudinal effect of rhythmic gymnastics participation at the competitive level on female bone mineral accrual before menarche (Jürimäe et al., 2018).

There are substantial data to suggest that body fat mass (FM) and fat free mass (FFM) are both positively associated with bone mineral accrual during puberty in girls with different training patterns (Gruodyte et al., 2010;

Jürimäe et al., 2021). A positive influence of FM on bone mineral accrual has been attributed to a mechanical loading (Jürimäe et al., 2018) and the impact of hormones linked to adipose tissue (Jürimäe, 2014), including leptin and adiponectin (Gruodyte et al., 2010; Vösoberg et al., 2016). Previously, leptin (Muñoz et al., 2004) and adiponectin (Gruodyte et al., 2010) have been linked to aBMD and BMC values at various skeletal sites, while other studies have not found relationships between these adipokine values and bone mineral accrual in adolescent female athletes with different training patterns (Maimoun et al., 2014). Mechanical loading factors such as weight-bearing and muscle forces are also important in bone mineral accrual, and FM together with FFM contribute to bone development by increasing compressive forces during skeletal loading (Ho and Kung, 2005). Accordingly, jumping ability seems to correlate with bone mineral accrual in maturing female athletes (Gruodyte et al., 2009; Jürimäe et al., 2021; Vösoberg et al., 2017). Various vertical jump tests have been used to evaluate jumping ability (Gruodyte et al., 2009; Kellis et al., 1999), and repeated jump tests, such as rebound jumps for 15 (RBJ15s) and 30 (RBJ30s) seconds and not single maximal countermovement jump (CMJ) test have previously been associated with different aBMD values in RG (Gruodyte et al., 2009; Vösoberg et al., 2017). It has also been suggested that the continuous number of jumps with changing directions is typical for rhythmic gymnastics training (Jürimäe et al., 2018). This kind of prolonged and intense gymnastics training in childhood can lead to a lowered body FM together with changes in blood adipokine values, which can negatively affect bone development in growing RG athletes (Jürimäe et al., 2018). In contrast, aBMD was not affected in adolescent elite RG, who had hypoleptinemia caused by intensive and stressful trainings in the presence of elevated energy expenditure, demonstrating that the mechanical loading of specific gymnastics activity overcomes the possible negative influence of high athletic activity (Courteix et al., 2007). To the best of our knowledge, no studies have been conducted to examine the longitudinal influence of body composition together with blood adipokine and jumping performance measures on bone mineral accrual in competitive premenarcheal RG. Accordingly, the aim of the present study was to investigate the longitudinal exposure to competitive rhythmic gymnastics training on bone mineral accrual, and to evaluate the possible influence of age, body composition, blood adipokine and jumping performance values on bone mineral accrual in premenarcheal female athletes.

## Methods

### Participants and study design

At the beginning of the study, 89 prepubertal girls were recruited, among them were 45 rhythmic gymnasts (RG) and 44 untrained controls (UC) with the mean age of  $8.0 \pm 0.6$  and  $8.2 \pm 0.6$  years, respectively. At the study entry, three cohorts were identified: 7, 8 and 9 years of age. At the first measurement occasion, all studied participants in both groups were prepubertal as they were all at Tanner stage I. It appeared that UC girls matured faster than RG as

