Bone Mineralization in Rhythmic Gymnasts Entering Puberty: Associations with Jumping Performance and Body Composition Variables

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Abstract
This study examined bone mineral density (BMD) accrual in prepubertal rhythmic gymnasts entering puberty and their age-matched untrained control girls, and associations with baseline jumping performance and body composition over the 3-year period. Whole body (WB) and femoral neck (FN) BMD, WB fat mass (FM) and fat free mass (FFM), countermovement jump (CMJ) and rebound jumps for 15 s (RJ15s) were assessed in 25 rhythmic gymnasts and 25 untrained controls at baseline and after 3-year period. The changes over this period were calculated (Δ scores). Pubertal maturation over the 3-year period was slower in rhythmic gymnasts compared to untrained controls, while no difference in bone age development was seen. WB BMD increased similarly in both groups, while the increase in FN BMD was higher in rhythmic gymnasts compared with untrained controls. In rhythmic gymnasts, baseline FFM was the most significant predictor of ΔWB BMD explaining 19.2% of the variability, while baseline RJ15s was the most significant predictor of ΔFN BMD explaining 18.5% of the variability. In untrained controls, baseline FM explained 51.8 and 18.9% of the variability in ΔWB BMD and ΔFN BMD, respectively. In conclusion, mechanical loading of high-intensity athletic activity had beneficial effect on BMD accrual in rhythmic gymnasts and may have counterbalanced such negative factors on bone development as slower pubertal maturation and lower body FM. Baseline FFM and repeated jumps test performance were related to BMD accrual in rhythmic gymnasts, while baseline FM was related to BMD accrual in untrained controls.

Key words: Rhythmic gymnasts, pubertal development, bone mineral accrual, body composition, jumping performance.

Introduction
Bone mineralization increases with age, height and body mass throughout childhood (Jürimäe, 2010), and maximal bone mineral density (BMD) accrual occurs in years surrounding puberty (Maimoun et al., 2013). The transition from prepuberty to 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 period (Hind and Burrows, 2007). Mechanical loading factors such as weight bearing and muscle forces are important in BMD accrual (Ho and Kung, 2005). Pubertal maturation also influences BMD accrual together with body composition factors (Cobayashi et al., 2005). Therefore, body fat mass (FM) and fat free mass (FFM) contribute to bone development by increasing compressive forces during skeletal loading (Ho and Kung, 2005). Accordingly, high impact mechanical loading is one of the most important factors determining BMD accrual during growth and maturation (Hind and Burrows, 2007), and young athletes whose skeletons are subjected to forces of high intensity, such as rhythmic gymnasts, present significantly higher BMD values at the load-bearing sites of the skeleton in comparison with athletes of low-impact activities (e.g., swimming) and healthy untrained controls (Gruodyte et al., 2009).

There are substantial data to support the view that body FM and FFM are both positively related to BMD in growing and maturing female athletes (Gruodyte et al., 2009). Various types of vertical jumps have been used to evaluate jumping ability in young athletes (Gruodyte et al., 2009; Kellis et al., 1999). It has been suggested that maximum vertical jump performed from the standing position with countermovement (CMJ) and also rebound jumps for 15 s (RJ15s) and 30 s (RJ30s) seconds represent well jumping abilities in young athletes (Kellis et al., 1999). However, to the best of our knowledge, no studies have been conducted to examine longitudinally the influence of body composition and jumping performance on BMD accrual in prepubertal rhythmic gymnasts entering puberty. Accordingly, the aim of the present 3-year longitudinal investigation was to study the increases in BMD values during pubertal maturation in rhythmic gymnasts and their age- and height-matched untrained control girls, and to evaluate the relationships between body composition and jumping performance parameters with increases in BMD values when entering from prepuberty to puberty. Therefore, in addition to whole body (WB) BMD, which is the most recommended measurement site for bone health in children and adolescents (Crabtree et al., 2014), BMD measurement was also done at the femoral neck (FN), since this skeletal site is associated with muscle power (Baptista et al., 2016) and used in rhythmic gymnastics trainings (Võsoberg et al., 2016).

