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Specific Bone Mass Acquisition in Elite Female Athletes

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Context: Cross-sectional studies have demonstrated that physical activity can improve bone mass acquisition. However, this design is not adequate to describe the specific kinetics of bone mass gain during pubertal development.

Objective: To compare the kinetics of bone mass acquisition in female adolescent athletes of sports that impose different mechanical loads and untrained controls throughout puberty.

Study Participants: A total of 72 girls with ages ranging from 10.8 to 18.0 years were recruited: 24 rhythmic gymnasts (RG, impact activity group), 24 swimmers (SW, no-impact activity), and 24 age-matched controls (CON).

Main Outcome Measures: Areal bone mineral density (aBMD) was determined using dual-energy x-ray absorptiometry and bone turnover markers were analyzed. All the investigations were performed at baseline and after 1 year.

Results: At baseline and after 1 year of follow-up, RG presented significantly greater aBMD adjusted for age, fat-free soft tissue, and fat mass compared with CON and SW, only at the femoral region. When aBMD variation throughout the pubertal period was modeled for each group from individual values, the aBMD at the femoral region was significantly higher in RG compared with the other 2 groups from 12.5 to 14 years, and this difference lasted up to 18 years. Moreover, the mean annual aBMD gain tended to be higher in RG compared with SW and CON only at the femoral region and this gain lasted longer in RG. Bone remodeling markers decreased similarly with age in the 3 groups.

Conclusions: This study, which was based on linear mixed models for longitudinal data, demonstrated that the osteogenic effect of gymnastics is characterized by greater bone mass gain localized at mechanically loaded bone (ie, the proximal femur) principally around the menarcheal period. Moreover, the bone mass gain lasts longer in gymnasts, which may be explained by the delay in sexual maturation.

It has been well established that puberty is associated with increases in bone mass over a relatively brief period (1, 2). The regular practice of physical activity improves bone mass acquisition, as demonstrated by observational (3) and interventional studies in young children (4–6). The beneficial effects of exercise on areal bone mineral density (aBMD), bone geometry, and, consequently, bone strength have been confirmed by cross-sectional

Abbreviations: aBMD, areal bone mineral density; BMD, bone mineral density; BMI, body mass index; CON, control; CTX, type I-C telopeptide breakdown products; FFST, fat-free soft tissue; FM, fat mass; OC, osteocalcin; PINP, procollagen type I N-terminal propeptide; RG, rhythmic gymnasts; SDS, standard deviation score; SW, swimmers.

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studies in young athletes (7–9). Nevertheless, the favorable effect seems to be exercise-dependent and only physical activity that generates high mechanical strain induces additional bone mass gain during growth (5, 6). Young elite female gymnasts practice an intensive, weight-bearing sport with well-described osteogenic activity, as opposed to swimming, a no-impact sport (7, 10, 11). The benefits to gymnasts have been observed despite the high prevalence of delayed menarche and/or secondary amenorrhea or oligomenorrhea (9, 11, 12), both factors known to have a deleterious effect on bone health and peak bone mass acquisition (13). In addition, the difference in aBMD between trained and untrained groups seems to be more marked in the late pubertal stages (9, 11, 14), even though a difference was also reported in the early period (15).

Due to the difficulties in following elite athletes, cross-sectional studies are generally carried out, but this design establishes only a limited causal relationship between exercise and bone density because self-selection may confound the athlete-control comparisons (14). Conversely, the longitudinal design, which is more appropriate in sports populations, has received limited attention (14–17). Moreover, these investigations have generally been conducted in a specific class of age or menarcheal status (15, 16) and thus have not provided data on the kinetics of bone mass acquisition. Nevertheless, such data might be interesting because acquisition is not linear over the peripubertal period (14, 17, 18). Last, the investigation of one sport (15–17) does not allow for generalization, as the bone mass gain is specific to the mechanical loading generated by the type of sport.

