Changes in bone density and bone markers in rhythmic gymnasts and ballet dancers: implications for puberty and leptin levels

Maria Teresa Muñoz, Concepción de la Piedra, Vicente Barrios, Guadalupe Garrido and Jesús Argente
Servicio de Endocrinología, Hospital Infantil Universitario Niño Jesús, Madrid, Spain, Laboratorio de Bioquímica, Sección de Fisiopatología Ósea, Fundación Jiménez Díaz, Madrid, Spain and Instituto Nacional de Educación Física, Departamento de Rendimiento Humano, Madrid, Spain
(Correspondence should be addressed to M T Muñoz Calvo, Servicio de Endocrinología, Hospital Infantil Universitario Niño Jesús, Avda Menéndez Pelayo, 65, 28009 Madrid, Spain; Email: munozmait@yahoo.es)

Abstract
Objective: Our aim was to compare physical activity and biochemical markers with bone mineral acquisition in rhythmic gymnasts and ballet dancers.
Methods: Weight, height, body mass index, nutritional intake, bone age and menstrual histories were analyzed in nine rhythmic gymnasts, twelve ballet dancers and fourteen controls. Bone mineral density (BMD) was assessed by X-ray absorptiometry at the lumbar spine, hip and radius. Bone alkaline phosphatase (bAP) and amino-terminal propeptide of procollagen I (PNIP) in serum and urinary α-isomer of the carboxy-terminal telopeptide of collagen I (α-CTX) were measured.
Results: Bone age was delayed 2 years and mean age at menarche was 15±0.9 years in rhythmic gymnasts and 13.7±1 years in ballet dancers, compared with 12.5±1 years in controls. Trocanteric and femoral neck BMD was significantly higher in rhythmic gymnasts compared with ballet dancers and controls. Right forearm (non-loaded zone) BMD was significantly decreased in rhythmic gymnasts and ballet dancers compared with controls. All subjects had normal bAP and PNIP levels, but the α-CTX/creatinine (Cr) ratio was increased in rhythmic gymnasts (P<0.001) with an inverse correlation between right forearm BMD and the α-CTX/Cr ratio (r=−0.74, P<0.001). Serum leptin levels were decreased in rhythmic gymnasts and ballet dancers. Rhythmic gymnasts had a positive correlation between right forearm BMD and leptin levels (r=0.85, P<0.001).
Conclusions: Decreased bone mass in rhythmic gymnasts could be partially explained by an increase in bone resorption. Serum leptin levels could be implicated in the pubertal delay and be a good marker of bone mass in these subjects.

Introduction
Bone mineral density (BMD) is modified by environmental factors such as exercise and dietary calcium intake. Several cross-sectional studies have shown that high impact weight-bearing activity is beneficial for the load-bearing sites of the skeleton (1, 2). An increase in bone mass due to augmentation of bone size, density of skeletal areas and bone turnover has been demonstrated in adolescents performing intensive exercise. Indeed strategies that enhance the acquisition of bone mass may be protective against osteoporosis (3).

Growth during puberty depends on genetic potential, nutritional status and hormonal regulation. Energy expenditure may modify the effects of these three factors on linear growth rate and the relative proportions of lean and fat body mass (4). In high-performance female gymnasts, a low body fat mass is favored for the esthetic appeal currently required for the complex movements performed. Optimal nutritional intake relative to physical training regimes is essential for normal pubertal development (5). Hence, this could be a problem in adolescents with strenuous training programs while trying to maintain a lean figure.

Although a high degree of exercise-induced osteopenia and amenorrhea are frequently found in elite athletes and performers, some studies suggest that the low estrogen levels may not be the cause of the low bone mass in these women (6). As bone mass is the net result of bone formation and resorption, an uncoupling between these two activities may be the cause of the osteopenia found in young athletes. Biochemical markers of bone remodeling can be used to determine the degree of bone formation and resorption. The number of such bone markers has expanded greatly during recent years. In a previous study, we demonstrate that...
the α-isomer of the carboxy-terminal telopeptide of collagen I (α-CTX), a biochemical marker of bone resorption, has a high level of sensitivity in the study of bone remodeling in adolescents (7). The bone isoenzyme of alkaline phosphatase (bAP) and amino-terminal propeptide of procollagen I (PNIP), both biochemical markers of bone formation, have a high sensitivity in the study of changes in bone remodeling associated with decreased estrogen levels, such as in postmenopausal osteoporosis (8).

There are few published data on the association between biochemical markers of bone formation and resorption and physical activity, especially in adolescents. The aims of this study were to analyze markers of bone formation and resorption in adolescent ballet dancers, rhythmic gymnasts and an age- and sex-matched control group and to determine whether the degree of osteopenia correlates with bone markers and leptin levels in these adolescent females.

**Subjects and methods**

**Subjects**

Thirty-five healthy girls, including nine rhythmic gymnasts, twelve ballet dancers and fourteen control adolescents of the same age were studied. The rhythmic gymnasts and ballet dancers performed intensive exercise for at least 20 h/week. At the beginning of the study, the rhythmic gymnasts and ballet dancers had a 5-year history of extensive exercise training. Adolescents of the control group performed less than 3 h of exercise per week. The three groups belonged to the same geographical area and social group.