Methods
Participants
A total of 50 Estonian prepubertal girls participated in this study, who were divided into rhythmic gymnasts (n = 25) and untrained control (n = 25) groups. They were followed for the next 3-year period, when they all had reached puberty. Rhythmic gymnasts were recruited from
locally trained groups and had usually trained 6-14 h/week (4-7 training sessions per week) for the past 2 years before starting the study. All RG had very similar training lessons, including rhythmic gymnastics, ballet and aerobatics. All RG were competing at the national level (Võsoberg et al., 2016). Controls had compulsory physical education classes twice a week at school. Participation only in school physical education classes was inclusion criteria for control subjects (Võsoberg et al., 2016). The groups were matched for age (±0.5 year) and height (±2 cm) at the beginning of the study (Jürimäe et al., 2016). All procedures were approved by the Medical Ethics Committee of the University of Tartu, Estonia and were explained to the children and their parents, who signed an informed consent form.

Height, body mass, body mass index (BMI), pubertal stage, bone age, body composition, BMD and jumping performance were obtained at baseline (T0) and after 3-years (T3). Therefore, the subjects were evaluated as close as possible to the baseline date at T3 measurement and within the same month of both measurements sessions. Both absolute values as well as changes between T3 and T0 (Δ scores) were used in the analyses (Võsoberg et al., 2016).

Materials and procedure

**Biological maturation:** Pubertal development of the participants was assessed by self-report using an illustrated questionnaire according to the Tanner classification method (1962), which has been used previously in our laboratory (Jürimäe et al., 2016; Parm et al., 2011; Võsoberg et al., 2016). Bone age was assessed with an X-ray of the left hand and wrist (Greulich and Pyle, 1959).

**Bone mineral density and body composition:** Bone mineral density (BMD; g·cm⁻²) of whole body (WB) and femoral neck (FN) were measured using dual-energy X-ray absorptiometry (DXA; DPX-IQ, Lunar Corporation, Madison, WI, USA) equipped with proprietary software, version 3.6. In addition, WB fat percentage (body fat%), fat mass (FM, kg) and fat free mass (FFM, kg) were also determined. Participants were scanned in light clothing while lying flat on their backs with arms at their sides. The fast scan mode and standard subject positioning were used for WB measurements and results were evaluated by the same examiner. To reduce the impact of the operator variability factor, one qualified observer analyzed all scans over the 3-year period. The precision of measurement expressed as coefficient of variation was less than 2% for all bone mineral and body composition measurements (Ivuskans et al., 2013; Parm et al., 2011).

**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) (Jürimäe et al., 2008). The participants performed two tests: 1) a countermovement jump (CMJ) from a standing position with a preliminary counter-movement, and 2) the rebound jump, with continuous countermovement jumps for 15 s (RJ15s) (Kellis et al., 1999). The participants were instructed and then verbally encouraged to jump as high and as rapidly they could. The hands remained on waist throughout both jumping tests to avoid upper extremities contribution to the jump height. The best value of three trials was used in the analysis (Kellis et al., 1999).

**Statistical analysis**

Statistical analyses were performed with SPSS version 20.0 for Window (SPSS Inc, Chicago, IL, USA), and the means and standard derivations (±SDs) were determined. Differences between gymnasts and controls at baseline (T0), after 3-years (T3) and the changes over time (Δ scores) were calculated using independent t-tests. Paired t-tests were performed to determine within-group changes in measured variables over the 3-year study period. Pearson correlation coefficients were computed to explore the relationship between baseline body composition and jumping performance indices with the increases in BMD values after the 3-year study period (Võsoberg et al., 2016). Partial correlation analysis was also applied to examine these relationships after adjusting for bone age (Baptista et al., 2016). Stepwise multiple regression analysis was performed to determine the possible independent associations of an increase in BMD parameters after the 3-year study with baseline age, bone age, BMI, FFM, RJ15s indices (Võsoberg et al., 2016). A significance level of p < 0.05 was used.

