In peripubertal girls, artistic gymnastic improves areal bone mineral density and femoral bone geometry without affecting the OPG/RANKL system

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ABSTRACT (250 words)

Purpose To evaluate the effect of artistic gymnastic (GYM) on the areal bone mineral density (aBMD), femoral bone geometry, bone remodelling and its relationship with the osteoprotegerin (OPG)/rank-ligand (RANKL) system in peripubertal girls.

Methods Forty-six girls (age 10-17.2 yr) were recruited for this study and consisted of 23 elite GYM trained from 12 to 30 h/wk and 23 age-matched (±6 months) controls (CON). The aBMD at whole body, total proximal femur, lumbar spine, mid-radius and skull was determined using dual-X-ray absorptiometry. Hip structural analysis (HSA software) was applied at the femur to evaluate the cross-sectional area (CSA, cm^2), cross-sectional moment of inertia (CSMI, cm^4), the section modulus (Z, cm^3) and buckling ratio at neck, intertrochanteric region and shaft of the femur. Moreover, markers of bone turnover as well as OPG/RANKL levels were paralleled analysed.

Results GYM had higher 5.5-16.4 % adjusted and non-adjusted aBMD at all bone sites, skull excepted and the difference increase with age. In the three regions of femur adjusting CSA (12.5-18%), CSMI (14-18%), Z (15.5-18.6%), mean cortical thickness (13.6-21%) are higher in GYM while buckling ratio (21.-27.1%) are lower than CON. Bone markers decrease with age in both groups, and GYM presented higher values than CON only in postmenarchal period. A similar increased of RANKL with age without variation of OPG levels was observed for both groups.

Conclusion GYM induces not only an increase of aBMD, but also an improvement of bone geometry and strength associated with an increase of bone remodeling. These adaptations seem to be independent of the OPG/RANKL system.

Keywords: Bone mass acquisition, intensive training, peripuberty, areal bone mineral density, bone strength, markers of bone turnover.

Mini abstract: (50 words)

Peripubertal artistic gymnasts had elevated areal bone mineral density at various and bone site, despite delayed menarche and high frequency of menstrual disorders, factors susceptible to compromise bone health. The concomitant improvement of femoral bone geometry and strength suggested that this type of physical activities may have favourable clinical impact.

INTRODUCTION

Physical activity during peripubertal period had long been recognized to improve bone mass gain in boys and girls and may be considered as a practical strategy for enhancing bone status during growth and then reduced osteoporosis risk fracture later in life {Rutherford, 1999 #130}. Results from interventional studies had demonstrated that children and adolescents practicing regular structured and controlled exercise that generated high mechanical constraints such as jumps or circuit training presented a higher areal bone mineral density (aBMD) that non-trained subjects {Hind, 2007 #127}. Moreover, this favourable effect was confirmed in young athletes and particularly in gymnasts which are submitted to high mechanical constraints and which presented higher aBMD than non-athletes or other athletes who are engaged in non-impact exercise like swimming {Courteix, 1998 #51}. In addition to bone density, bone geometry is an important component of bone strength and fragility risk. According to a recent study, gymnastic induced also a positive adaptation of bone microarchitecture and geometry at tibia evaluated by periphereal quantitative computed tomography (pQCT) {Tournis, #126}. Other methods, using conventional dual energy X-ray absorptiometry (DXA) images, had been proposed to be valuable tools for the estimation of the bone structure and strength at the proximal femur {Beck, 1990 #122},{Nikander, 2005 #129}, known as a relevant bone site in gymnasts because it is mechanically solicited in this case {Courteix, 1998 #51}. However, in our knowledge, the use of hip structure analysis (HAS) in athletes had receive a limited attention in adults {Nikander, 2005 #129} as well as in children {Faulkner, 2003 #128}. Moreover, in this last study only premenarchal gymnasts were studied {Faulkner, 2003 #128} and as the response of skeleton to mechanical constraints may be also dependent to age and the hormonal status {Hind, 2007 #127}, an evaluation of these parameters in this population throughout the peripubertal period is required.

We have recently reported that various hormonal parameters such as sex hormones and IGF-1/IGFBP-3 ratio have been found to be positively correlated with bone mass accrual in peripubertal rhythmic gymnasts {Maimoun, #123}, {Maimoun, #104}. Others bone related cytokines, such as osteoprotegerin (OPG) and rank-ligand (RANKL), scarcely evaluated in sports {Herrmann, 2004 #72}, {Ziegler, 2005 #82}, {Kerschan-Schindl, 2009 #76}, may give some interesting information on the mechanism of bone adaptation to mechanical constraints. Actually, the OPG/RANKL system has been demonstrated to be a paracrine mediator of mechanical strain on bone metabolism {Rubin, 2000 #80},{Kobayashi, 2000 #77}. RANKL is expressed by osteoblasts, which binds to its receptor, RANK, on the osteoclasts surface and their precursors. This process regulates the differentiation of precursors into multinucleated osteoclasts, as well as osteoclasts activation and survival. OPG, which is also secreted by osteoblasts, protects the skeleton from excessive bone resorption by binding to RANKL and preventing it from interacting with RANK {Lacey, 1998 #86}, {Hofbauer, 2000 #75}.

The aim of this study was to characterise the bone status of peripubertal artistic gymnast girls which performed a sport discipline that requiring high training level and high mechanical constraints. The bone adaptation was evaluated by bone mineral density, by the estimated strength of the femur and by bone remodelling markers, respectively throughout the peripubertal period and particularly before and after menarche. Moreover, we report for the first time, the effect of intense physical activity on OPG/RANKL system in young athletes.