indicated by the changes in Tanner stages over the next measurement occasions (Table 1). Pubertal development was assessed by self-report using an illustrated questionnaire according to the Tanner classification method (1962), which has been used previously in our laboratory (Gruodyte et al., 2010; Jürimäe et al., 2016). Data were collected annually for the next 3-year period (not all participants were measured at every occasion). As there were overlaps in ages between the clusters, it was possible to assess a consecutive 5-year developmental pattern (7 to 12 years) over a shorter 3-year follow-up period (Erlandson et al., 2011; Gruodyte-Raciene et al., 2013; Jackowski et al., 2015). All RG were recruited from the local training groups and had trained 10–14 h per week for the past 2 years before starting the study and were competing at the national level in Estonia (Vösoberg et al., 2014). All RG had very similar training lessons, including rhythmic gymnastics, ballet and acrobatics and during the 3-year follow-up period they continued with the same training lessons and similar weekly training volume (Vösoberg et al., 2014). Untrained control subjects were recruited from local secondary schools and participation only in compulsory physical education classes (2–3 times of 45 min each) was the inclusion criteria for UC subjects (Vösoberg et al., 2014). A questionnaire was used to identify that none of the participants was receiving any medications or had a history of bone or renal diseases. In addition, all RG remained premenarcheal over the 3-year study period. Throughout the study period, no restrictions were placed on dietary intake and participants consumed their everyday diet (Gruodyte et al., 2010; Jürimäe et al., 2016). The study protocol was approved by the Medical Ethics Committee of the University of Tartu (Estonia), and was explained to the girls and their parents, who signed a written informed consent form.

**Table 1.** Mixed longitudinal study design with numbers of rhythmic gymnasts (untrained controls) measured at each test year by age and biological maturation categories.

	Test year				Total
	1st year	2nd year	3rd year	4th year	
<b>Age</b>					
<b>7</b>	14 (4)				14 (4)
<b>8</b>	20 (23)	11 (2)			31 (25)
<b>9</b>	11 (17)	16 (20)	10 (2)		37 (39)
<b>10</b>		12 (17)	16 (20)	9 (2)	37 (39)
<b>11</b>			10 (14)	17 (19)	27 (33)
<b>12</b>				9 (14)	9 (14)
<b>Total</b>	45 (44)	39 (39)	36 (36)	35 (35)	155 (154)
<b>Tanner stage</b>					
<b>I</b>	45 (44)	39 (30)	32 (10)	5 (4)	121 (88)
<b>II</b>		0 (9)	4 (19)	26 (17)	30 (45)
<b>III</b>			0 (6)	4 (10)	4 (16)
<b>IV</b>			0 (1)	0 (4)	0 (5)
<b>Total</b>	45 (44)	39 (39)	36 (36)	35 (35)	155 (154)

### Bone mineral content and body composition

Whole body (WB), femoral neck (FN) and lumbar spine (LS) bone mineral content (BMC, in g) were measured by dual-energy x-ray absorptiometry (DXA) using the DPX-IQ densitometer (Lunar Corporation, Madison, WI, USA)

equipped with proprietary software, version 3.6. Data for different aBMD values for this cohort have been previously published (Vösoberg et al., 2016, 2017). In addition, WB fat mass (FM, in kg) and fat free mass (FFM, in kg) were also determined. 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 WB measurements, and were analyzed using the extended analysis option. To reduce the impact of the operator variability factor, the same qualified examiner analyzed all scans over the 3-year period. The precision of measurement expressed as coefficient of variation (CV%) was less than 2% for all bone mineral and body composition measurements (Vösoberg et al., 2016, 2017).

### Jumping performance

The maximal vertical height (in cm) of two-footed hands-on-the-hips vertical jumps were measured using a contact mat (Newtest OY, Finland) (Grudyte et al., 2009; Kellis et al., 1999). The girls performed two jumping tests: 1) a countermovement jump (CMJ) from a standing position with a preliminary countermovement; and 2) the rebound jumps with continuous countermovement jumps for 15 seconds (RBJ15s). The best jumping height out of three attempts was recorded in CMJ. The average jumping height of RBJ15s was calculated according to the jumping results within 15 seconds (Grudyte et al., 2009; Kellis et al., 1999). All participants were instructed and verbally encouraged to jump as high and as rapidly as they could. The hands remained on hips throughout both jumping tests to avoid upper extremities contribution to the jump height (Grudyte et al., 2009; Kellis et al., 1999).

### Blood analysis

Venous blood samples were taken between 7:30 and 8:30 a.m. after an overnight fast from an antecubital vein with the participant sitting in the upright position. Samples from one individual were run in the same assay (Vösoberg et al., 2016). Leptin concentration was determined by radioimmunoassay (RIA) (Mediagnost GmbH, Reutlingen, Germany). This assay has intra- and interassay CV%*s* 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 CV%*s* were less than 7 %, and the least detection limit was 1 µg/mL (Vösoberg et al., 2016).