**Results**

Baseline age, bone age and FFM were not different between rhythmic gymnasts and untrained controls, and increased similarly after the 3-year study period (Table 1). Pubertal maturation was slower in gymnasts in comparison with controls. All studied girls were prepubertal at the beginning of study. After the 3-year study period, 21 rhythmic gymnasts were at pubertal stage 2 and four gymnasts at pubertal stage 3. In control group, 13 girls were at pubertal stage 2, nine girls at pubertal stage 3 and three girls at pubertal stage 4 after the 3-year study period. The studied groups had similar baseline body height and body mass values, while the increase in body height (p = 0.017) and body mass (p = 0.002) was lower in gymnasts compared to controls after the 3-year period. Rhythmic gymnasts had lower (p < 0.05) BMI, body fat% and FM in comparison with untrained controls at both measurement times, and the increase in BMI (p = 0.008), body fat% (p = 0.022) and FM (p = 0.0001) over the 3-year study period was also lower in gymnasts compared to controls. Baseline WB BMD was higher (p = 0.012) in gymnasts when compared with controls. No difference (p > 0.05) in WB BMD was found after the 3-year study period between groups. However, WB BMD increased significantly after the 3-year study in both groups. Baseline FN BMD was also higher (p = 0.013) in rhythmic gymnasts when compared with untrained controls, and the increase in FN BMD was higher (p = 0.036) in gymnasts in comparison with controls after the 3-year study period. Rhythmic gymnasts also presented higher CMJ and RJ15s values when compared with controls at both measurement times (Table 1).
In rhythmic gymnastics, the only baseline body composition and jumping performance measure that correlated with increases in BMD indices was baseline FFM, which was positively related to ∆WB BMD (r = 0.48; p = 0.016) value (Table 2). The relationship between FFM and ∆WB BMD remained significant (r = 0.44; p = 0.030) after controlling for bone age. In addition, baseline FFM (r = 0.45; p = 0.027) and RJ15s (r = 0.48; p = 0.016) were correlated with ∆FN BMD when adjusting for bone age in gymnasts. Stepwise multiple regression analyses showed that baseline FFM was the most significant predictor of ∆WB BMD explaining 19.2% of the variability, while baseline RJ15s was the most significant predictor of ∆FN BMD explaining 18.5% of the variability.

In untrained controls, baseline bone age (r = 0.62; p = 0.001), BMI (r = 0.58; p = 0.002), FM (r = 0.73; p = 0.0001) and FFM (r = 0.61; p = 0.001) were correlated with ∆WB BMD (Table 2). The relationships of BMI (r = 0.44; p = 0.032), FM (r = 0.62; p = 0.001) and FFM (r = 0.43; p = 0.034) with ∆WB BMD remained significant after adjusting for bone age. Only baseline FM (r = 0.47; p = 0.017) was correlated with AFN BMD, while no correlations (p > 0.05) with AFN BMD were observed when controlling for bone age. Stepwise multiple regression analyses showed that baseline FM value was the most significant predictor of ∆WB BMD and ∆FN BMD values, explaining 51.8% and 18.9% of the variability, respectively.

**Discussion**

Different jumping performance and body composition parameters are related to BMD accrual in pubertal girls with different physical activity levels (Gruodyte et al., 2009; 2010). Our 3-year prospective study demonstrated that baseline body composition parameters were significantly associated with increases in BMD values when entering from prepuberty to puberty in girls with different physical activity patterns, while jumping performance was related to BMD accrual at the weight-bearing site.

**Table 1.** Mean (±SD) clinical characteristics of rhythmic gymnasts (RG) and untrained controls (UC), and their changes over the 3-year period (Δ score).