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 children and her parents gave written informed consent before entering the study. A total of 46 peripubertal girls with ages ranging from 10 to 17.2 years were recruited for this study, consisted of 23 elite artistic gymnasts and 23 age-matched (± 6 months) controls were investigated. The control group consisted of subject who performed only leisure physical activity less than 3 hours per week. None had obvious signs of acute or chronic illness known to affect bone health and no recent long term periods of immobilisation or fractures in the previous 12 months. The gymnasts had begun training at 5.3 ± 1.3 years and trained 12 to 30 hours per week (mean 19.9 ± 4.1 $h.wk^{-1}$), depending on age. Most training sessions lasted 4 hours and consisted of a warm-up, routine training, and strength and stretching exercises.

Methods

This study used a cross-sectional 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 square of height (m). Pubertal development was assessed by breast stages (I to V) according to the Tanner classification {Tanner, 1962 #37} by an experienced paediatric endocrinologist. Skeletal age was determined using the Greulich and Pyle method {Greulich, 1959 #39}.

Information regarding pubertal onset of family members was obtained from a standardised 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. Moreover, the target height of the gymnasts was calculated with the following equation:

Target height = ([father's height – mother's height]/ 2)-6.5.

Medical and menstrual histories

Each subject also responded to a medical questionnaire designed to assess general medical and menstrual history with questions concerning the age of menarche and the presence of menstrual cycle disorders.

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 (0900-11h00) 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 min at 4°C. Plasma 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 analysed in a single session. In pubertal girls, the date of the last menses was not recorded, thus hormonal values were obtained at an unsynchronised menstrual stage.

Concerning bone metabolism, plasma samples were assayed for osteocalcin (OC;????), ??? (PINP, ???) and type I-C telopeptide breakdown products (CTX????). The inter-assay CVs of OC, PINP, CTX variation were, respectively, ?, ?, and ?%. The intra-assay were, respectively, ? , ? and ?%.

The intra- and inter-assays CVs respectively for OPG (OPG ELISA, Immunodiagnostic Systems, Boldon, UK) and RANKL (ampli sRANKL human ELISA, Immunodiagnostic Systems, Boldon, UK) 10%, 7% and 8%, 6 %

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

DXA (Hologic QDR-4500A, Hologic, Inc., Waltham, MA) was used to measure the areal bone mineral density (BMD; g/cm^2) of the whole body (WB), the antero-posterior lumbar spine (L1-L4), the dominant arm radius, the proximal part of the left femur (TPF), and specific sites of the femoral neck, the trochanteric and intertrochanteric areas. The soft tissue body composition (fat mass [FM, kg], percent 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 insure 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.

Bone geometry of the proximal femur

Besides conventional aBMD analysis of the total proximal femur, the HAS software originally developed by Beck et al., {Beck, 1990 #122} was used to assess structure characteristics of three regions of the proximal region: at femoral neck, intertrochanteric region and femoral shaft. At these three femoral sites, various parameters had been determined: cross sectional area occupied by bone mineral (CSA) [an index of axial strength, which is equivalent to cortical area], width, endocortical diameter, mean cortical fitness, cross-sectional moment of inertia (CSMI), the section modulus (Z) [an index of bone strength against bending] and buckling ratio. All the values determined were secondary adjusted to body weight and body height. One doctor conducted all software analyses.

Statistical analysis

The characteristics of the young athletes entered in the present study are described with proportions for categorical variables and with means and standard deviation values for continuous variables (age, weight, aBMD, biochemical markers, etc.). The distributions were tested with the Shapiro-Wilk statistic. The comparisons of means among the gymnasts and controls (or subgroup according to menarchal status) were performed using Student's t-test when data distribution was normal and Mann-Whitney rank sum test if continuous variables were skewed. For each BMD sites, adjusted means for age, FM and FFST were computed and compared between groups using multivariate linear regression analysis. Geometry and bone strength calculated values at the femur adjusted for weight and height were also compared. Spearman correlations and Spearman partial correlations controlling for height and weight, were used to summarise the relationships between the various continuous variables.

Statistical analyses were performed at the conventional two-tailed α level of 0.05 using SAS version 9.1 (SAS Institute, Cary, North Carolina).

RESULTS

The anthropometric characteristics, the familial and birth data and training status of the 23 GYM and 23 CON are summarised in Table 1. The age distribution ranged from 10 to 17.2 years in both groups, with a mean age of 13.6 and 13.2 years, respectively for GYM and CON. Concerning anthropometric data, there were no significant differences between the two groups with regard to weight, BMI and body fat free soft tissue, while body fat mass (kg and %) were significant lower in GYM ($p<0.01$ and $p<0.001$, respectively). GYM tended to be smaller than CON, but the values did not reach significance ($p=0.08$). However, when height standard deviation score (height SDS) and weight standard deviation score (weight SDS) were calculated according to the French standard curves, GYM presented lower values than CON ($p<0.001$ and $p=0.08$, respectively). No difference was observed for bone age, however, when the difference between chronological age and bone age (Δ age – bone age) was calculated, GYM presented a significantly higher values (0.68 vs. -0.02 yr; $p<0.05$). The age of menarche was significantly delayed ($p<0.001$) in GYM (13.8 ± 1.2 yr) compared to CON (12 ± 0.9 yr) and despite values in GYM tended to be higher than his mother (mean difference 0.745 ± 1.3 yr) the significance not reaching the statistical level ($p=0.1$, data not shown). Similarly, no difference concerning the age of menarche was demonstrate in CON and his mother (mean difference -0.717 ± 1.8 yr; $p=0.3$, data not shown). However, when a comparison between the difference of age of menarche between adolescent and their mother in both groups was performed, the difference tended to be significant ($p=0.07$). When 18 months post-menarche was used as cut-off, corresponding in clinic to a period of normalisation of menstrual cycle and maturation of the hypothalamic-pituitary-ovarian axis, a high prevalence of oligomenorrhea menstrual irregularity was observed in GYM (42.8%: 3 out of 7) compared to CON (0%: 0 out of 9). In each group, two adolescents take oral contraceptives.

