Bone mineral density acquisition in peripubertal female rhythmic gymnasts is directly associated with plasma IGF1/IGF-binding protein 3 ratio

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Abstract

Objectives: Intense physical activity in peripubertal girls may delay menarche and cause menstrual disorders and estrogen deficiency, particularly in sport disciplines that require strict weight control. It may also have a deleterious effect on bone mass acquisition. The aim of this study was to determine the time-course of bone mass accretion in peripubertal elite female rhythmic gymnasts (FRGs) over a 1-year period, as well as the anthropometric and hormone parameters that could be helpful for predicting bone mineral density (BMD) gain.

Methods and design: We conducted a 1-year follow-up study in 29 FRGs (10.7–16.1 years old). Whole body composition and BMD of the whole body, proximal femur, lumbar spine, mid-radius, and skull were measured by dual energy X-ray absorptiometry (DXA). Moreover, baseline growth- and bone metabolism-related hormones such as IGF1, IGF-binding protein 3 (IGFBP3), leptin, and bone markers were measured.

Results: BMD increased significantly at all bone sites throughout puberty, particularly between Tanner stages II and IV–V (P < 0.001). The IGF1 level, IGF1/IGFBP3 ratio, and leptin level were higher in late pubertal stages (i.e. IV–V) compared with early stage (i.e. I). In simple and multivariate analyses, only the IGF1/IGFBP3 ratio was strongly correlated with the BMD change at all bone sites (r = 0.49, P < 0.02 to r = 0.77, P < 0.0001).

Conclusion: This 1-year follow-up study of peripubertal FRGs showed that BMD gain was maximal around Tanner stage III. The plasma IGF1/IGFBP3 ratio was associated with bone mass acquisition in this period, and it may thus serve as a surrogate marker of bone mass gain in this population.

Introduction

Growth is a critical time for bone mass accrual. In a longitudinal study, Theintz et al. (1) reported that the bone mass gain in girls was particularly marked between 11 and 14 years at the lumbar spine and femoral neck, with the increment falling dramatically 2 years after menarche. Various biological and environmental parameters influence the acquisition of peak bone mass, which is known to be a major factor in the development of osteoporosis later in life (2). Physical activity and in particular weight-bearing activity improve bone mass acquisition (3, 4). However, many adolescent athletes have delayed menarche, and develop menstrual disorders such as oligomenorrhea and amenorrhea (5, 6) associated with an estrogen deficit known to have a deleterious effect on bone density. Nevertheless, we reported in a previous cross-sectional study that bone mineral content (BMC) and bone mineral density (BMD) increased throughout growth in young female rhythmic gymnasts (FRGs), particularly between Tanner stages II and IV, with variable bone accretion time lags depending on the bone site (7). Despite menstrual disorders, the older athletes (i.e. Tanner stage V, mean age 15.8 years) presented higher BMD compared with bone mass acquisition in this period, and it may thus serve as a surrogate marker of bone mass gain in this population.
associated with bone mass (8, 9), while the effects of other hormones like leptin remain more questionable (8, 10, 11). These relationships may be modified in athletes, who present body composition variations and hormonal adaptations. In a cross-sectional study, growth factors have also been associated with bone mass in athletes (12), confirming the strong effect of IGF1 on bone mass acquisition. However, this study design cannot be used to investigate the effects of anthropometric parameters and hormones on bone mass gain in the same subject over an extended period.

A better understanding of the factors associated with the great increase in bone mass that occurs in puberty would improve the clinical management of adolescent girls at risk of developing menstrual disorders and hypoestrogenism, such as gymnasts. The aim of this study was to determine the time-course of bone mass accretion in peripubertal elite FRGs over a 1-year period. From a large palette of anthropometric and hormone factors, we also sought to determine whether any would be helpful for predicting BMD gain.