<table>
<thead>
<tr>
<th>Variable</th>
<th>RG (n = 25)</th>
<th>UC (n = 25)</th>
<th>Δ RG (n = 25)</th>
<th>Δ UC (n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>T0 8.0 (0.6)</td>
<td>T0 8.2 (0.6)</td>
<td>T0 3.0 (0.1)</td>
<td>T0 3.4 (0.2)</td>
</tr>
<tr>
<td>Bone age (yrs)</td>
<td>T0 10.9 (0.6)</td>
<td>T0 11.2 (0.5)</td>
<td>T0 3.2 (0.8)</td>
<td>T0 3.4 (0.7)</td>
</tr>
<tr>
<td>Body height (m)</td>
<td>T0 0.43 (0.05)</td>
<td>T0 1.30 (0.05)</td>
<td>T0 0.17 (0.02)</td>
<td>T0 0.19 (0.02)</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>T0 27.0 (3.4)</td>
<td>T0 28.9 (5.0)</td>
<td>T0 10.2 (2.8)</td>
<td>T0 13.7 (4.5)</td>
</tr>
<tr>
<td>BMI (kg·m⁻²)</td>
<td>T0 15.9 (1.3)</td>
<td>T0 17.0 (2.2)</td>
<td>T0 1.1 (0.9)</td>
<td>T0 2.1 (1.7)</td>
</tr>
<tr>
<td>Body fat %</td>
<td>T0 19.8 (5.3)</td>
<td>T0 26.0 (6.2)</td>
<td>T0 1.4 (3.3)</td>
<td>T0 4.1 (4.8)</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>T0 5.1 (1.9)</td>
<td>T0 7.4 (2.7)</td>
<td>T0 2.4 (1.5)</td>
<td>T0 5.4 (2.9)</td>
</tr>
<tr>
<td>Fat free mass (kg)</td>
<td>T0 20.2 (2.0)</td>
<td>T0 20.1 (2.5)</td>
<td>T0 7.1 (2.0)</td>
<td>T0 7.6 (2.3)</td>
</tr>
<tr>
<td>WB BMD (g·cm⁻³)</td>
<td>T0 .88 (.04)</td>
<td>T0 .84 (.05)</td>
<td>T0 .08 (.03)</td>
<td>T0 .09 (.02)</td>
</tr>
<tr>
<td>FN BMD (g·cm⁻³)</td>
<td>T0 .78 (.07)</td>
<td>T0 .72 (.009)</td>
<td>T0 .14 (.06)</td>
<td>T0 .10 (.06)</td>
</tr>
<tr>
<td>CMJ (cm)</td>
<td>T0 23.2 (2.4)</td>
<td>T0 19.2 (2.0)</td>
<td>T0 3.1 (2.5)</td>
<td>T0 4.2 (2.3)</td>
</tr>
<tr>
<td>RJ15s (cm)</td>
<td>T0 20.2 (1.8)</td>
<td>T0 16.2 (2.3)</td>
<td>T0 2.8 (2.1)</td>
<td>T0 3.4 (2.4)</td>
</tr>
</tbody>
</table>

**Table 2.** Pearson correlation coefficients between changes (Δ scores) in bone mineral values over the 3-year period with baseline body composition and jumping performance variables.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Rhythmic gymnasts (n = 25)</th>
<th>Untrained controls (n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>.28</td>
<td>.32</td>
</tr>
<tr>
<td>Bone age (yrs)</td>
<td>.19</td>
<td>.62 *</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>.13</td>
<td>.58 *</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>.07</td>
<td>.73 *</td>
</tr>
<tr>
<td>Fat free mass (kg)</td>
<td>.48 *</td>
<td>.61 *</td>
</tr>
<tr>
<td>CMJ (cm)</td>
<td>.11</td>
<td>.22</td>
</tr>
<tr>
<td>RJ15s (cm)</td>
<td>.29</td>
<td>.33</td>
</tr>
</tbody>
</table>