No difference was reported for weight and height birth and father's height between both groups, while mother's height and target height were significantly lower ($p<0.001$ and $p<0.05$, respectively) in GYM group. No significant differences in mean Tanner stages of breast development existed between both groups.

On average, GYM initiated participation in the sport at age 5.3 yr (range 4-8) and trained an average of 19.9 $h.wk^{-1}$ (range 12-30) according to their age. CON were normally active and practice only leisure physical activity under 3 $h.wk^{-1}$. The activities the controls most frequently participated in were rhythmic and artistic gymnastic, tennis, judo, volley-ball, basket-ball, hand-ball, judo and karate.

Bone characteristics

Areal bone mineral density

Table 2 presents the non-adjusted aBMD for both groups at various bone sites. Compared with CON, GYM presented a noticeably greater aBMD at all bone sites evaluated, WB, TPF, L2-L4 and radius axial, skull excepted. The differences between the two groups ranged between 8.8 % for the WB and 16.4% for the trochanter region.

When both groups were subdivided according to their menarchal status (Fig. 1), aBMD values in premenarchal and postmenarchal GYM remains significantly higher at all bone sites, pelvis excepted for premenarchal group ($p=0.12$) and skull excepted for pre-and postmenarchal than CON. In addition, the degree of significance appeared systematically higher between GYM and CON at postmenarchal than premenarchal stage. Moreover, the use of regression model including age, sport, as well as interrelation between sport and age, demonstrated that GYM presented an aBMD gain more rapidly with age than CON at arms, pelvis, legs, femoral neck, intertrochanteric region and radius and more slowly aBMD gain at skull. Globally, in both groups, aBMD were significantly and positively correlated to chronological and bone age, weight, height, FFST and FM.

Table 3 reported the BMD adjusted for age, fat-free soft tissue and fat mass. In parallel with the results observed for the non-adjusted BMDs, the adjusted BMD values measured at WB, femoral region, L2-L4 and mid-radius were higher in GYM group, skull and pelvis excepted. The differences between the two groups ranged between 5.5% at WB and 11.5% at the intertrochanteric region.

Bone geometry of the proximal femur

Results for comparison of the bone geometry of proximal femur are presented in Table 4. After adjustment for height and weight, the calculated values in the three regions of femur at femoral neck, intertrochanteric region and femoral shaft are significantly higher in GYM for CSA (+16.5%, +18%, +12.5 %), CSMI (+11.4%, femoral neck non significant (NS), +14%, +18%), Z (+16%, +18.6%, +15.5%), mean cortical thickness (+21%, +17.2%, +13.6%) while buckling ratio (-27.1%, -21.7%, -6.4% femoral shaft NS) are significantly reduced than CON. No difference was observed for endocortical diameter and width whatever the femoral region.

A positive correlation between bone geometry parameters at the three sites of the femur and age (CSA, r= 0.28 to r=0.46; p=0.05 to p= 0.002; mean cortical thickness, r=0.39 to r=0.48; p=0.01 to p=0.001) and negative correlation for buckling ratio (r= -0.38 to r= -0.41; p=0.01 top=0.006) were found when global population was studied (n=46). These relationships disappeared when evaluation was performed in subgroup (GYM and CON), excepted in CON for CSA (r=0.46, p=0.04), cortical thickness (r=0.55, p=0.01) and buckling ratio (r=-0.49, p=0.02) at the femoral shaft.

Bone biochemical markers and OPG/RANKL system

Concerning the biochemical markers of bone turnover (Table 5), no significant difference for markers of bone formation (OC, P1NP) were observed between groups, while marker of bone resorption (sCTX) tended to be higher (p=0.07) in GYM. When parameters were analysed in subgroups, according to the menarchal status, OC and sCTX (Fig 2. A and C), where found higher in GYM ($p=0.029$ and $p=0.024$) compared to CON, only in postmenarchal period. PINP (Fig 2. B) tended to be also higher in GYM ($p=0.07$) during this same period. All the bone biochemical markers OC, PINP, CTX decreased significantly with age in similar profile in GYM ($r = -0.65, -0.47, -0,52$; $p<0.001$, $p<0.05$, $p<0.01$, respectively) and CON ($r = -0.78, -0.71, -0,64$; $p<0.001$ for all, respectively).

No difference for OPG/RANKL concentrations between groups was reported (Table 5) whatever the menarchal status (Fig 2. D and E). RANKL levels increased significantly with age for both groups ($r=0.77$, $p <0.001$ for GYM and $r=0.79$, $p <0.001$ for CON) while no variation was reported for OPG with age.

DISCUSSION

In this study, we report elevated values of aBMD in elite GYM compared to age-matched less active CON independently of pubertal status. Beside, intense training induced an improvement of bone structure and strength at femur as well as an intense bone remodelling while OPG/RANKL ratio is not modified.