**Subjects and methods**

**Subjects**

The study protocol was reviewed and approved by the Regional Research Ethics Committee (CPP Sud-Mediterranee IV, Montpellier, France), and each gymnast and her parents gave written informed consent before entering the study. Twenty-nine peripubertal FRGs from a group of 43 previously described gymnasts (7) were followed at a 1-year interval. The participants’ ages at the start of the study ranged from 10.7 to 16.1 years, and the girls were classed according to the Tanner stage (Tanner I, n = 6; Tanner II, n = 9; Tanner III, n = 6; and Tanner IV–IV, n = 8). Tanner stages IV and V were combined because these late pubertal stages have shown no differences in anthropometric parameters, BMC, BMD (7, 9), or biological parameters, particularly leptin (7). None of the participants had obvious signs of acute or chronic illness. The gymnasts had begun training at 6.9 ± 1.5 years and trained for a mean of 21.2 ± 4.6 h per week in stage I (mean age 11.4 ± 0.4 years) to 23.3 ± 1.7 h per week in stages IV–V (mean age 14.3 ± 1.4 years). Most training sessions lasted 4 h and consisted of a warm-up, routine training, and strength and stretching exercises. All subjects were competing at club level.

**Methods**

This study used a 1-year follow-up design. Standing height was measured with a stadiometer to the nearest 0.1 cm. Weight was determined using a weight scale with a precision of 0.1 kg. Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m). Pubertal development was assessed by breast stage (I–V) according to the Tanner classification (13) by an experienced pediatric endocrinologist. Bone age was determined using the Greulich & Pyle method (14).

Information regarding pubertal onset of family members was obtained from a standardized questionnaire (menarche of mothers). Height SDS and weight SDS were calculated according to the French standard curves.

**Medical and menstrual histories**

Each subject also responded to a medical questionnaire designed to assess general medical and menstrual history with questions concerning age of menarche and periods of amenorrhea. The mean age of menarche was 14.0 ± 1.3 years, and the number of girls with menarche by Tanner stage was as follows: stage I: n = 0 out of 6; stage II: n = 1 out of 9; stage III: n = 2 out of 6; and stages IV–V: n = 8 out of 8. A high prevalence of menstrual irregularity was observed: stage II: n = 6 out of 6; stage III: n = 3 out of 6; and stages IV–V: 7 out of 8.

**Physical activity determination**

Detailed information about training history was collected, including data on starting age of intensive training, years of active sport-specific training, and number of training sessions per week, training hours per week, and training months per year. Other physical activities were documented with a training recall diary covering the previous 3 years.

**Assays**

Blood samples (25 ml) were collected in the morning (0900–1100 h) in sterile chilled tubes by standard venipuncture technique. The samples were allowed to clot at room temperature and were then centrifuged at 750 g for 10 min at 4 °C. Serum samples were stored at −80 °C until analysis. All samples were run in duplicate and, to reduce inter-assay variation, all the plasma samples were analyzed in a single session. The date of the last menses was not recorded for the pubertal girls, and hormonal values were thus obtained at an unsynchronized menstrual stage.

E2 (Biomerieux, Marcy-l’Etoile, France), testosterone (Immunotech, Marseille, France), IGF1 (Diagnostic Systems Laboratories, Webster, TX, USA), IGF-binding protein 3 (IGFBP3, IRMA, Immunotech), and leptin (Active human leptin IRMA DSL-23100, Diagnostic Systems Laboratories) were analyzed. The inter-assay coefficients of variation (CV) for E2, testosterone, IGF1, IGFBP3, and leptin were 5.1, <4, <8, 8.2, and 6.6% respectively. The intra-assay CV were 3.9, 7, <4, <8, and 4.9% respectively. Markers of bone remodeling, osteocalcin (OC; Elsa-OST-NAT, CIS
Biointernational, Gif/Yvette, France), bone alkaline phosphatase (B-ALP; Tandem-R Ostase Hybritec, Inc., San Diego, CA, USA), and type I-C telopeptide breakdown products (CTX; CrossLaps ELISA, OSTEOMETER A/S, Rodovre, Denmark), were also analyzed. The inter-assay CV of OC, B-ALP, and CTX variation were < 5, < 9, and < 13% respectively. The intra-assay CV were < 5, < 7, and < 10% respectively. Intact parathyroid hormone (iPTH, N-tact PTH SP, Diasorin, Stillwater, MN, USA), 25-hydroxyvitamin D (25(OH)D, 25-hydroxyvitamin D RIA kit, Nichols Institute Diagnostics, Paris, France), and calcitriol (1.25(OH)2D3, 1.25 dihydroxyvitamin D RIA kit, Nichols Institute Diagnostics) were additionally analyzed. The inter-assay CV of iPTH, 25(OH)D, and 1.25(OH)2D3 variation were 8, 5, and 5% respectively. The intra-assay CV were 3.6, 8.1, and < 10.8% respectively.