Individuals who achieve high peak bone mass may be less susceptible in later life to osteoporosis and fracture (19). Consequently, a better understanding of the factors that influence bone gain in early life, like the type of physical activity or the period when bone is most responsive to mechanical loading, may be helpful to develop programs to optimize peak bone mass in young girls. Such data would be useful in building preventive strategies to reduce the risk of osteoporotic fractures later in life (20).

The aim of this study was to compare the effects of 2 intense physical activities (ie, swimming and rhythmic gymnastics) that generate specific mechanical loads on bone. We followed girls with ages ranging from 10 to 18 years for 1 year, and thus, the entire peripubertal period when bone mass undergoes its greatest gains could be investigated.

### Subjects and Methods

#### Subjects

The study protocol was reviewed and approved by the Regional Research Ethics Committee (Comité de Protection des Personnes Sud-Mediterrane IV, Montpellier, France), and each child and her parents gave written informed consent before entering the study. A total of 72 peripubertal girls with ages ranging from 10.8 to 18.0 years (mean 14.2 ± 1.7) were recruited for this study: 24 rhythm gymnasts (RG), 24 swimmers (SW), and 24 controls (CON). All the athletes and controls were age-paired (±7 mo) and the age distribution was comparable in the 3 groups (P = .414 data not shown). For RG, SW, and CON, the values were, respectively, 12.4, 12.8, and 13.1 for Q25; 14.7, 13.7 and 14.4 for the median; and 15.9, 15.1 and 15.7 for Q75. The 2 sports groups were composed of girls training more than 8 hours per week (23.0 ± 2.7 for RG and 14.4 ± 4.7 for SW) and who had been practicing their sport for more than 5 years (start of training for RG: 6.8 ± 1.3 y and 6.6 ± 2.2 y for SW). The control group consisted of subjects who performed only leisure physical activities for fewer than 3 hours per week. None had obvious signs of acute or chronic illness known to affect bone health and no long periods of immobilization or fractures within the previous 12 months. None of the participants used calcium or vitamin D supplements or declared taking any illicit substances.

#### Materials and Methods

This study used a 1-year follow-up design. In each participant, 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), and percentile values are given according to the French standard curves. Pubertal development was assessed by breast stage (I to V) according to the Tanner classification (21) by an experienced pediatric endocrinologist. Bone age was determined using the Greulich and Pyle method (22).

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

#### Medical and menstrual histories

Each subject or her parents also responded to a medical questionnaire designed to assess general medical and menstrual history from questions concerning the age of menarche.

#### Physical activity determination

Detailed information about training history was collected, including data on starting age of intensive training, years of active sport-specific training, 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 AM) in sterile chilled tubes by standard venipuncture technique. The samples were allowed to clot at room temperature and were then centrifuged at 2500 rpm for 10 minutes at 4°C. Serum samples were stored at −80°C until analysis. All samples were run in duplicate and, to reduce interassay 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 bone marker values were thus obtained at an unsynchronized menstrual stage.

Concerning bone metabolism, plasma samples were assayed by Cobas 6000 (Roche Diagnostic, Mannheim, Germany) for osteocalcin (OC), procollagen type I N-terminal propeptide (PINP), and type I-C telopeptide breakdown products (CTX). The inter- and intra-assay CVs for the 3 parameters were lower than 7%.

Bone mineral density, body fat, and fat-free soft tissues

Dual-energy x-ray absorptiometry (Hologic QDR-4500A; Hologic, Inc, Waltham, Massachusetts) was used to measure the bone mineral density (BMD; g/cm²) of the whole body, the anteroposterior lumbar spine (L2–L4), the dominant arm radius, the total proximal left femur, and specific sites of the femoral neck and the trochanteric areas. The soft tissue body composition (fat mass [FM, kg], percentage body fat mass [%FM], and fat-free soft tissue [FFST, kg]) was derived from the whole-body 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 whole body, 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 (23).