Areal bone mineral density

GYM demonstrated a higher aBMD values than controls in the WB, TPF, L2-L4 and mid-radius. The mean difference ranged between 8.8 to 16.4%. It is interesting to note that the higher aBMD values were observed both in bone domain constituted mainly to trabecular bone such as L2-L4 as well as cortical bone such as TPF. It was suggested that the response of the bone to mechanical constraints induced by strong muscle contractions and impact generated by weight bearing activity are an adaptive biological response {Frost, 1992 #121}. The potential osteogenic effect of gymnastic may be particularly exacerbated, since this sport discipline involves a variety of exercises with a high impact on the bones and muscle contractions and ground reaction forces until 10-12 times of body weight {Robinson, 1995 #32} {Faulkner, 2003 #128}. Moreover, the mechanical forces are present in both upper and lower limbs as well as the trunk, given its unique mechanical character of this physical activity {Proctor, 2002 #105}. In our study, this was expressed by a concomitant higher aBMD in arms (+14.3 %), as well as in legs (+11.6%). Several studies have reported in competitive artistic gymnasts a high aBMD {Robinson, 1995 #32},{Helge, 2002 #90},{Proctor, 2002 #105} {Faulkner, 2003 #128} and a total trabecular and cortical volumetric density ($g.cm^{-3}$) {Tournis, #126}. However, the age groups was generally relatively closed and limited to a specific period of life such as premenarche {Faulkner, 2003 #128}, {Tournis, #126} postmenarche {Helge, 2002 #90} or young adults {Robinson, 1995

#32},{Proctor, 2002 #105}. Their design did not allow to evaluate the osteogenic effect of this sport throughout the peripubertal period. We also reported for the first time that whatever the age of the subject or the pubertal status, aBMD in GYM was higher than CON. In the premenarchal group, our data suggest that a minimal amount of training during introductory gymnastic classes is sufficient to increase aBMD, as previously reported {Dowthwaite, 2006 #119},{Erlandson, #112}. Nevertheless, the difference between both groups seems to be exacerbated in postmenarchal compared to premenarchal period (8.8% vs. 7.6% for WB, 12.6% vs. 11.1% for L1-L4, 13.4% vs. 9.9% for TPF, 12% vs. 7.6% for radius). Then, the early stage of puberty may be particularly optimal for bone adaptation to loading and this may be due to the velocity of bone growth and to the well known concomitant endocrine variations (estrogens, androgens, growth factors: IGF-1) {Hind, 2007 #127}. Estrogen may indeed reduce the set point for bone mass adaptation to mechanical loads {Frost, 1992 #121} and the increase of estrogen in postpubertal period may increase the sensitive feature to mechanical stimuli on the skeleton. This leads to probable synergy action of mechanical loading and sex hormones. The optimal gain that occurs after menarche result may be due partly to the cumulative effects of training on bone that continues as the gymnasts advance in pubertal stage and more advances maneuvers {Laing, 2002 #110}. In elite rhythmic gymnasts (hours of training > 20h/wk), we recently reported than optimal aBMD gain occur between Tanner stages II and IV (12.5-15.8 yrs) {Maimoun, #123},{Maimoun, #104}.

Bone geometry parameters

In addition to aBMD gain, we observed also a beneficial effect of elite gymnastic on bone geometry evaluated by HAS method. The main morphological variation was an increase of cross sectional area occupied by bone mineral **(**CSA) (an index of axial strength), without modification of width. These may be explained either by an increase of mean cortical thickness probably due to corticalization of the trabecular structure beneath the endocortical surface rather than an enlargement of periosteal dimensions {Nikander, 2005 #129} or a reduced endosteal resorption {Petit, 2002 #136}. The cortical compartment plays a crucial role in the mechanical resistance of bone and low cortical thickness have been reported to constitute a higher risk of hip fracture in elderly women {Szulc, 2006 #131}. This bone adaptation was associated with an increase in section modulus by 15.5% to 18.6% (an index of bending strength) and a decrease of buckling ratio by -6.4% to -27.1% at the three femoral sites evaluated. The increase of strength of bone may thus have favourable clinical impact. In premenarchal gymnasts, other techniques, such as pQCT has demonstrated that this positive adaptation of the skeleton was also observed especially in cortical bone at the tibia level {Tournis, #126}. The beneficial effects of mechanical loading on bone geometry found here are in agreement with those reported in physically active girls (5 to 11 yr) {Janz, 2007 #132}, in prepubertal gymnasts {Faulkner, 2003 #128} as well as in adult athletes which perform high-impact sports activity such as volleyball {Nikander, 2005 #129}. Conversely, in prepubertal girls, exercise interventional program don't seem to have a measurable effect {Alwis, 2008 #135}, {Petit, 2002 #136}. These difference may be mainly attributed to the age of the participants {Petit, 2002 #136}, but also to the intensity and the type of the exercise performed {Nikander, 2005 #129}.

We did not find a specific structural adaptation within the three region of the femur despite it is well known that long bone adaptation to mechanical loading is site specific and the magnitudes, directions, and types of load vary along its length {Petit, 2002 #136}. Previous interventional study reported a predominantly action of exercise on cancellous bone sites of the femur (neck and intertrochanteric regions) compared to femoral shaft {Petit, 2002 #136}, but the magnitude and the numbers of mechanical constraints were lower (3.5-5 times body weight) {Petit, 2002 #136}. Moreover, the bone adaptation to mechanical loading is not homogeneous but depends on the maturity of the subjects {Petit, 2002 #136}.

We observed a delayed menarche (13.7 *vs.* 12.0 yr) as well as high percentage of oligomenorrhea and secondary amenorrhea are frequently reported in gymnasts {Erlandson, 2008 #134} and may be considered as contributing factors that compromise bone health {Christo, 2008 #118; Chevalley, 2009 #46} {Hind, 2006 #74}. However, this don't seems to have the same negative effects on bones as that observed in endurance athletes such as endurance runners submitted to repetitive and low level of stress (2-5 times body weight) {Robinson, 1995 #32} {Hind, 2006 #74}. However, Kirchner et al., {Kirchner, 1995 #120} reported in a limited number of gymnasts that aBMD was unrelated to menstrual status. It would be interesting to confirm these data in a large group of GYM, in the aim to determine since a synergic action between sex hormones and mechanical constraints is present.