BMD, body fat, and fat-free soft tissues

Dual energy X-ray absorptiometry (DXA) (Hologic QDR-4500A, Hologic, Inc., Waltham, MA, USA) was used to measure the BMD (g/cm²) of the whole body (WBBMD), the antero-posterior lumbar spine (L2–L4), the dominant arm radius, the total proximal left femur (TPF), and specific sites of the femoral neck and the trochanteric areas. The soft tissue body composition (FM (kg), percent body FM (% FM), and fat-free soft tissue (FFST, kg) was derived from the WB scan. All scanning and analyses were performed by the same operator to ensure consistency, after following standard quality control procedures. Quality control for DXA was checked daily by scanning a lumbar spine phantom consisting of calcium hydroxyapatite embedded in a cube of thermoplastic resin (DPA/QDR-1; Hologic x-caliber anthropometrical spine phantom). For BMD, the laboratory precision error was defined by the CV of repeated measurements; this was found to be 1% at the lumbar spine and < 1% at the femoral neck, < 1% at the forearm, < 0.5% for the WB, and < 1% for FFST and FM. Identical and accurate positioning of the region of interest was ensured by superimposing the image from the very first session on the image of the explored bone area; this initial image thus served as the visual reference (15).

Statistical analysis

The characteristics of the young athletes entered in the present study are described with proportions for categorical variables and with means and s.d. values for continuous variables (age, weight, BMD, etc.). The distributions were tested with the Shapiro–Wilk statistic. The comparisons of means according to the Tanner stage were performed using the Kruskall–Wallis test. Pairwise comparisons were made using the Bonferroni post hoc test. The relationships between continuous parameters were assessed using the Spearman correlation coefficient. A multivariate analysis using the linear regression was performed to determine the factors independently associated with the BMD gain at 1 year evaluated at each bone site. Variables significantly associated with BMD gain at 1 year in the univariate analysis were entered into the model. A backward selection was used to conserve the variables significantly associated with a P multivariated < 0.20 in the model. A P < 0.05 was considered as statistically significant. SAS software, version 9.1 (SAS Institute, Cary, NC, USA), was used for the statistical analysis.

Results

Parameters at baseline

The anthropometric characteristics and the BMD values from the bone sites of the 29 FRGs are reported in Table 1. Height, weight, BMI, FFST, and absolute FM (kg) increased with Tanner stages, while no variation was observed for relative FM (%). The SDS for height and weight were within ± 2, indicating that the FRGs were within the normal range for French girls in all groups. A significant difference was observed between chronological age and bone age (P < 0.05) in Tanner stages I and II only. This difference remained significant 1 year later (P < 0.05) for both groups (−2.1 ± 0.6 for stage I and −1.1 ± 0.7 for stage II). The increase in height (cm/year) for each Tanner stage group at 1 year was 4.7 ± 2.1 for stage I, 5.9 ± 1.7 for stage II, 4.8 ± 2.7 for stage III, and 2.5 ± 1.5 for stages IV–V. BMD increased significantly at all bone sites throughout puberty (P = 0.025 to P < 0.001). This was observed for the WB, at weight-bearing bone sites (TPF), and at less mechanically solicited (lumbar spine) and no-impact (skull) sites. The BMD difference was consistently marked between stages IV–V and I and II. The BMD gain between Tanner stages IV–V and I ranged between 17.8% for the WB and 32.5% for the lumbar spine.

