Effect of rhythmic gymnastics on volumetric bone mineral density and bone geometry in premenarcheal female athletes and controls.

Tournis S.[1], Michopoulou E.[2], Fatouros I.G.[2], Paspati I[1], Michalopoulou M[2], Raptou P.[1], Leontsini D.[2], Avlonitou A[2]., Krekoukia M.[2], Zouvelou V.[1], Galanos A.[1], Aggelousis N[2]., Kambas A. [2], Douroudos I[2], Lyritis G.P[1], Taxildaris K.[2], Pappaioannou N.[1].

1. Laboratory of Research of Musculoskeletal System “Th. Garofalidis”, University of Athens, KAT Hospital, 14561, Athens, Greece.
2. Department of Physical Education and Sports Science, Democritus University of Thrace, 69100, Komotini, Greece.

Abbreviated title: Rhythmic gymnastics and bone geometry

Key words: rhythmic gymnastics, bone geometry, peripheral quantitative computerized tomography.

PRECIS: Rhythmic gymnastics in premenarcheal girls induces positive adaptations in cortical bone, while increased duration of exercise is associated with a positive response of bone geometry.

Address for correspondence and requests for reprints:
Symeon Tournis M.D.,
Laboratory of Research of Musculoskeletal System “Th. Garofalidis”
University of Athens, KAT Hospital
10 Athinas Str., Kifissia, PC: 14561, Athens, Greece.
Tel: +302108018123
Fax: +302108018122.
E-mail: stournis@med.uoa.gr
Word Count: 3581
Figures: 0
Tables: 3
Disclosure Statement: The authors have nothing to disclose

Abstract

Context and Objective: Weight-bearing exercise during growth exerts positive effects on the skeleton. To test the hypothesis that long-term elite rhythmic gymnastics exerts positive effects on volumetric bone mineral density (vBMD) and geometry and whether exercise-induced bone adaptation is associated with increased periosteal bone formation or medullary contraction using tibial peripheral quantitative computed tomography (pQCT) and bone turnover markers (BTM).

Design and Setting: Cross-sectional study at a tertiary center.

Subjects: Twenty-six elite premenarcheal female rhythmic gymnasts (RG) and 23 female controls (C), aged 9–13 years.

Intervention: None

Main Outcome Measure: We measured bone age, vBMD, bone mineral content (BMC), cortical thickness, cortical and trabecular area and polar stress strength index (SSIp) by pQCT of the left tibia proximal to the distal metaphysis (trabecular), at 14%, 38% (cortical) and 66% (muscle mass) from the distal end and BTM.

Results: The two groups were comparable according to height, chronological and bone age. After weight adjustment, cortical BMC, area and thickness at 38% were significantly higher in RG ($p<0.005$-0.005). Periosteal circumference, SSIp, and muscle area were higher in RG ($p<0.01$-0.001). Muscle area was significantly associated with cortical BMC, area and SSIp, while years of training showed positive association with cortical BMC, area and thickness independent of chronological age.

Conclusions: RG in premenarcheal girls may induce positive adaptations on the skeleton, especially in cortical bone. Increased duration of exercise is associated with a positive response of bone geometry.

Introduction

Regular physical exercise is a modifiable lifestyle factor that might exert favorable effects on the skeleton. The inconsistency between studies[1] lies on the fact that the skeletal response to exercise[1, 2] depends on both training-related factors, such as type of exercise, intensity and duration of the training program and individual-related factors such as baseline training status, nutritional and hormonal factors and most importantly age at which training intervention is started. Importantly, the most consistent favorable results are reported in children and adolescents following weight-bearing exercise, in which bone adaptation to applied loads is associated with gains in areal bone mineral density (aBMD)[1,2] and positive changes in size and geometry[3].

Contrary to low or moderate intensity exercise, participation in elite artistic (AG) and rhythmic gymnastics (RG) might result in a more favorable skeletal response due to the high-volume, high-impact training and the involvement at an early age. In particular cross-sectional [4,5,6,7] and longitudinal studies[4, 7, 8] in AG show positive results of both aBMD and bone geometry[9, 10]. Although the same responses concerning aBMD can be observed in RG, at least in cross-sectional studies[11, 12], the two types of gymnastics are quite different in terms of degree, type of impact loading and possibly individual-related factors such as nutrition and growth potential[13]. Generally RG requires less arm and body strength, loading mostly the lower limbs, whereas AG imposes a greater mechanical load in the upper and lower limbs and the trunk[14]. Accordingly, a recent study comparing RG with AG showed higher regional aBMD and muscle mass in AG, despite delayed pubertal development[15].