Genetic predisposition

We can not exclude the hypothesis that some degree of self-selection exist in the difference for the aBMD and bone geometry between trained and untrained groups. The cross-sectional design used in this study and the absence of parental bone mass evaluation cannot allow to establish a specific relationship between exercise and bone density {Bennell, 1997 #108}. Nevertheless, the investigation of different age groups of GYM and CON allowed to apprehend the bone variation throughout the peripubertal period. Moreover, the similar aBMD at the skull, a bone site not affected by mechanical strain {Morel, 2001 #89}, adds considerable support to the hypothesis that physical activity is responsible for the bone accretion rather than genetic predisposition {Maimoun, 2008 #109}. In addition, the greater rate of aBMD gain among young gymnasts compared with non-gymnasts previously reported {Nickols-Richardson, 1999 #53}, {Laing, 2002 #110} reinforces the strong effect of physical activity on bone mass acquisition. Nevertheless, the lower target height of the GYM compared to CON, which is mainly explained by their lower mother's height, may highlight the implication of some potential genetic predisposition for the practice of this sports discipline. In our knowledge, this information was only reported in a study that compared various types of sport and a lack of a control group of nonathletes limited its interpretation {Erlandson, 2008 #134}.

Bone remodelling

The impact of exercise on variation of bone turnover markers in the growing skeleton has received little attention. Moreover, the results remain controversial and this may be related to the age of the subjects. Then, growth may partially mask the effect of physical activity on bone remodelling {van Coeverden, 2002 #31} {Maimoun, #124}. In our study, markers of bones formation (PINP, OC) as well as bone resorption (sCTX) decreased significantly with age independently to the training status. These results confirm the reduction of bone remodelling and bone mass gain in pospubertal period as previously reported in elite rhythmic gymnasts {Maimoun, #123} and controls {van Coeverden, 2002 #31}. Moreover, GYM presented higher levels of bone markers than CON only in postpubertal period, when bone remodelling tends to decrease. These results may explain why no difference was reported between gymnasts and less active controls in premenarchal period {Tournis, #126} compared to older subjects {Courteix, 2007 #7}. These results suggest that bone remodelling are noticeable affected by physical activity only in the context of reduced bone remodelling from advanced pubertal stages. In this period, a higher bone turnover in favour of bone formation may partly explained the higher bone mass gain observed in GYM and previously in an other group of rhythmic gymnasts {Courteix, 2007 #7}.

OPG/RANKL system

In this study, we reported for the first time the OPG/RANKL variations in young athletes. A similar increase of RANKL levels throughout the peripubertal period associated with a lack of variation of OPG levels was observed in the two groups. The reduction of OPG/RANKL ratio may partly explain the reduced bone mass gain in young athletes with pubertal stage {Maimoun, #123}. To date, few data dealing with the change of OPG/RANKL during peripubertal period in non-athlete population are available and additionally these results are rather controversial. Then, no variation of OPG {Wasilewska, 2009 #88} and a higher levels in infancy (1-4 yr) or prepubertal period (6-8 yr) {Buzi, 2004 #83}, {Gajewska, 2006 #87} than adult has been reported {Buzi, 2004 #83}, {Szulc, 2001 #84}. In agreement with our results, an increase of RANKL with age in children was reported {Wasilewska, 2009 #88}, while others study didn't demonstrate any variation {Buzi, 2004 #83}. The demonstration of similar levels in both GYM which presented improved aBMD and bone geometry parameters are somewhat unexpected because several experimental studies have implicated the OPG/RANKL system as a paracrine mediator of mechanical strain on bone metabolism {Rubin, 2000 #80},{Kobayashi, 2000 #77}. However, in human, the effect of physical activity on OPG/RANKL system is only reported in adults, but remains questionable. Some results showed no difference of OPG/RANKL concentrations in basal condition between runners and non-active subjects {Herrmann, 2004 #72}, a higher OPG and a lower RANKL levels {Ziegler, 2005 #82} associated with a variation after a intense running race (marathon and 246-km){Ziegler, 2005 #82; Kerschan-Schindl, 2009 #76}. The authors hypothesized that the positive effects of long-distance running on skeletal mass may be mediated by the OPG/RANKL system {Ziegler, 2005 #82}. Unfortunately, this assumption may be inappropriate, because a lower aBMD has been generally reported in highly trained runners of both sexes compared to age-matched controls {Hind, 2006 #74}. In our study, the absence of relationship between OPG/RANKL and aBMD or bone geometry status did no exclude a direct action of OPG/RANKL on the bone in athletes, because it remains unclear whether circulating concentration of OPG and RANKL reflect faithfully its activity in the microenvironment of bone cells {Buzi, 2004 #83}.

In conclusion, we reported that whatever the age or the menarchal status, female artistic gymnasts present a higher aBMD in proximal femur, lumbar spine and radius compared to less-active controls. Moreover, in addition to the well known effect on bone density, exercise improves also femoral geometry and strength and induces an intense bone remodelling. This favourable bone adaptation seems not related to the OPG/RANKL system and is present despite the presence of delayed menarche and menstrual disorders. These results may give some bases for future exercise intervention program to improve bone accretion.

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CONFLICT OF INTEREST
No Disclosures

REFERENCES

TABLE 1. Age, anthropometric, body composition, birth and familial data and training status of the gymnasts and controls.