Hormonal variations are presented in Table 2. E2 and testosterone levels increased significantly with pubertal stage (P = 0.002 and P < 0.001 respectively). The mean age of recalled menarche was 14.0 ± 1.3 years (n = 11; range 12–16.3) in FRGs, and was not different (P = 0.35) from the mothers’ mean age of menarche, which was 13.4 ± 1.8 years (range 11–17). The IGF1 levels and the IGF1/IGFBP3 ratio were higher in the late pubertal stages (i.e. IV–V) compared with the early stage (i.e. I). The leptin increase was more than threefold between Tanner stage I (3.6 ± 1.7 ng/ml) and stages IV–V (11.6 ± 5.1 ng/ml), and the value remained doubled when leptin concentrations were adjusted to WB FM (0.571 ± 0.234 vs 1.102 ± 0.250 ng/ml per kg).

The biochemical markers of bone turnover and calciotropic hormones at each stage of puberty are shown in Table 3. Markers of bone formation (OC and B-ALP) and resorption tended to decrease with Tanner stage, but the values did not reach significance.

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This was probably due to the large dispersion of individual values. Similarly, $1.25(\text{OH})_2\text{D}_3$ tended to increase ($P=0.064$), while no variation was observed for iPTH and 25(OH)D.

**BMD changes at 1 year**

The mean BMD change in FRGs over 1 year is presented in Fig. 1. The increase in BMD at WB and skull was maximal in Tanner stages IV–V, and the gain was significantly higher for stages IV–V and III compared with stage I. Concerning the other bone sites (i.e. TPF, L2–L4, and mid-radius), the maximal BMD gain was observed for stage III, and the gain remained significantly higher for stages IV, III, and II compared with stage 1. When percentage change by year was calculated (data not shown), the gain ranged from 0.8–4.5% at WB (stage I versus stages IV–V), 0.45–9.8% at skull (stage I versus stages IV–V), 0.8–11.8% at L2–L4 (stage I versus stages IV–V), 0.45–9.8% at TPF (stage I versus stage III), and 0.24–6.1% at mid-radius (stage I versus stage III).

**Table 2** Initial hormonal profile of rhythmic gymnasts stratified by pubertal stage ($n=29$). Values are presented as mean±s.d.

<table>
<thead>
<tr>
<th>Tanner stages</th>
<th>I (n=6)</th>
<th>II (n=9)</th>
<th>III (n=6)</th>
<th>IV–V (n=8)</th>
<th>Global $P$ value</th>
<th>Bonferroni's post hoc test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex hormones</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estradiol (pg/ml)</td>
<td>9.6±0.9</td>
<td>14.3±9.5</td>
<td>46.0±52.7</td>
<td>77.2±90.4</td>
<td>0.002</td>
<td>(V–IV) vs II–I</td>
</tr>
<tr>
<td>Testosterone (ng/ml)</td>
<td>0.21±0.02</td>
<td>0.21±0.06</td>
<td>0.30±0.08</td>
<td>0.45±0.13</td>
<td>0.001</td>
<td>(V–IV) vs II–I</td>
</tr>
<tr>
<td>Growth factors</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGF1 (μg/l)</td>
<td>168.3±40.0</td>
<td>219.8±86.7</td>
<td>291.6±128.4</td>
<td>290.3±24.6</td>
<td>0.020</td>
<td>(V–IV) vs I</td>
</tr>
<tr>
<td>IGFBP3 (μg/l)</td>
<td>3239.4±589.7</td>
<td>3007.1±662.8</td>
<td>2989.8±847.6</td>
<td>3295.8±346.2</td>
<td>0.457</td>
<td>NS</td>
</tr>
<tr>
<td>IGF1/IGFBP3 ratio</td>
<td>5.3±1.1</td>
<td>7.2±1.5</td>
<td>9.4±1.8</td>
<td>8.9±1.0</td>
<td>&lt;0.001</td>
<td>(V–IV) vs I</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>3.6±1.7</td>
<td>4.0±2.3</td>
<td>6.2±2.7</td>
<td>11.6±5.1</td>
<td>0.002</td>
<td>(V–IV) vs I–II</td>
</tr>
<tr>
<td>Ratio leptin/body fat mass (ng/ml per kg)</td>
<td>0.571±0.234</td>
<td>0.552±0.229</td>
<td>0.727±0.285</td>
<td>1.102±0.250</td>
<td>0.009</td>
<td>(V–IV) vs I–II</td>
</tr>
</tbody>
</table>

IGF1, insulin-like growth factor 1; IGFBP3, insulin-like growth factor-binding protein 3.
Calciotropic hormones at TPF (r observed between CTX and any of the bone sites (Table 4).