This study was approved by the local ethics committee and permission was obtained from the subjects and their parents. None of the subjects had any known disease or had ever used medication known to affect bone health.

**Methods**

Nutritional state was determined by the following anthropometric measurements: height, measured using a stadiometer (Holtain Ltd., Crymych, UK), weight on a SECA scale (Germany) and body mass index (BMI), calculated as body weight (kg) divided by the squared standing height (m²). These parameters are expressed in s.d. for age and sex for the Spanish population (9). Subjects were seen individually for a medical examination to determine pubertal maturation assessed according to the Tanner stage. Menstrual history with questions concerning the age of menarche and periods of oligomenorrhea.

Food intake was evaluated by using a 5-day (including Sunday) recall survey. Nutrient composition data were analysed with the software package Nutritionist IV (San Bruno, CA, USA). Bone age was measured by X-ray of the left hand and wrist and determined by the method of Greulich & Pyle (10).

BMD, expressed in g/cm², was measured at the following sites: lumbar vertebrae (L2–L4), dominant hip (including neck and femoral trochanter) and the right forearm (total radius). Measurements were performed by dual energy X-ray absorptiometry (Hologic QDR 1000; Hologic, Waltham, MA, USA). The in vivo coefficient of variation using this technique was less than 1% at the specific regional sites.

Blood samples were obtained in the morning from fasting subjects. Serum was separated by centrifugation at 4°C no more than 1 h after sample collection and frozen at −80°C until assayed. Two-hour fasting morning urine samples (after the first voiding was discarded) were collected and aliquots were kept at −80°C.

Serum levels of bAP were quantified by an immunoradiometric assay (Tandem R-Ostase; Hybritech, Liège, Belgium) using two monoclonal antibodies specific for bAP over liver AP. Intra- and interassay coefficients of variation were less than 4% and 7% respectively and sensitivity was 2.3 μg/l. PNIP was quantified by RIA (Orion Diagnostica, Espoo, Finland). Intra- and interassay coefficients of variation were less than 6% and 10% respectively and sensitivity was 2 μg/dl.

Urinary α-CTX levels were determined by RIA (α-Crosslaps; Osteometer Biotech, Austin, TX, USA). This method uses a tube coated with monoclonal antibody raised to an eight amino acid sequence of the C-telopeptides of the α1-chain of type I collagen (EKAHD-α-GGR, α isomer). Intra- and interassay coefficients of variation were less than 6% and 11% respectively and sensitivity was 40 μg/l. α-CTX levels are expressed as urinary creatinine ratio (α-CTX/Cr).

Leptin levels were determined by RIA (Linco, St Charles, MO, USA). Intra- and interassay coefficients of variation were less than 5% and 8% respectively and sensitivity was 0.5 μg/l.

**Statistics**

Results are expressed as the mean±S.D. Data were analyzed using one-way ANOVA, followed by Scheffe’s F test. P < 0.05 was chosen as the level of significance.

**Results**

**Anthropometric results**

The characteristics of the groups are shown in Table 1. There were no significant differences between groups, with regard to age, weight or height. The rhythmic gymnasts and ballet dancers had BMIs within the normal range. In rhythmic gymnasts and ballet dancers, bone age was significantly delayed compared with controls, with a mean delay of 2 years (Table 1).

The caloric intake in rhythmic gymnasts was 1828±500 Kcal/day and in ballet dancers 1946±639 Kcal/day. This intake is insufficient for the level
of physical exercise performed by both groups taking the guidelines of the Food and Nutrition Board (Institute of Medicine of the National Academy, USA) into account. In all groups, calcium intake was below the recommended levels and no significant difference between the groups was found (Table 1). The mean age at menarche was 15±0.9 years and 13.7±1 years respectively, compared with 12.5±1 years in controls (P < 0.01). During the first years after menarche, the percentage of rhythmic gymnasts and ballet dancers with oligomenorrhea was 45% and 74% respectively. All control girls had normal menstrual cycles.

**Bone measurements**

The BMD in lumbar spine was normal in all groups. As shown in Table 2, in rhythmic gymnasts, BMD in the trocanteric and femoral neck was significantly higher than in ballet dancers and the control group. In contrast, a significant decrease was found in BMD at the right forearm (non-loaded zone) in rhythmic gymnasts compared with the control group (Table 2).

**Biochemical analyses**

All groups had normal bAP and PNIP levels (Table 3). In contrast, rhythmic gymnasts had an increase in the α-CTX/CR ratio (P < 0.001) and an inverse correlation between right forearm BMD and α-CTX/CR ratio (r = −0.74, P < 0.001) (Fig. 1).

Serum leptin concentrations were significantly increased in rhythmic gymnasts and ballet dancers as compared with controls (Table 3). In the rhythmic gymnasts, a positive correlation between BMD in the right forearm and serum leptin levels (r = 0.85, P < 0.001) was found (Fig. 2).