### Statistical analysis

All statistical analyses were performed using SPSS software version 21.0 package for Windows (Chicago, IL, USA). Standard statistical methods were used to calculate means and standard deviations ( $\pm$ SD). Group differences (RG versus UC) for height, body mass, FM, FFM, leptin, adiponectin, CMJ and RBJ15s were assessed in each age category by analysis of variance. Group differences (RG vs UC) for measured BMC variables at the first testing occasion were assessed using analysis of covariance (covariates: age, height, body mass and RBJ15s). For longitudinal analyses, multilevel modelling approach was used to build hierarchical mixed-effects models (Erlandson et al., 2011; Grudyte-Racine et al., 2013; Jackowski et al., 2015). The

bone mineral accrual was measured repeatedly in individuals (level 1 of hierarchy) and between individuals (level 2 of hierarchy). Within-individual variance (level 1) and between-individual variance (level 2) were estimated using unstructured matrix with random intercepts and slopes. Height, body mass, leptin, adiponectin, CMJ, RBJ15s, age centered around 7 years and group (RG or UC) were considered as fixed effects in the models. In addition, interaction terms (group x age centered) were created to assess whether changes in bone mineral accrual parameters through time differed significantly between groups. Time component (age centered around 7 years) and subjects were used as random effects in the model. We used stepwise procedure to construct models and log likelihood ratio statistic Akaike Information Criteria (AIC) were used to judge the relevance of a model. The level of significance was set at  $p < 0.05$ .

### Results

Measured anthropometric, body composition, blood adipokine and jumping performance characteristics of RG and UC from 7 to 12 years of age are presented in Table 2. All measured parameters were similar ( $p > 0.05$ ) between RG and UC groups at the youngest age category (i.e., 7-year-old girls). There were also no significant differences ( $p > 0.05$ ) between RG and UC groups for height, body mass and adiponectin values, except for the 11-year-old groups, where RG had significantly lower body mass and higher adiponectin values in comparison with UC group. In addition, from the age of 8 years, RG presented significantly lower values for FM and leptin in comparison with UC girls. Similarly, CMJ and RBJ15s values were higher ( $p < 0.05$ ) in RG when compared with UC girls starting from the age of 8 years. Unadjusted (data not shown) and adjusted BMC values at all sites measured were significantly higher ( $p < 0.05$ ) in RG when compared with UC at the first testing occasion (Table 3).

Table 4 summarizes the multilevel mixed-models for WB, FN and LS BMC development. Fixed effects of age centered, height and body mass were significant predictors ( $p < 0.05$ ) of the development of WB BMC. Height and body mass also significantly predicted the development of FN BMC, whereas height, body mass and RBJ15s were observed to be independent significant predictors of LS BMC. However, leptin and adiponectin were not found to be independent significant predictors ( $p > 0.05$ ) to any BMC values measured (Table 4). When comparing the development of BMC between RG and UC, it was observed that RG had  $71.9 \pm 12.0$  g more WB BMC,  $0.23 \pm 0.11$  g more FN BMC and  $1.39 \pm 0.42$  g more LS BMC values compared with UC. Interaction terms (group x age centered) as fixed effects showed that WB BMC ( $19.4 \pm 6.09$  g), FN BMC ( $0.9 \pm 0.2$  g) and LS BMC ( $0.9 \pm 0.2$  g) increased differently ( $p < 0.05$ ) between RG and UC (Table 4; Figure 1).

The random-effects coefficients describe the two levels of variance (Table 4). The significant variances at level 1 (within individuals) of the all three BMC models indicate that BMC was increasing significantly at each measurement occasion within individuals ( $p < 0.05$ ). The

significant variance at level 2 (between-individuals) for each BMC model demonstrated that individuals had significantly different ( $p < 0.05$ ) BMC growth curves in terms of

both their intercepts and slopes. The variances of these intercepts and slopes were not correlated ( $p > 0.05$ ) in any of these models.