Bone markers

<table>
<thead>
<tr>
<th>Bone markers</th>
<th>Stage I (n=6)</th>
<th>Stage II (n=9)</th>
<th>Stage III (n=6)</th>
<th>Stage IV–V (n=8)</th>
<th>Global P value</th>
<th>Bonferroni’s post hoc test</th>
</tr>
</thead>
<tbody>
<tr>
<td>OC (ng/ml)</td>
<td>54.3±12.7</td>
<td>56.6±17.0</td>
<td>66.4±17.0</td>
<td>37.0±21.0</td>
<td>0.074</td>
<td>NS</td>
</tr>
<tr>
<td>B-ALP (ng/ml)</td>
<td>62.4±22.6</td>
<td>85.3±35.6</td>
<td>73.9±28.0</td>
<td>47.2±18.3</td>
<td>0.188</td>
<td>NS</td>
</tr>
<tr>
<td>CTX (pmol/l)</td>
<td>13 398±3885</td>
<td>13 162±5393</td>
<td>13 450±2871</td>
<td>8679±4461</td>
<td>0.296</td>
<td>NS</td>
</tr>
<tr>
<td>iPTH (pg/ml)</td>
<td>26.2±4.6</td>
<td>27.6±11.7</td>
<td>22.2±8.7</td>
<td>23.8±7.2</td>
<td>0.675</td>
<td>NS</td>
</tr>
<tr>
<td>25(OH)D (pg/ml)</td>
<td>23.1±10.3</td>
<td>20.0±5.4</td>
<td>27.4±5.5</td>
<td>25.8±7.2</td>
<td>0.163</td>
<td>NS</td>
</tr>
<tr>
<td>1.25(OH)2D3 (pg/ml)</td>
<td>63.5±24.1</td>
<td>50.4±7.3</td>
<td>70.8±13.5</td>
<td>77.2±19.5</td>
<td>0.064</td>
<td>NS</td>
</tr>
</tbody>
</table>

Calcitropic hormones

| Relationships between BMD changes at 1 year and basal biological parameters |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| In univariate analysis, weight, height, and WB FFST measured at baseline were significantly correlated with ΔBMD at WB (r=0.51, 0.58, and 0.54 respectively), mid-radius (r=0.55, r=0.51, and r=0.55 respectively), and skull (r=0.67, r=0.69, and r=0.46 respectively). WB FM was correlated with ΔBMD at mid-radius and skull (r=0.43, P=0.018 and r=0.64, P=0.0001 respectively).
| ΔBMD at L2–L4 was significantly correlated with the baseline plasma concentrations of OC (r=0.47, P=0.020) and was borderline with B-ALP (r=0.38, P=0.06). B-ALP was significantly correlated with ΔBMD at TPF (r=0.53, P=0.009). No relationship was observed between CTX and any of the bone sites (Table 4).
| The baseline plasma leptin and E2 levels were significantly correlated with ΔBMD at WB (r=0.39 and 0.41 respectively) and mid-radius (r=0.43 and 0.59 respectively), while testosterone tended to be associated with ΔBMD at mid-radius (r=0.40, P=0.06). E2 was also correlated with ΔBMD at skull (r=0.42, P=0.04).
| IGF1 and the IGF1/IGFBP3 ratio were significantly correlated with ΔBMD at WB, skull, L2–L4, TPF, and mid-radius. Nevertheless, when multiple regression analysis was performed, only the IGF1/IGFBP3 ratio appeared as an independent factor influencing bone mass acquisition at all bone sites (WB, P=0.0008; L2–L4, P<0.0001; TPF, P=0.014; and mid-radius, P=0.0002), skull excepted. The other independent factors associated with bone mass variation were height (P=0.005) for skull, E2 (P=0.006) for L2–L4, B-ALP (P=0.05) for TPF and weight (P=0.0046), and leptin (P=0.0032) and iPTH (P=0.002) for mid-radius.