WB, whole body; BMC, bone mineral content; BMD, bone mineral density; FN, femoral neck; CMJ, countermovement jump; RJ15s, the rebound jumps for 15 seconds. * Statistically significant differences between RG and UC; p < 0.05. # Statistically significant changes between T3 and T0; p < 0.05. ‡ Statistically significant differences between Δ RG and Δ UC; p < 0.05.
only in athletic girls. Furthermore, while the increase in WB BMD was similar in rhythmic gymnasts and untrained controls, the increase in FN BMD was significantly higher in gymnasts when compared with controls, indicating that the weight-bearing bones are more sensitive to chronic exercise in prepubertal girls entering from prepuberty to puberty (Gruodyte et al., 2009; Maimoun et al., 2010). These results suggest that different body composition and jumping performance parameters are associated with BMD accrual in girls entering into puberty depending on specific physical activity patterns in studied girls. In addition, mechanical loading of high-intensity athletic activity has positive influence on BMD accrual and may have counterbalanced such negative factors on bone development as slower pubertal development and lower body FM values in early pubertal rhythmic gymnasts.

Measured BMD values increased significantly after the 3-year study period in both groups despite different physical activity patterns. However, BMD accrual at the FN site of the skeleton was more sensitive to the specific physical activity pattern compared to WB BMD accrual in prepubertal gymnasts entering into puberty. Specifically, the increase in FN BMD was significantly higher over 3-year period in gymnasts when compared with controls, while WB BMD increased similarly in both groups (see Table 1). Accordingly, it could be suggested that BMD accrual at FN experienced greater mechanical loading during high-impact activities than WB BMD in rhythmic gymnasts. Furthermore, this chronic mechanical loading of high-impact athletic activity appears to be more important factor for BMD accrual than relatively low body FM values, which could be indicative of inadequate energy intake of chronic athletic activity (Courteix et al., 2007). Similarly, other studies have demonstrated significant increases of measured BMD values despite lowered body FM during prepuberty (Parm et al., 2011) and puberty (Maimoun et al., 2010, 2013) in girls with intensive gymnastics trainings. In our study, the increases in the measured BMD values occurred despite slower pubertal progression in rhythmic gymnasts in comparison with untrained control girls. It has been argued that because of the slower pubertal maturation bone mineral accrual lasts longer in elite gymnasts (Maimoun et al., 2013), a high peak bone mass is achieved (Maimoun et al., 2014). The results of our study also demonstrated that there was not any delay in bone age maturation in the transition from prepuberty to early puberty in studied rhythmic gymnasts after the 3-year period. It appears that bone age maturation may be delayed during prepuberty (Parm et al., 2011), and pubertal maturation may be shifted to a later age in elite gymnasts (Maimoun et al., 2010; 2014). However, this slower pubertal maturation did not affect BMD accrual in our gymnasts as regular high-impact weight bearing physical activity appears to be the most important determinant of bone development during maturation in these athletes (Maimoun et al., 2014).

The results of current study demonstrated that while both baseline FM and FFM parameters were correlated with WB BMD accrual in untrained controls, FFM was the only body composition parameter that was associated with WB BMD accrual in rhythmic gymnasts. Furthermore, regression analyses indicated that while baseline FM was the most important parameter that was associated with WB BMD accrual in controls, baseline FFM was the most important parameter that was associated with WB BMD in prepubertal gymnasts entering into puberty. There is a disagreement in the literature regarding the relative contributions of FM and FFM values on bone mineral development in growing children (Ivuskans et al., 2013). The association of FM and FFM indices with BMD accrual could be dependent on maturation (Co-bayashi et al., 2005), weight status (Ivuskans et al., 2013) and/or physical activity pattern (Parm et al., 2011) of the studied children. Similarly to our results, positive effect of FM on BMD accrual has been demonstrated in prepubertal normal weight untrained girls (Parm et al., 2011). It has been suggested that bone development may be mediated by the increasing synthesis of estrogen in the adipose tissue that promotes bone mass accrual (Cobayashi et al., 2005). In contrast, it has also been suggested that FFM is better determinant of BMD accrual in normal weight children (Ivuskans et al., 2013), which is in line with the results observed in our gymnasts. Accordingly, it can be argued that intense athletic activity has an influence on the relationship between body composition and BMD increment in gymnasts. The negative effect of reduced FM on BMD accrual may be compensated by the mechanical loading generated by high-impact weight bearing exercise in gymnasts (Maimoun et al., 2014).