**Table 2.** Mean ( $\pm$ SD) descriptive characteristics for chronological age-related anthropometry, body composition, adipokines, and jumping performance data for rhythmic gymnasts and untrained controls.

	Age categories (years)					
	7	8	9	10	11	12
<b>Rhythmic Gymnasts</b>						
N	14	31	38	36	27	9
Height (cm)	129.4 $\pm$ 4.8	130.7 $\pm$ 6.5	134.9 $\pm$ 5.3	141.5 $\pm$ 6.0	145.5 $\pm$ 5.3	151.0 $\pm$ 5.4
Body mass (kg)	27.1 $\pm$ 3.2	27.0 $\pm$ 3.6	29.0 $\pm$ 2.9	32.6 $\pm$ 3.9	34.8 $\pm$ 3.9*	39.8 $\pm$ 3.7
Fat mass (kg)	5.0 $\pm$ 1.9	4.9 $\pm$ 1.9*	5.4 $\pm$ 1.6*	6.2 $\pm$ 1.8*	6.6 $\pm$ 2.1*	8.5 $\pm$ 2.4
Fat free mass (kg)	20.6 $\pm$ 2.1	20.5 $\pm$ 2.3*	22.0 $\pm$ 2.1	24.6 $\pm$ 2.8*	26.0 $\pm$ 2.5	29.1 $\pm$ 2.3
Leptin (ng/mL)	2.6 $\pm$ 2.1	2.1 $\pm$ 1.0*	2.3 $\pm$ 1.5*	2.7 $\pm$ 1.4*	2.8 $\pm$ 1.9*	2.5 $\pm$ 0.8*
Adiponectin ( $\mu$ g/mL)	8.8 $\pm$ 3.3	9.9 $\pm$ 3.3	13.4 $\pm$ 7.8	13.6 $\pm$ 7.1	12.6 $\pm$ 5.4*	15.8 $\pm$ 7.3
CMJ (cm)	24.2 $\pm$ 2.1	23.7 $\pm$ 3.5*	24.2 $\pm$ 2.6*	25.9 $\pm$ 2.5*	26.1 $\pm$ 2.3*	27.6 $\pm$ 2.8*
RBJ15s (cm)	20.5 $\pm$ 2.3	21.4 $\pm$ 2.7*	21.3 $\pm$ 2.5*	21.5 $\pm$ 2.9*	22.0 $\pm$ 2.7*	22.7 $\pm$ 2.5*
<b>Untrained Controls</b>						
N	4	26	39	38	33	14
Height (cm)	126.9 $\pm$ 6.7	128.4 $\pm$ 4.5	133.4 $\pm$ 5.6	139.2 $\pm$ 6.0	146.0 $\pm$ 6.9	150.8 $\pm$ 6.4
Body mass (kg)	23.6 $\pm$ 3.5	27.1 $\pm$ 4.1	30.1 $\pm$ 4.9	34.3 $\pm$ 6.8	39.4 $\pm$ 8.2	43.3 $\pm$ 10.1
Fat mass (kg)	3.9 $\pm$ 1.5	6.2 $\pm$ 2.5	7.6 $\pm$ 3.0	9.3 $\pm$ 4.3	11.3 $\pm$ 4.9	12.3 $\pm$ 6.1
Fat free mass (kg)	18.3 $\pm$ 2.4	19.2 $\pm$ 1.7	21.2 $\pm$ 2.4	23.2 $\pm$ 3.0	26.1 $\pm$ 3.9	28.9 $\pm$ 4.9
Leptin (ng/mL)	1.7 $\pm$ 0.8	3.7 $\pm$ 2.3	4.7 $\pm$ 2.9	6.3 $\pm$ 3.9	7.7 $\pm$ 4.2	7.9 $\pm$ 4.8
Adiponectin ( $\mu$ g/mL)	10.3 $\pm$ 2.2	11.1 $\pm$ 4.8	10.7 $\pm$ 3.6	11.3 $\pm$ 3.5	9.7 $\pm$ 4.2	10.3 $\pm$ 4.7
CMJ (cm)	19.5 $\pm$ 2.4	19.8 $\pm$ 2.3	19.5 $\pm$ 2.0	21.5 $\pm$ 2.3	22.5 $\pm$ 2.7	23.0 $\pm$ 3.4
RBJ15s (cm)	17.0 $\pm$ 2.6	16.7 $\pm$ 2.2	16.5 $\pm$ 2.5	17.8 $\pm$ 2.7	18.8 $\pm$ 3.0	18.9 $\pm$ 2.3