Discussion

In this study, we report the progressive bone mass accretion in peripubertal FRGs who train more than 20 h per week, according to the pubertal stage.

We previously reported that the maximal gain in BMD between two consecutive Tanner stages occurred between stages II and III or between stages III and IV, depending on the bone site (7). However, these data were obtained with a cross-sectional study design, and variations may have been influenced by individual characteristics such as genetic predisposition, nutrition, or training status. This 1-year follow-up reinforces the previous findings, and underlines that the BMD gain in FRGs was maximal around Tanner stage III for L2–L4, TPF, and mid-radius, while minor BMD variation was observed for the prepubertal period (Tanner stage I). In an earlier study, Lehtonen-Veromaa et al. (4) reported similar findings in athletes from various sports (i.e. gymnasts and runners), as well as controls, and specified that the mean increase in BMD in the femoral region and L2–L4 was highest in early puberty (Tanner stages II–III). Our study also showed that for WB and skull, the maximal gain was later, at stages IV–V. This suggests different time lags in acquisition, depending on the bone site and its specific characteristics, such as composition (i.e. cortical or trabecular) and the applied mechanical constraints (weight-bearing or not).
weight-bearing bone). However, the similar time-course and absolute BMD gain in L2–L4, a trabecular and very low weight-bearing bone, and TPF, a cortical and heavy weight-bearing bone, suggests that a common factor is responsible for major bone acquisition. Among the causes, bone growth appeared to be the most determinant factor. Nevertheless, it is possible that the high impact exercise of gymnastics training induces a positive adaptive response in the growing skeleton, particularly at TPF, which undergoes far more repetitive loading than the lumbar spine (4). The benefits of physical activity may thus be accentuated during the growth spurt or Tanner stages III–IV (16), as observed in our population.

The concerted actions of GH, IGF1, and sex steroids are responsible for the timing of the bone modeling process and bone mass acquisition through a complex sequence of variations that interact with nutritional and environmental factors (17). This study showed an increase in IGF1 levels and the IGF1/IGFBP3 ratio with pubertal stage, which confirms an earlier large cross-sectional study in non-active girls (18). We recently reported that univariate analysis revealed correlations at baseline between BMD and IGF1 and BMD and the IGF1/IGFBP3 ratio in various bone sites of these athletes (7). Now, in this study, we demonstrate for the first time the predictive value of the basal IGF1/IGFBP3 ratio for BMD gain at 1 year. The correlation between IGF1/IGFBP3 ratio and BMD gain at all bone sites, in both univariate analysis and stepwise regression models, suggests a systemic effect of growth factors on bone mass acquisition. IGF1 is an important regulator of bone turnover; it enhances osteoblast proliferation to stimulate type-I collagen production and BAP activity and to modulate osteoblast–osteoclast interactions (19, 20). Moreover, an experimental study demonstrated that circulating levels of IGF1 directly regulate bone growth and density, suggesting that it plays an important role in the acquisition of peak bone mass and strength (21). In a cross-sectional study in humans, Snow et al. (12) observed concomitantly higher IGF1 levels and BMD values in young adult gymnasts compared with controls, and IGF1 was found to be an independent predictor of bone mass and lean body mass. The same authors further suggested that IGF1 might be a potential mediator of muscle–bone interaction (12). The high levels observed by this group may accentuate the effect of IGF1 on bone tissue. However, normal (22) and low IGF1 values have also been reported in gymnasts, and these discrepant findings may be due to the differences in the timing of investigation (23) or in nutritional status. The effect of IGF1 or IGFBP3 on bone mass acquisition was very recently supported by the results of Gruodyte et al. (24), who also reported that the femoral neck and lumbar spine BMD were correlated with the IGF1/IGFBP3 molar ratio in rhythmic gymnasts, after adjusting for age, body height, and body mass. Interestingly, these relationships were not observed in sedentary controls or other athletes, such as swimmers, team-sport players, sprinters, and cross-country skiers.