Parameters	Gymnasts	Controls	p-value
Number of subjects	23	23	
Age (yr)	13.4 ± 2.2	13.2 ± 2.2	0.80
Bone age (yr)	12.7 ± 2.5	13.2 ± 2.4	0.46
Δ Age – bone age (yr)	0.68 ± 1.05	-0.02 ± 0.99	**<0.05**
Tanner stages	I:6, II:1, III:3, IV:1, IV: 12	I:5, II:5, III:3, IV:0, IV: 11	
Age of menarche (yr)	13.8 ± 1.2 (n=11)	12.0 ± 0.9 (n=11)	**<0.001**
Anthropometric data			
Weight (kg)	43.6 ± 9.2	45.2 ± 11.2	0.61
Weight SDS	0 ± 0.6	0.5 ± 1.3	0.08
Height (cm)	149.3 ± 9.3	154.3 ± 9.8	0.08
Height SDS	-0.5 ± 0.7	0.5 ± 0.9	**<0.001**
BMI ($kg.m^{-2}$)	19.3 ± 2.0	18.7 ± 2.9	0.39
Target height (cm)	160.5 ± 3.9	164.4 ± 4.5	**<0.05**
Body fat mass (kg)	7.5 ± 2.1	10.9 ± 4.7	**<0.01**
Body fat mass (%)	17.1 ± 2.3	23.3 ± 5.5	**<0.001**
Body fat free soft tissue (kg)	34.4 ± 7.1	32.8 ± 6.9	0.41
Birth data			
Birth weight (kg)	3.09 ± 0.25	3.06 ± 0.48	0.65
Birth height (cm)	48.9 ± 1.8	48.3 ± 2.0	0.25
Familial data			
Father's height (cm)	173.1 ± 5.8	176.0 ± 6.5	0.207
Mother's height (cm)	160.8 ± 4.2	165.7 ± 4.1	<0.001
Mother age of menarche (yr)	12.9 ± 1.3	12.7 ± 1.6	0.61
Training status			
Hours/weeks	19.9 ± 4.1	2.4 ± 0.5	<0.001
Age at start of training (yr)	5.3 ± 1.3	6.4 ± 2.1	<0.05

TABLE 2. Non-adjusted areal bone mineral density at various bone sites in gymnasts and controls.

Bone mineral density (g.cm^{-2})	Gymnasts	Controls	Difference (%)	p-value
Whole body	1.009 ± 0.127	0.927 ± 0.103	8.8	**<0.05**
Arm BMD	0.729 ± 0.101	0.638 ± 0.067	14.3	**<0.001**
Leg BMD	1.062 ± 0.162	0.952 ± 0.111	11.6	**<0.05**
Pelvis BMD	1.122 ± 0.207	1.009 ± 0.143	11.2	**<0.05**
Skull BMD	1.646 ± 0.211	1.651 ± 0.320	0.3	0.953
Femoral region				
Femoral neck	0.879 ± 0.152	0.745 ± 0.104	18	**<0.001**
Trochanter	0.753 ± 0.142	0.647 ± 0.107	16.4	**<0.01**
Intertrochanteric region	1.069 ± 0.200	0.936 ± 0.104	14.2	**<0.01**
Total proximal femur	0.945 ± 0.167	0.838 ± 0.116	12.8	**<0.05**
Lumbar spine (L1-L4)	0.912 ± 0.174	0.803 ± 0.145	13.6	**<0.05**
Radius	0.536 ± 0.079	0.483 ± 0.057	13.6	**<0.05**

TABLE 3. Adjusted areal bone mineral density according to age, fat-free soft tissue and fat mass at various bone sites in gymnasts and controls.

Bone mineral density (g.cm^{-2})	Gymnasts	Controls	Difference (%)	p-value
Whole body	0.995 ± 0.081	0.940 ± 0.081	5.5	<0.01
Arm BMD	0.716 ± 0.061	0.651 ± 0.061	9.1	<0.001
Leg BMD	1.041 ± 0.101	0.972 ± 0.101	6.6	<0.01
Pelvis BMD	1.095 ± 0.142	1.035 ± 0.142	11.5	0.08
Skull BMD	1.640 ± 0.285	1.656 ± 0.285	1	0.80
Femoral region				
Femoral neck	0.857 ± 0.135	0.767 ± 0.135	10.5	<0.01
Trochanter	0.735 ± 0.142	0.664 ± 0.142	9.6	<0.05
Intertrochanteric region	1.051 ± 0.169	0.953 ± 0.169	11.5	<0.01
Total proximal femur	0.923 ± 0.135	0.853 ± 0.135	7.6	<0.05
Lumbar spine	0.894 ± 0.108	0.821 ± 0.108	8.2	<0.01
Radius	0.529 ± 0.058	0.489 ± 0.058	7.6	<0.01

TABLE 4. Biomechanical calculation of femur adjusted for body weight and body height