Statistical analysis

The characteristics of the young girls entered in the present study are described with proportions for categorical variables and with means and SD values for continuous variables (age, weight, aBMD, etc). The distributions were tested with the Shapiro-Wilk statistic. The comparisons of means among the 3 groups were performed using ANOVA when data distribution was normal and the Kruskall-Wallis test if continuous variables were skewed. For each BMD site, adjusted means for age, FM, and FFST were computed and compared between groups using multivariate linear regression analysis.

The 1-year relative variations at each BMD site were compared between groups after adjustment for age using a multivariate linear regression model. We then determined whether the 1-year relative variation at each BMD site differed from the whole-body BMD in each group. This analysis was performed using a linear mixed model to account for correlations between the measures of different BMD sites of each subject. This model included a subject-specific random intercept, and the fixed effects were age, BMD (with “whole body” as the reference), group, and the interaction between the BMD site and group.

The changes in BMD with age were modeled using the mixed model with a subject-specific random intercept; the fixed effects were group, age, age², age³, and the interaction between age, age², age³, and group. We chose a third-degree polynomial model to obtain a flexible curve with 2 potential inflections. A polynomial with a higher degree did not show a better fit of the data.

Last, we modeled the yearly aBMD change in the 3 groups using a thin plate regression spline model. The results are expressed graphically with their 95% Bayesian confidence interval.

In all these analyses, Tukey’s multiple comparison procedures were used to control for the familywise error rate when groups were compared in pairs. Statistical analyses were performed at the conventional two-tailed level of 0.05 using SAS version 9.1 (SAS Institute, Cary, North Carolina).

Results

The anthropometric characteristics, body composition, and bone metabolism of the athletes and controls at inclusion and after 12 months of follow-up are described in Table 1.

The 3 groups did not differ in terms of age. Weight, weight SDS, BMI, and BMI percentile were significantly lower in RG compared with CO and SW. RG was shorter than SW, but height remained within the normal French standard curves as demonstrated by the SDS (0.1 ± 0.8). FM (kg) was significantly lower in both groups of athletes compared with CO, whereas body FM (%) was only lower in RG and FFST was higher in SW compared with the other 2 groups. Bone age and Tanner stages were significantly delayed in RG compared with the other 2 groups. The number of subjects with menarche was reduced (P = .016) and the age of menarche was significantly delayed in RG compared with the other 2 groups (P = .003). The mean age of menarche was 14.4 ± 1.1 years for RG, 12.8 ± 1.2 years for SW, and 12.3 ± 1.4 years for CO. The mean hours of training per week was 23 ± 2.7 for RG and 14 ± 4.7 for SW and the mean age of start of training was 6.8 ± 1.3 years and 6.6 ± 2.2 years, respectively.

Areal BMD

At baseline, RG presented noticeably greater aBMDs adjusted for age, FFST, and FM measured at whole body and the femoral region (femoral neck and trochanter), compared with CON and SW. At the lumbar spine, a higher aBMD value was observed in RG compared with SW only, whereas no difference between groups was demonstrated for radius. No difference was observed between CON and SW for any bone site.
The aBMD was adjusted for age, FFST, and FM. Values are presented as means ± SD.
Significant difference between rhythmic gymnasts and controls: *P < .05; †P < .01; ‡P < .001.
Significant difference between rhythmic gymnasts and swimmers at the same time: ††P < .001; †‡P < .05; ††‡P < .01.

After 1 year of follow-up, a significant increase was observed at all bone sites in the 3 groups. The difference between RG and the other 2 groups persisted at the total proximal femur, a weight-bearing bone site, whereas at the lumbar spine, a less mechanically solicited bone site, no difference between groups was observed. At whole body, aBMD remained higher in RG only compared with SW.