During growth, estrogen is a major regulator of the IGF1 increase (25), but it also has direct action on bone mineralization (26). In this study, the increases in serum E2 levels with pubertal stage were significantly associated in univariate analysis with the increment in BMD values at WB and mid-radius, while testosterone was not involved in bone accretion. Adolescent gymnasts generally undergo intensive training, with considerable stress and strict weight control. These factors, along with low estrogen levels, may delay both skeletal maturation and menarche (5). In this study, we confirmed the delayed age of menarche (mean age: 14 vs 12.6 years in the French population) (27) and the high prevalence of menstrual disorders. Conversely, no alteration in either peak bone mass acquisition or the IGF1/IGFBP3 ratio was observed, in agreement with previous reports (12, 22). The multivariate analysis suggested that the plasma E2 level may not be a strong determinant of BMD acquisition, except at the lumbar

**Table 4** Relationship between bone mineral density changes at 1 year and basal biological parameters.

<table>
<thead>
<tr>
<th>Parameters VarDMO WB</th>
<th>VarDMO skull</th>
<th>VarDMO L2–L4</th>
<th>VarDMO TPF</th>
<th>VarDMO mid-radius</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>r = 0.47; P = 0.0097</td>
<td>r = 0.65; P = 0.001</td>
<td>r = 0.05; P = 0.781</td>
<td>r = 0.06; P = 0.756</td>
</tr>
<tr>
<td>Weight</td>
<td>r = 0.51; P = 0.0047</td>
<td>r = 0.67; P &lt; 0.0001</td>
<td>r = 0.25; P = 0.189</td>
<td>r = 0.23; P = 0.226</td>
</tr>
<tr>
<td>Height</td>
<td>r = 0.58; P = 0.0009</td>
<td>r = 0.69; P &lt; 0.0001</td>
<td>r = 0.24; P = 0.216</td>
<td>r = 0.18; P = 0.336</td>
</tr>
<tr>
<td>WB FM</td>
<td>r = 0.25; P = 0.181</td>
<td>r = 0.46; P = 0.020</td>
<td>r = 0.07; P = 0.699</td>
<td>r = 0.12; P = 0.531</td>
</tr>
<tr>
<td>WB FFST</td>
<td>r = 0.54; P = 0.0023</td>
<td>r = 0.64; P &lt; 0.0001</td>
<td>r = 0.22; P = 0.249</td>
<td>r = 0.18; P = 0.347</td>
</tr>
<tr>
<td>OC</td>
<td>r = 0.18; P = 0.397</td>
<td>r = 0.07; P = 0.760</td>
<td>r = 0.47; P = 0.020</td>
<td>r = 0.33; P = 0.106</td>
</tr>
<tr>
<td>B-ALP</td>
<td>r = 0.17; P = 0.438</td>
<td>r = 0.09; P = 0.680</td>
<td>r = 0.38; P = 0.066</td>
<td>r = 0.53; P = 0.009</td>
</tr>
<tr>
<td>CTX</td>
<td>r = 0.06; P = 0.808</td>
<td>r = 0.38; P = 0.110</td>
<td>r = 0.12; P = 0.611</td>
<td>r = 0.02; P = 0.903</td>
</tr>
<tr>
<td>Leptin</td>
<td>r = 0.39; P = 0.053</td>
<td>r = 0.32; P = 0.130</td>
<td>r = 0.13; P = 0.546</td>
<td>r = 0.08; P = 0.689</td>
</tr>
<tr>
<td>Testosterone</td>
<td>r = 0.19; P = 0.387</td>
<td>r = 0.25; P = 0.260</td>
<td>r = 0.01; P = 0.958</td>
<td>r = 0.19; P = 0.399</td>
</tr>
<tr>
<td>Estradiol</td>
<td>r = 0.41; P = 0.045</td>
<td>r = 0.42; P = 0.040</td>
<td>r = 0.31; P = 0.127</td>
<td>r = 0.32; P = 0.121</td>
</tr>
<tr>
<td>IGF1</td>
<td>r = 0.48; P = 0.018</td>
<td>r = 0.50; P = 0.010</td>
<td>r = 0.42; P = 0.038</td>
<td>r = 0.44; P = 0.029</td>
</tr>
<tr>
<td>IGF1/IGFBP3 ratio</td>
<td>r = 0.62; P = 0.0013</td>
<td>r = 0.49; P = 0.020</td>
<td>r = 0.64; P = 0.0008</td>
<td>r = 0.54; P = 0.006</td>
</tr>
</tbody>
</table>