CMJ, countermovement jump; RBJ15s, rebound jumps for 15 seconds. \*Significant difference between rhythmic gymnasts and untrained controls at the specified chronological age category;  $p < 0.05$ .

**Table 3.** Adjusted mean ( $\pm$ SE) bone mineral content values for rhythmic gymnasts and untrained controls at the first testing occasion (covariates: age, height, body mass, RBJ15s).

	Rhythmic Gymnasts (n = 45)	Untrained Controls (n = 44)
WB BMC (g)	1010.3 $\pm$ 12.9	942.9 $\pm$ 12.9*
FN BMC (g)	2.9 $\pm$ 0.1	2.7 $\pm$ 0.1 *
LS BMC (g)	17.7 $\pm$ 0.4	15.9 $\pm$ 0.4 *

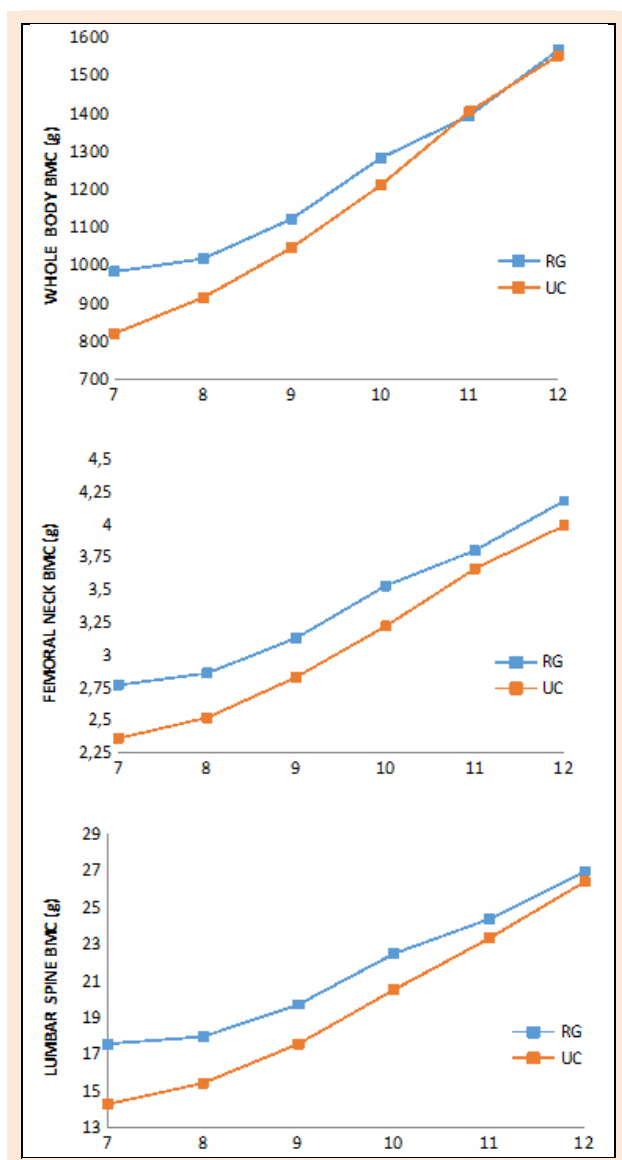
WB, whole body; FN, femoral neck; LS, lumbar spine; BMC, bone mineral content. \* Significantly different between groups;  $p < 0.05$ .