When the mean percentage of the aBMD change was compared in athletes and controls over 1 year, RG presented significantly (P < .05 to P < .01) higher values at the total proximal femur (+5.8%) and the trochanter (6%) and introchanter (6.5%) subregions than SW (3.6%, 2%, 4%) and CO (2.9%, 3%, 2.9%). At whole body and other bone sites like L1–L4 and the radius, the changes in aBMD were not significantly different between groups. When the relative variation in aBMD (%) at various bone sites was compared at 1 year with the variation in whole-body aBMD (%) (Figure 1), the gain was significantly higher in the femoral region than at whole body only in RG by approximately 2% to 3.2%. In the 3 groups, the relative gain in aBMD (%) at L1–L4 was higher than at whole body (mean 2.1 to 3.7%), whereas the mean gain at the radius was comparable to the mean whole body gain and similar between groups.

Figure 2 shows the modeling of the aBMD variation throughout the peripubertal period. At the femoral region, aBMD was significantly higher in RG compared with the other 2 groups from 12.5 years at the femoral neck and from 14 years at the trochanter and at the total proximal femur until 18 years. At whole body, the difference between RG and the other 2 groups tended to be higher from approximately 16.8 years. At the radius and lumbar spine, no difference was demonstrated between groups. SW and CO presented similar aBMD variations at all bone sites.

Figure 3 shows the modeling of the mean annual gain in aBMD at various bone sites. For the femoral neck, trochanter, total proximal femur, and lumbar spine, the mean annual aBMD gain decreased with age in each group. Al-
though the mean annual gain tended to be higher in RG compared with SW and CO at each age, the difference did not reach significance due to the wide dispersions in the values. For RG, the optimal gain at the radius and whole body was observed between 14 to 16 years and 13 to 15 years, respectively, whereas the value decreased from 12 to 18 years in SW and CO. Moreover, for every bone site except the radius, the gain in aBMD tended to be maintained over time in RG compared with the other 2 groups.

Markers of bone turnover

The concentrations in the biochemical markers of bone turnover are shown in Table 1 and Figure 4. No difference was observed between groups for markers of bone formation (OC and PINP) or bone resorption (CTX) at basal evaluation. After 1 year of follow-up, only OC levels were significantly higher in gymnasts than swimmers. Moreover, all the markers decreased in swimmers and controls, but not in gymnasts (data not shown). Figure 4 presents
the modeling of bone marker concentrations throughout the growth period and shows a similar dramatic decrease with age for all markers in every group.

Discussion

Only a few groups have reported that intense exercise improves aBMD and bone geometry during the growth period, on the basis of cross-sectional studies (7, 9, 10). In this work, athletes in 2 sports, each inducing a specific pattern of mechanical load on the skeleton (ie, rhythmic gymnastics, a weight-bearing activity, and swimming, a non-weight-bearing activity), were compared with untrained subjects. We confirm that only high-impact and weight-bearing activities induce positive adaptive responses in the growing skeleton (7, 9–11). Our longitudinal evaluation reinforces the cross-sectional observations and demonstrates specific bone-site adaptations with different time lags.

The results observed at baseline and after 1 year of follow-up confirm the beneficial effect of rhythmic gymnastics on skeletal regions specifically submitted to high-impact and repetitive mechanical loads, such as the proximal femur (14). The localized effect of exercise was confirmed by the lack of difference between groups at less mechanically solicited bone sites, such as the lumbar spine and radius. Moreover, the comparable aBMDs in swimmers and controls suggest that a minimum level of strain is necessary to induce noticeable bone adaptation (7, 11, 24). The persistence of higher aBMD at the femoral region in the gymnasts may be explained by a specific model characterized by a significantly higher mean yearly aBMD percentage change associated with a faster increase in the femoral/whole-body aBMD ratio. Conversely, the aBMD values at the lumbar spine and radius at 1 year, the variation in aBMD, and the aBMD ratio at these sites/whole body were not significantly different between groups. All these data indicate that the osteogenic effect of exercise is