WB, whole body fat mass; WB FFST, whole body fat-free soft tissue; OC, osteocalcin; B-ALP, bone alkaline phosphatase; CTX, type I-C telopeptide breakdown products; IGF1, insulin-like growth factor 1; IGFBP3, insulin-like growth factor-binding protein 3.
spine, a trabecular bone site that is more sensitive to estrogen deficiency (28). This finding is not in agreement with reports in non-active girls, for whom E2 was found to be an independent predictor of WB and lumbar spine BMD (9) and the WB bone mass gain (29). When multiple regression analysis was performed, only the IGF1/IGFBP3 ratio remained an independent predictor of 1-year BMD gain at different sites.

We also report here a decrease in bone accretion in the later stages of puberty, which coincided with the reduction in bone modeling/remodeling indicated by the gradual reduction in markers of bone formation and resorption. Bone marker values are maximal in mid-puberty (Tanner stages II–III), which coincides with the growth spurt in girls, and the values decrease thereafter until the postpubertal period, when bone mineral consolidation occurs and adult values are reached (30, 31). We found that the biochemical markers of bone turnover showed little predictive value for bone mass gain, and only a modest univariate correlation was found between bone formation markers (OC and B-ALP) and △BMD at the lumbar spine and TPE. Until now, the association between bone mass gain and bone markers in peripubertal girls has remained questionable (29), probably because bone markers reflect global bone remodeling while bone acquisition is bone site dependent. Moreover, it is possible that intensive physical training modifies bone turnover, although the data remain controversial (31, 32).

Low leptin levels have been reported in athletes in relation to reduced body FM (22) and as a direct result of chronic exercise (22). Although a concomitant increase in BMD and leptin concentration has generally been observed during growth (8), the implication of leptin in bone mass accretion remains questionable in both young athletes (6, 22, 33) and controls (8, 10). In our earlier cross-sectional study, we reported that the basal leptin level was not an independent predictor of BMD (7). This 1-year follow-up study confirms that basal leptin is not associated with BMD gain.

Lastly, bone mass acquisition in FRGs follows pubertal development, as suggested by the concomitant increase in plasma E2 and IGF1, and bone age rather than chronological age. These results were also reported in a large cohort of artistic gymnasts (34).

Our results were obtained in a relatively small number of FRGs, who were nevertheless quite homogeneous in terms of pubertal development and training characteristics, and these data thus unquestionably add to our knowledge on bone mass acquisition and hormonal adaptations related to intense training during the peripubertal period. However, in the absence of a control group, our data cannot be generalized in all adolescent girls. Future prospective studies of a larger number of untrained young girls may help to draw conclusions about whether the IGF1/IGFBP3 ratio plays a crucial role in BMD change only in FRGs or in untrained subjects, as well. Such studies would also address the question of whether estrogen and testosterone have similar effects on pubertal bone in FRGs, who frequently present menstrual disorders, and controls. Lastly, current DXA technology provides a real rather than a volumetric density measurement. It thus does not take into account all the parameters related to the growing skeleton, particularly the changes in bone size that occur during normal growth (35). This is one of the major limitations of pediatric DXA (35). More recent technologies such as peripheral quantitative computed tomography (pQCT), which directly evaluates volumetric density, may be very useful in analyzing the real effect of intense training on bone mass acquisition, but this technology is not widely accessible at this time.

In conclusion, a 1-year follow-up study of high-training peripubertal FRGs showed that BMD gain is maximal around Tanner stage III, and that the acquisition of bone mass is dependent on various parameters, depending on the bone site. Nevertheless, the plasma IGF1/IGFBP3 ratio is strongly associated with bone mass acquisition in this period, and it may thus serve easily as a surrogate marker of bone mass gain during follow-up in this population. Longer follow-up in a variety of sport disciplines would be helpful to more clearly define the role of these parameters and to determine the specific role of mechanical constraints.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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