Jumping performance parameters were better in rhythmic gymnasts in comparison with untrained control girls in both measurement times, which is probably due to the high-impact weight-bearing athletic activity in gymnasts (Gruodyte et al., 2009). Furthermore, baseline jumping performance (RJ15s) was the most important predictor of FN BMD accrual in studied gymnasts. In accordance, it has been shown that vertical jump is a strong and positive determinant of bone health in prepubertal children (Baptista et al., 2016). Vertical jump power and force have also distinct associations with cortical bone parameters in individuals with high bone mass (Hardcastle et al., 2014). Similarly, jumping performance was correlated with higher ∆FN BMD values in gymnasts but not in controls. It has been shown that chronic exercise has more effect on BMD at mechanically loaded cortical bone sites, while hormonal factors influence more trabecular bone (Maimoun et al., 2014). Accordingly, BMD accrual increased similarly in WB BMD in both groups, while the increase in FN BMD was higher in gymnasts when compared with controls after the 3-year study period (see Table 1). It appears that jumping performance is related to bone accretion at cortical bone sites only in prepubertal girls with previous athletic activity and with higher BMD values.

In contrast to other studies (Baptista et al., 2016; Hardcastle et al., 2014), single vertical jump (CMJ test) performance did not appear to be a significant predictor of ∆FN BMD in rhythmic gymnasts. The results of our study demonstrated that repeated jumps (RJ15s test) performance better characterized ∆FN BMD in gymnasts, which is similar to the results of a previous study in pubertal girls with different physical activity patterns (Gruodyte et
al., 2009). In addition, it has also been reported that CMJ test may not be a significant predictor of BMD at the legs region of the skeleton in prepubertal boys and girls (Jürimäe et al., 2008). It has been suggested that the continuous loading of the legs is more important than the absolute height of the single jump to influence regional BMD in growing children (Jürimäe et al., 2008). Similarly to our results, CMJ was not correlated with FN BMD in pubertal athletes representing different sport events with different impact characteristics to the growing bones (Gruodyte et al., 2009). Taken together, the results of current study demonstrate that repeated jumps test (RJ15s) should be measured instead of a single jump test (CMJ) to evaluate the association between muscle performance and BMD accrual in prepubertal athletes entering puberty.

Some limitations of the current study include a lack of dietary information, the use of two-dimensional DXA technology and the relatively long observation period between two measurement times. In the future, RG should be evaluated at shorter intervals to more precisely monitor pubertal maturation. However, the longitudinal design indicated positive effects of regular weight-bearing athletic activity on bone mineralization in a specific group of growing and maturing athletes despite their low body fat values.

Conclusion

In conclusion, while the WB BMD accrual over 3-year period was similar in both groups, continuous high-impact mechanical loading had additional beneficial effects on the FN BMD accrual in prepubertal rhythmic gymnasts entering into puberty. Jumping performance and FFM values predicted BMD accrual only in girls with previous athletic activity, while FM was associated with BMD accrual in prepubertal untrained control girls entering into puberty. Finally, only repeated jumps test (RJ15s), but not single jump test (CMJ) was related to bone development in rhythmic gymnasts.

Acknowledgments

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References


Key points

- Study examined bone mineralization in prepubertal rhythmic gymnasts entering puberty and their age-matched untrained control girls, and associations with baseline jumping performance and body composition.
- Jumping performance and fat free mass values predicted bone mineral accrual in rhythmic gymnasts.
- Fat mass predicted bone mineral accrual in untrained control girls.
- Repeated jumps test, but not single jump test was related to bone mineralization in rhythmic gymnasts.

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