**Discussion**

In our study, a higher bone mass at the trocanteric and femoral neck was found in rhythmic gymnasts compared with ballet dancers and controls. In contrast, a significant decrease was found in BMD of the right forearm in rhythmic gymnasts and ballet dancers. Daly et al. (11) showed that during training rhythmic gymnasts experience frequent high-impact stress on the upper and lower extremities. These findings suggest that weight-bearing exercise during the pre- and peripubertal period may enhance the mechanical competence of the skeleton (11). The BMD heterogeneity may be explained mainly by the direct mechanical effects of weight bearing at weight-bearing sites. Nevertheless, other factors such as the amount of cortical and trabecular bone content, the hypogonadism, exercise, as well as body weight, could affect the BMD (12, 13).

Nguyen et al. (14) showed that adequate dietary calcium intake and maintaining a physically active lifestyle in the later decades of life could potentially translate into a reduction in the risk of osteoporosis. The rhythmic gymnasts and ballet dancers studied here had a deficient energy and calcium intake for the physical exercise they performed (15). Subjects with deficient caloric intake run the risk of stunted growth, delayed puberty, menstrual irregularities, decreased physical output, increased numbers of lesions, and increased possibilities of suffering from eating disorders (16, 17).

Elite female rhythmic gymnasts and ballet dancers exhibit a specific pattern of growth characterized by a marked delay in skeletal maturation and pubertal development (18), as found in our subjects. Georgopoulos et al. (19) demonstrated that the elite rhythmic gymnasts compensate for their loss of the pubertal growth spurt by a late acceleration of linear growth. Despite this delay in skeletal maturation, the genetic predisposition of growth is not only preserved, but even exceeded (20). The percentage of subjects with menstrual alterations was increased in rhythmic gymnasts and ballet dancers with respect to the normal
population and is in agreement with data reported in elite athletes and performers (21). The etiology of these alterations is multifactorial and includes the physical and emotional stress associated with the lifestyle of elite performers and athletes (competitions, trips, etc.), low caloric intake, low percentage of body fat and periods of very intensive physical training (22).

Puberty is associated with the highest levels of biochemical markers of bone turnover. Mora et al. (23) found that the concentrations of these biochemical markers reach a zenith at pubertal stage 2 and that serum bAP and osteocalcin levels correlate inversely with BMD. Furthermore, they showed that markers of bone resorption were related to bone volume. Borderie et al. (24) found that values of bone biochemical markers correlate with the change in BMD at the lumbar spine, but not at the femoral neck. This might be due to the higher biological activity of trabecular bone than that of cortical bone since the lumbar spine consists mostly of trabecular bone (25).

The effect of physical activity on bone turnover markers in the growing skeleton has been poorly studied and its effects on bone formation markers are controversial. Some studies report no effect of exercise on the level of serum osteocalcin and total AP (26), while others have shown either significantly higher or lower bone formation marker concentrations in exercising subjects compared with controls (27). Bass et al. (28) reported that prepubertal gymnasts had significantly lower serum concentrations of osteocalcin and bAP than sedentary controls. This may have been due to delayed growth as a result of strenuous exercise (28).

In the present study, we found no significant difference in the levels of bAP or PNIP between the gymnasts and ballet dancer groups and controls. However, rhythmic gymnasts had a significantly higher $\alpha$-CTX/Cr ratio compared with ballet dancers and control adolescents. It is possible that the higher $\alpha$-CTX levels in rhythmic gymnasts result from an increased turnover of bone tissue including resorption of the newly formed bone. Moreover, mean levels of $\alpha$-CTX were also higher in ballet dancers than in controls, although this did not reach statistical significance. An increase in bone resorption with no change in bone formation could explain the osteopenia found in the total radius (unloaded bone) of these girls.

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**Figure 1** $\alpha$-CTX serum levels and BMD correlation. BD = ballet dancers; RG = rhythmic gymnasts. ***$P < 0.01$.

**Figure 2** Leptin levels and BMD correlation. BD = ballet dancers; RG = rhythmic gymnasts. ***$P < 0.01$.  

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Leptin, a hormone secreted by fat cells, is disproportionately lowered by fasting and is an independent regulator of metabolic rate (29). Leptin receptors have been found in bone, suggesting that this hormone may be involved in skeletal regulation (30). In our study, serum leptin levels were significantly decreased in rhythmic gymnasts and ballet dancers, as compared with controls. Furthermore, in rhythmic gymnasts a positive correlation was found between BMD of the right forearm and serum leptin levels. Estrogen deficiency may also be responsible for the low BMD seen in women whose amenorrhea is associated with caloric deficiency, nutritional imbalances and exercise (31).

Different studies performed in elite women athletes and performers show low leptin levels, which may be related to an insufficient nutritional intake, although this decrease could be influenced directly by the physical activity (32). Normal or decreased BMD has been described in athletes, showing a direct relationship with leptin values. Some studies indicate that osteopenia could be related to the amenorrhea suffered by these women athletes (33).

In conclusion, the decrease in bone mass in rhythmic gymnasts could be explained, at least in part, by the increase in bone resorption indicated by increased α-CTX levels. Serum leptin levels appear to be a good marker of bone mass in these subjects and could also be implicated in the delay of puberty.

References


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