Figure 4. Modeling of changes in bone biochemical markers (A: osteocalcin; B: procollagen type 1 N-terminal propeptide; C: type-I collagen C-telopeptide breakdown products) in rhythmic gymnasts (RG, green), swimmers (SW, black), and controls (CO, red). The dashed curves with the same color represent the 95% confidence interval.
region dependent and load dependent. This 1-year follow-up of the same participants reduced the potentially confounding role of genetic predisposition, such as a higher basal aBMD, and environmental factors and demonstrates the causal effect of exercise by excluding a selection bias (16). Longitudinal studies, which have mostly focused on artistic gymnasts, have been few, probably because these young female athletes follow an extremely demanding regimen (intense training, various competitions, stress, trainer and familial constraints, etc), but they all found a favorable effect of physical activity on bone mass acquisition (14–16, 25). Only Nickols-Richardson et al (15) found similar 1-year changes in femoral and whole-body aBMD in prepubertal gymnasts and controls. The inclusion of athletes with different training status (years or duration of training), initial aBMD values, and particularly age or pubertal stage (14), may explain the divergent results (15).

The mean variations in aBMD observed during this 1-year follow-up or in previous studies (14–16, 25) are not sufficient to provide great detail on the kinetics of bone mass acquisition throughout the pubertal period. Various phases with different rates of aBMD gain have been described in both untrained (1, 26, 27) and athletic girls (14, 17, 18) during the growth period. Moreover, bone tissue response may differ according to age or pubertal status (3, 28, 29). Nevertheless, our analysis models derived from mixed longitudinal data, including a broad age range from 10.8 to 18 years, are unique and, for the first time, can be used to determine the bone mass acquisition in elite athletes throughout the pubertal period according to the type of sport performed. Our results show that bone mass acquisition tends to stabilize at about 18 years for most of the bone sites in controls and swimmers, except for the radius, where aBMD tends to increase over this period only in swimmers. Moreover, our data confirm that the dramatic increase in aBMD is observable during the pre-and perimenarcheal periods, as previously reported in sedentary (1, 26, 27, 30) and trained young girls (14, 17, 18). Recently, Baxter-Jones et al (27) demonstrated that a bone mass plateau was reached in untrained girls at 18.8 years for whole body, at 16.8 years for the lumbar spine, and at 14.8 years for the femoral neck. Interestingly, although the swimmers trained more than 14 hours per week, they presented a profile of bone mass acquisition similar to that of the controls. The nonostogenic effect of swimming observed here is in line with previous cross-sectional studies in prepubertal girls (7, 11, 31), as well as in adult female and male swimmers (32, 33).

Conversely to swimmers, the rhythmic gymnasts presented a specific bone mass acquisition profile. Beyond 14 years, aBMD at the proximal femur was increased compared with controls and swimmers. Moreover, this difference appeared to be accentuated with time, probably due to a cumulative effect of continuous higher annual gain and the persistence of this gain at least up to 18 years. No difference in bone mass acquisition was identified at the radius or lumbar spine, however, and the difference between gymnasts and the other 2 groups at whole body was significant only beyond 16 years. It has been reported that exercise during adolescence has the greatest impact on bone accrual in bone that is mechanically solicited, such as the femoral neck as opposed to the lumbar spine (3, 14, 16). Our data further suggest specific patterns of change in bone sites depending on localization (axial or appendicular) (19, 27), composition (cortical or trabecular), and the applied mechanical constraint (weight-bearing or not) (17).

The reduction in bone mass gain with age in the 3 groups was associated with a concomitant reduction in bone modeling/remodeling, as demonstrated by the decrease in the concentration of bone markers. Nevertheless, no specific variation was observed according to the type of sport, probably because in these young populations, the modification in bone markers induced by growth (34) may partially mask the effect of physical activity (35). This lack of specific bone marker profiles may also be explained by the fact that markers of bone formation and resorption represent an average of the bone turnover in all skeletal sites, and localized BMD gain observed only at mechanically loaded sites would not be reflected by a variation in these markers (35). The few studies that have longitudinally evaluated bone turnover markers in young athletes (14, 15) reported similar decreases in concentrations with advancing pubertal stages but no differences between controls and athletes.