**Table 4.** Hierarchical mixed-models with fixed and random effects for whole body, femoral neck and lumbar spine bone mineral content (BMC) development.

Variable	Whole body BMC		Femoral neck BMC		Lumbar spine BMC	
<b>Fixed effects</b>						
Constant	-1112.2 $\pm$ 168.5		-1.1 $\pm$ 0.6		-31.6 $\pm$ 5.1	
Age centered	18.7 $\pm$ 6.1		NS		NS	
Height	12.8 $\pm$ 1.3		0.02 $\pm$ 0.01		0.30 $\pm$ 0.05	
Body mass	16.9 $\pm$ 1.6		0.03 $\pm$ 0.01		0.27 $\pm$ 0.07	
Leptin	NS		NS		NS	
Adiponectin	NS		NS		NS	
CMJ	NS		NS		NS	
RBJ15s	NS		NS		0.10 $\pm$ 0.04	
RG	71.9 $\pm$ 12.0		0.23 $\pm$ 0.11		1.39 $\pm$ 0.42	
RG*Age centered	19.4 $\pm$ 6.09		0.9 $\pm$ 0.2		0.45 $\pm$ 0.22	
<b>Random effects</b>						
<i>Level 1</i>						
Constant	774.6 $\pm$ 101.2		0.02 $\pm$ 0.03		1.29 $\pm$ 0.19	
<i>Level 2</i>						
	Constant	Age centered	Constant	Age centered	Constant	Age centered
Constant	4499.8 $\pm$ 970.4	120.6 $\pm$ 239.0	0.05 $\pm$ 0.02	0.003 $\pm$ 0.004	2.14 $\pm$ 0.91	0.14 $\pm$ 0.29
Age centered	120.6 $\pm$ 239.0	421.7 $\pm$ 105.5	0.003 $\pm$ 0.004	0.004 $\pm$ 0.002	0.14 $\pm$ 0.29	0.48 $\pm$ 0.15

Fixed effect values are estimates  $\pm$  SEE (standard error of estimate) of bone mineral content (g). Random effects values are estimates. Age centered is age in years centered around 7 years of age (years). Height (cm), body mass (kg), leptin (ng/mL), adiponectin ( $\mu$ g/mL), CMJ (countermovement jump, cm), RBJ15s (rebound jumps for 15 s, cm), RG, rhythmic gymnasts. UC, untrained controls. All numerical values are reported as significant,  $p < 0.05$ . NS, not significant.





**Figure 1.** Predicted values of whole body (WB), femoral neck (FN) and lumbar spine (LS) bone mineral content (BMC) development for rhythmic gymnasts (RG) and untrained controls (UC) from 7 to 12 years of age drawn from hierarchical mixed-models with random intercepts and slopes.

## Discussion

The purpose of the current longitudinal investigation was to study whether prolonged competitive rhythmic gymnastics training is associated with the bone mineral accrual in premenarcheal girls. It was observed that being exposed to chronic competitive rhythmic gymnastics trainings conferred skeletal advantages to WB, FN and LS BMC values in RG compared with UC girls. This is the first study, to our best of knowledge that has investigated the prolonged effects of competitive rhythmic gymnastics training in premenarcheal athletes reaching early puberty. It has been suggested that early puberty seems to be the most sensitive period for maximizing bone mineral gain (Jürimäe et al., 2018). According to the results of present study, it can be suggested that competitive rhythmic gymnastics training has a positive effect on bone mineral accrual in studied premenarcheal RG, which overcame the possible negative

influence of high athletic training activity and lowered body FM that could be associated with lower bone mineral accrual in growing female athletes (Maimoun et al., 2014).