Parameters	Gymnasts (n=23)	Controls (n=23)	Difference (%)	p-value
HAL (mm)	104.8 ± 6.4	102.5 ± 6.4	+2.2	0.12
Shaft neck angle (°)	131.1 ± 8.1	129.3 ± 8.1	+1.2	0.33
Femoral neck				
Cross-sectional area (CSA; cm^2)	2.962 ± 0.440	2.473 ± 0.440	+16.5	**<0.001**
Cross-sectional moment of inertia (CSMI; cm^4)	1.998 ± 0.570	1.770 ± 0.570	+11.4	0.08
Section modulus (Z; cm^3)	1.355 ± 0.247	1.137 ± 0.247	+16.0	**<0.001**
Endocortical diameter (cm)	2.387 ± 0.417	2.545 ± 0.417	-6.6	0.26
Buckling ratio	7.042 ± 2.535	8.947 ± 2.535	-27.1	**<0.01**
Mean cortical thickness (cm)	0.219 ± 0.040	0.173 ± 0.040	+21.0	**<0.001**
Width (cm)	2.825 ± 0.372	2.892 ± 0.372	-2.4	0.42
Intertrochanteric region				
Cross-sectional area (CSA; cm^2)	4.703 ± 0.692	3.858 ± 0.692	+18.0	**<0.001**
Cross-sectional moment of inertia (CSMI; cm^4)	8.542 ± 2.275	7.345 ± 2.275	+14.0	**<0.05**
Section modulus (Z; cm^3)	3.298 ± 0.651	2.685 ± 0.651	+18.6	**<0.001**
Endocortical diameter (cm)	3.739 ± 0.550	3.975 ± 0.550	-6.3	0.06
Buckling ratio	5.821 ± 1.651	7.087 ± 1.651	-21.7	**<0.001**
Mean cortical thickness (cm)	0.453 ± 0.086	0.375 ± 0.086	+17.2	**<0.001**
Width (cm)	4.646 ± 0.491	4.726 ± 0.491	-1.7	0.47
Femoral shaft				
Cross-sectional area (CSA; cm^2)	3.492 ± 0.406	3.053 ± 0.406	+12.5	**<0.001**
Cross-sectional moment of inertia (CSMI; cm^4)	2.216 ± 0.522	1.817 ± 0.522	+18.0	**<0.01**
Section modulus (Z; cm^3)	1.611 ± 0.231	1.361 ± 0.231	+15.5	**<0.001**
Endocortical diameter (cm)	1.439 ± 0.507	1.496 ± 0.507	-4	0.61
Buckling ratio	2.548 ± 0.898	2.710 ± 0.898	-6.4	0.42
Mean cortical thickness (cm)	0.566 ± 0.141	0.489 ± 0.141	+13.6	**<0.05**
Width (cm)	2.571 ± 0.263	2.474 ± 0.263	+3.8	0.10

TABLE 5. Bone biochemical markers and OPG/RANKL system in gymnasts and controls

Parameters	Gymnasts (n=23)	Controls (n=23)	p-value
Bone biochemical markers			
PINP ($ng.ml^{-1}$)	610.6 ± 342.2	519.2 ± 373.3	0.39
OC ($ng.ml^{-1}$)	134.9 ± 75.8	104.4 ± 61.3	0.14
CTx ($ng.ml^{-1}$)	1.447 ± 0.65	1.128 ± 0.545	0.07
OPG ($pmol.l^{-1}$)	3.5 ± 0.7	3.3 ± 0.7	0.19
RANKL ($pmol.l^{-1}$)	0.46 ± 0.27	0.45 ± 0.24	0.89

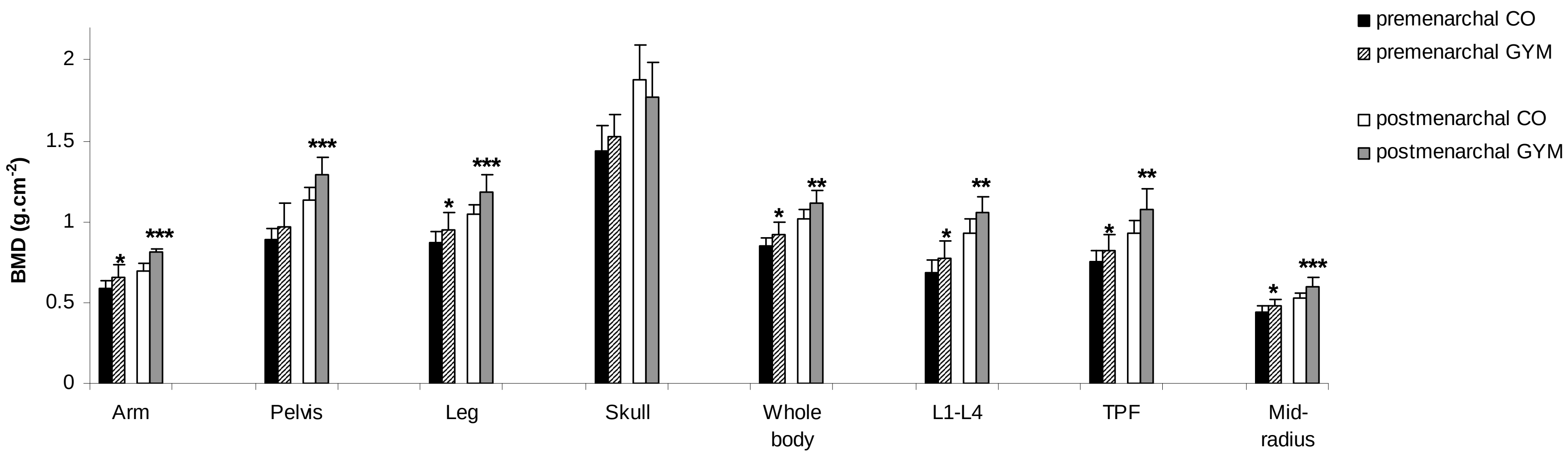

Figure 1. aBMD comparison between gymnasts and controls within premenarchal and postmenarchal subgroups.

* indicates a significantly higher BMD for gymnasts vs. controls for the same menarchal status, $p<0.05$; ** for $p<0.01$ and *** for $p<0.001$.