The greater bone mass gain of gymnasts was observed despite delays in the age of menarche, pubertal development (Tanner stages), and bone age, all well described in elite rhythmic gymnasts (36, 37). This suggests retarded sexual and auxological maturation in these athletes. It has been demonstrated that the onset and length of puberty have strong effects on bone mass acquisition (2, 13, 38). Also, an inverse relationship between the timing of puberty and bone mass in early adulthood has been reported (2, 13, 38, 39), suggesting that the time of exposure to estrogen from prepuberty to peak bone mass is an important factor of bone mass acquisition (40). A more recent study nevertheless suggests that the bone mass difference between healthy girls with earlier vs later menarche is already present at Tanner stage P1 (41). In our study, the delayed sexual maturation did not seem to have a noticeable negative effect on bone mass acquisition because, from 12.4 years—that is, 1.8 years before menarche—the
rhythmic gymnasts already presented higher aBMD at the femoral neck and normal values at the other, less mechanically loaded bone sites (ie, the lumbar spine and radius). This difference appeared more marked at the femoral neck beyond 14 years, a period that corresponds to menarche in gymnasts (mean age 14.4 ± 1.1 y). Various authors have reported that the peaks of bone gain and bone calcium deposition occur around menarche (ie, −0.6 to −0.8 mo before) and decrease afterward (ie, 2 y later) (1, 26, 42). The increase in IGF-1 and estradiol during this period has an essential concerted action on direct bone development in peripuberty (43). Moreover, the increase in estradiol may reduce the set point of the bone mechanostat and thus affect the relation between mechanical loading and bone strength (44, 45). Although we cannot predict the final bone mass, it is probable that the difference in early adulthood is exacerbated because rhythmic gymnasts present late catch-up growth (36, 46). This was confirmed in our study by the maintenance of aBMD gain in the late pubertal stages compared with the other 2 groups. Another element in favor of higher peak bone mass in gymnasts is the systematically higher bone mass in retired gymnasts compared with controls (16, 47, 48).

Although the results presented here are unique, 1 year of follow-up in 3 groups of peripubertal girls with a wide age range (10–18 y) does not necessarily reflect the variation in bone mass that would be observed by longitudinal evaluation. However, it is extremely difficult to follow young elite athletes with highly demanding schedules (training, competition, traveling, etc) for 8 years. Nevertheless, despite the variability in growth and aBMD development between individuals of the same chronological age, elite athletes represent a highly select group of girls with similar anthropometric characteristics (36), who have been exposed to similar constraints (intense training, nutritional control, stress, etc) since a young age. Therefore, the changes in bone mass observed in this study may adequately reflect the kinetics of bone mass gain in these specific sports, but they cannot be generalized to other trained populations. The bone kinetics in the controls, which were similar to those in previous cross-sectional and longitudinal studies (1, 26, 27, 30), and the inclusion of 3 groups of 6-month age-matched peripubertal girls strongly reinforce the credibility of this study. In the future, a study with a broader range of ages may help to specify the entire bone mass acquisition period further in gymnasts because the improvement in bone mass gain may start earlier (15) and may be delayed compared with the general population (49, 50).

**Conclusion**

This study, which was based on linear mixed models for longitudinal data, describes for the first time bone mass acquisition during the pubertal period in 3 groups of girls: those heavily involved in weight-bearing activity, those involved in non-weight-bearing activity, and controls. The osteogenic effect of gymnastics is characterized by greater bone mass gain localized at mechanically loaded bone, principally around the menarcheal period. Moreover, the bone mass gain lasts longer in gymnasts, which may be explained by the delay in sexual maturation. These data strongly suggest that physical exercise that generates high mechanical loading, such as rhythmic gymnastics, should be encouraged during the growth period to optimize peak bone mass and subsequently reduce fracture risk later in life.

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