It has been demonstrated that RG in comparison with UC girls present significantly higher BMC values already prepubertal years (Jürimäe et al., 2018). In accordance, a cross-sectional comparison at the first measurement occasion in 7 to 9 years old girls showed that RG presented significantly higher BMC values in all skeletal sites measured compared to UC girls (see Table 3). These results demonstrate that two years of competitive training may be enough to produce higher bone mineral accrual when compared with UC girls of similar age, but more similar studies are needed to claim this assumption. Similarly, Dowthwaite et al. (2006) reported higher WB BMC values in artistic gymnasts at Tanner stage II after two years of specific gymnastics trainings when compared with age- and maturity-matched UC group. The greater bone mineral accrual achieved by prepubertal gymnasts is a result of high impact training, repetitive series and dynamic loading associated with the gymnastics participation (Burt et al., 2013). In contrast, a group of 4 to 7 years old precompetitive, recreational gymnasts, who had trained at least four months with a mean of 1.5 hours per week presented no differences in measured bone mineral values in comparison with children from other recreational sport programs (Erlandson et al., 2011). It was argued that a longer duration of gymnastics stimulus is required to change bone parameters in these early year children (Erlandson et al., 2011). In addition, a dose-response relationship between gymnastics exposure (i.e., hours and years of training) and bone mass increment has been suggested (Georgopoulos et al., 2004; Laing et al., 2005). Our first measurement year findings demonstrate that at least two years of participating in competitive rhythmic gymnastics trainings is required to influence bone mineral accrual in premenarcheal girls.

The present investigation observed that participating in competitive rhythmic gymnastics training, in which premenarcheal girls were exposed to on average of 10-14 hours per week of gymnastics-type activities in childhood over a three year period up to 12 years of age provided benefits to WB, FN and LS BMC development. These findings demonstrated that for every year an individual is exposed to competitive training in rhythmic gymnastics, they benefit from 72, 0.23 and 1.39 g greater WB, FN and LS BMC development, respectively. This magnitude of benefits is similar to the effects of gymnastics participation that has previously been observed in BMC and aBMD at the WB, FN and LS skeletal sites when compared with non-gymnasts (Erlandson et al., 2011; Gruodyte-Raciene et al., 2013; Laing et al., 2002; Zanker et al., 2003). For example, Laing et al. (2002) reported that competitive female artistic gymnasts with a mean age of 11 years had a greater rate of bone mineral accrual at WB and LS BMC following three years period. Likewise, Erlandson et al. (2011) found that on average as little as 1-4 hours per week of recreational gymnastics activities over a three years period produced significantly higher increases in WB and FN BMC by 3% and 7%, respectively, in initially 4 to 7 years old precompetitive, recreational gymnasts compared with the same age non-gymnasts. However, increments in LS BMC

values were not different between recreational gymnasts and non-gymnasts participating in other recreational sports (Erlandson et al., 2011). In addition to higher and longer exposure to gymnastics activities in our studied competitive RG compared with the recreational gymnasts of Erlandson et al. (2011) investigation, the different tempo of skeletal growth reported between axial and appendicular sites at slightly different ages in girls may have also contributed to the differences in BMC increment (Moyer-Mileur et al., 2003) between these studies. It is known that appendicular skeletal sites such as at FN bone mineral accrual occurs more rapidly before puberty at younger ages, while axial skeletal sites such as LS bone mineral accrual occurs more rapidly during puberty (Bass et al., 1999). Accordingly, the gymnastics loading amount in our studied premenarcheal RG was sufficient to increase LS BMC increment more intensively compared to the same-age but faster maturing UC girls, while the loading experienced from low-level gymnastics exposure was insufficient to increase LS BMC increment in recreational gymnasts compared to non-gymnasts in Erlandson et al. (2011) study. In addition, the development of BMC values between RG and UC appears to be more similar after the age of 10 years (see Figure 1), despite high gymnastics-type activities of RG. It appeared that more UC girls were older and more mature at the 4th year of measurement in comparison with RG group. It is well known that RG may mature at slower rate in comparison with UC (Jürimäe et al., 2018) and skeletal maturation could be delayed about 1-3 years in RG (Maimoun et al., 2014). Accordingly, despite the fact that bone mineral accrual is proportional to the development of puberty in RG (Jürimäe et al., 2018), the results of present study demonstrate that long-term participation in competitive rhythmic gymnastics training is effective to increase bone mineral accrual at the whole body and different regional sites of the skeleton in premenarcheal girls during the transition from prepuberty to early puberty.