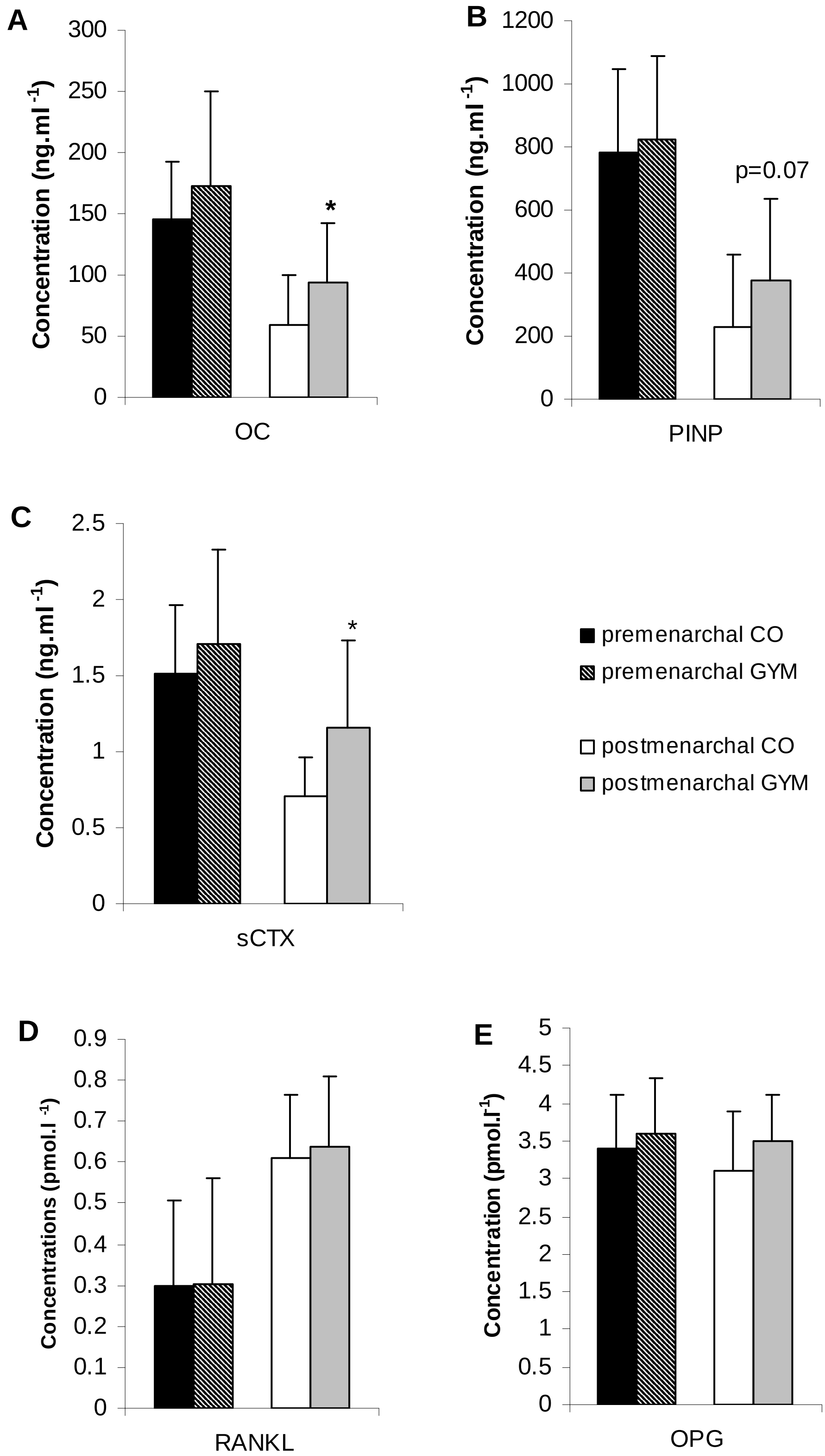

Figure 2. Comparison between gymnasts and controls within premenarchal and postmenarchal subgroups for osteocalcin : OC (A), procollagen type I N-terminal propeptide : PINP (B), type I-C telopeptide breakdown products: sCTX (C), rank-ligand: RANKL (D) and osteoprotegerin: OPG (E).

* indicates a significantly higher BMD for gymnasts vs. controls for the same menarchal status, p<0.05

Tables and legends

TITLE: TABLE 1. Descriptive characteristics of gymnasts and controls
LEGEND: Values are presented as mean (SD); BMI: body mass index

TITLE: TABLE 2. Bone mineral density ($g.cm^{-2}$) in gymnasts and controls
LEGEND: Values are presented as means (SD); TB BMD: total body BMD; Z-score is defined as a difference (%) with a gender- and age-matched population.

TITLE: TABLE 3. Adjusted bone mineral density in gymnasts and controls according to weight, whole body fat-free soft tissue and whole body fat mass.

LEGEND: Values are presented as means ± SD; TB BMD: total body BMD

TITLE: TABLE 4. Biomechanical calculation of femur adjusted for body weight and body height**. LEGEND:** Values are presented as means ± SD; TB BMD: total body BMD

TITLE: TABLE 5. Bone biochemical markers and OPG/RANKL system in gymnasts and controls.
LEGEND: Values are presented as means ± SD; PINP: Procollagen type I N-terminal propeptide; OC: osteocalcin; CTx: type I-C telopeptide breakdown products, OPG: osteoprotegerin; RANKL: Rank-ligand.

Figures and Legends

TITLE Figure 1. aBMD comparison between gymnasts and controls within premenarchal and postmenarchal subgroups.

LEGEND * indicates a significantly higher BMD for gymnasts vs. controls for the same menarchal status , $p<0.05$; ** for $p<0.01$ and *** for $p<0.001$.

TITLE AND LEGEND: Figure 2. Comparison between gymnasts and controls within premenarchal and postmenarchal subgroups for OPG: osteoprotegerin (A), rank-ligand (B), procollagen type I N-terminal propeptide :PINP (C), osteocalcin (D) and type I-C telopeptide breakdown products: sCTX (E)

* indicates a significantly higher BMD for gymnasts vs. controls for the same menarchal status, $p<0.05$