Both measured blood adipokine values, leptin and adiponectin did not influence the development of WB, FN and LS BMC in premenarcheal girls. In accordance, it has been argued that leptin and adiponectin are not related to bone mineral gain in the presence of elevated energy expenditure and lowered FM in growing and maturing RG (Jürimäe et al., 2018). However, positive correlations of leptin with bone mineral values in pubertal RG have been reported (Muñoz et al., 2004), while another study found no direct role for leptin in bone mass accrual in elite RG during puberty (Maimoun et al., 2010). In the current study, leptin values together with body FM were significantly lower at all age categories from the 8 years of age in RG compared with UC. Relatively low leptin concentrations have reported in young female athletes in relation to their reduced body FM (Jürimäe, 2014). Leptin levels only rise in parallel with the increase in FM in highly trained RG even with a reduced amount of adipose tissue during further pubertal maturation (Maimoun et al., 2010). Our results showed that leptin and adiponectin levels remained relatively stable from 7 to 12 years of age in premenarcheal RG. In agreement with leptin results in RG (Jürimäe, 2014), adiponectin was associated with weekly training

volume but not with bone mass acquisition in elite RG (Roupas et al., 2014). The longitudinal results of present study confirm that leptin and adiponectin are not directly involved in bone mineral accrual in premenarcheal RG.

Jumping performance results were better in RG in comparison with UC girls in all age categories starting from the age of 8 years. The difference in this outcome between studied groups was expected and is a result of the high-impact weight-bearing athletic activity in RG (Jürimäe et al., 2018). Furthermore, repeated jumps test (RBJ15s) and not single jump test (CMJ) has previously been related to aBMD values in RG (Gruodyte et al., 2009; Vösoberg et al., 2017). Similarly, our results demonstrated that RBJ15s is associated with bone mineral accrual at LS BMC site of the skeleton, but not in the models of WB and FN BMC development in premenarcheal girls. In accordance, Kontulainen et al. (2002) found that 9-month jumping intervention increased LS BMC accrual. In addition, it has been found that jumping training, similar to rhythmic gymnastics training, produces ground reaction forces two to six times of the body mass and therefore has a substantial positive effect on bone mass accrual at the LS site of the skeleton (Heinonen et al., 1996, Kontulainen et al., 2002). Therefore, the continuous number of jumps with varying directions and forces such as seen during gymnastics training may play role in development of LS BMC in premenarcheal RG during early puberty (Jürimäe et al., 2018).

There are some limitations in our study that should be considered. The observational mixed longitudinal study design does not allow for any cause-effect assessment of current or rhythmic gymnastics participation on bone parameters (Erlandson et al., 2011). Controlled prospective investigations starting before the initiation of rhythmic gymnastics training are necessary to answer definitively whether cumulative rhythmic gymnastics exposure is responsible for the observed greater bone mineral accrual in studied RG group. Furthermore, our findings are limited to a specific group of Caucasian females with specific age, body composition and athletic activity just before menarche. On the other hand, we present longitudinal data for the healthy population with well-defined biological maturation, body composition and specific mechanical loading pattern on bone during the period of growth where maximal increases in bone mineral accrual occur.

## Conclusion

The prolonged exposure to competitive rhythmic gymnastics trainings in premenarcheal girls is associated with greater bone mineral accrual despite lower body fat mass and leptin values between 7 to 12 years of age. This prolonged athletic activity during growth may reduce the risk of fragility fracture and osteoporosis later in life.

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### Key points

- Study examined long-term association between the exposure to competitive rhythmic gymnastics training and bone mineral accrual at the whole body, femoral neck and lumbar spine sites of the skeleton in premenarcheal rhythmic gymnasts.
- Prolonged exposure to competitive rhythmic gymnastics training before menarche provides skeletal benefits to bone mineral accrual at all measured skeletal sites despite lowered body fat mass and leptin values.

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