Dual-energy x-ray absorptiometry (DXA) is the most widely used technique for measuring bone mineral content (BMC) and BMD in children due to its low cost, low radiation exposure and availability. However, evaluation of bone strength in the growing skeleton, especially under the influence of exercise, requires information about both bone size and geometry, data that cannot be reliably derived from planar DXA images. Thus although during growth aBMD[16] increases, volumetric BMD (vBMD) remains constant or slightly increases, due to the proportional rise of both BMC and bone size[17]. Moreover DXA is unable to separate cortical from trabecular bone, components that respond differently to loading[18]. In contrast peripheral quantitative computed tomography (pQCT) has the advantage of simultaneous separate assessment of trabecular and cortical components as well as geometric characteristics of the peripheral skeleton, thus being suitable for the evaluation of training-induced skeletal adaptation[19, 20] at least in research settings due to the relative radiation exposure. Given that the processes underlying bone geometry adaptation, especially at cortical sites may include not only increased periosteal apposition, but also increased endosteal apposition or decreased endosteal resorption[1, 3], the concomitant estimation of pQCT derived variables with bone turnover markers (BTM) might give some insight of the underlying mechanism, although data relating pQCT indices and fracture risk in children are scarce.

The present study was designed to test the hypothesis that long-term elite rhythmic gymnastics in premenarcheal females exerts positive effects on vBMD and geometry and examine whether exercise-induced bone adaptation is associated with increased periosteal bone formation or medullary contraction using tibial pQCT and BTM.

Methods

Participants were recruited from eight gymnastics centres and schools within the district of Attika over a 12-week period from January to March 2008. Inclusion criterion was premenarcheal females aged 9–13 years. Elite rhythmic gymnasts were included if they had been training for at least 2 years. Gymnastics instructors identified children who exercised at least 24h per week and were the most promising athletes of their centre. The parents of 53 gymnasts were approached and 30 consented to participate. Controls were female premenarcheal school children, having only physical-education related activity, matched with the athletes by age and height. Controls participating in other athletic activities, assessed by questionnaire, were excluded from the study (Physical activity Score < 40 METs/day). All participants were Caucasian, clinically healthy, were not receiving medication known to affect bone metabolism or had any immobilising surgery or fracture in the previous 12 months. The study was approved by the Ethics committee of KAT hospital and was conducted in accordance with the Declaration of Helsinki. Informed written consent was obtained from the parents or legal guardians of each child and each child gave verbal assent to participate in the study.

Anthropometric measurements and maturity assessment

Standing height was measured without shoes using a stadiometer to the nearest 0.1 cm (SECA 220) and weight using an electronic scale to the nearest 0.1 Kg (Soehnle 7840), both in duplicate one week apart (CVs' 0.97 and 0.98, respectively). Body mass index (BMI) was calculated as weight (Kg) per squared standing height (m^2). Skinfold sum is the sum of skinfold thickness (in mm) measured sequentially, in duplicate, using a skinfold calliper, at biceps, triceps, subscapula, abdomen, suprailiac, calf, and thigh.

Skeletal age was calculated from left hand and wrist radiograph using the Greulich Pyle[21] method by two independent experienced pediatric radiologists, blinded to the status of the participant. In case of difference of more than 6 months, x-rays were reevaluated blindly by two M.D.'s (S.T. and I.P.) and an agreement was reached. Pubertal stage was self assessed using Tanner diagrams of five pubertal stages of breast development[22].

Physical activity and dietary assessment

Based on their training logs, athletes performed two training sessions/day, 6 days/week, with a total training volume of > 20,000 foot-contacts/year. Controls participated only in physical education classes and had minimal physical activity weekly as reported in a 4-day physical activity questionnaire (METs/day) [23]. In order to determine nutrient intakes, 5-d diet (three week-days and week-end) recalls were completed. Subjects' parents were taught how to complete diet recall questionnaires and determine food serving and sizes. Diet records were analyzed using the computerized nutritional analysis system Science Fit Diet 200A (Science Technologies, Athens, Greece) [24].

vBMD and geometric properties estimation by pQCT

pQCT bone mineral measurements and analyses were performed at the left tibia, using XCT-3000 device (Stratec Gmbh, Pforzheim, Germany) as previously described[25]. Participants were asked about dominance of hand and the limb to be measured was the contralateral leg. All subjects were right-handed, so the left tibia was measured in all. A single energy X-ray source was used and all computed tomography scans had a slice thickness of 2.4 mm and a voxel size of 0.5 mm^3. The distal end of the tibia was used as an anatomical marker; the bone cross sectional area (CSA) was imaged at 10mm proximal to the distal surface of the distal metaphysis and 14%, 38% and 66% of the total tibia length. Analyzing each slice vBMD (mg/cm^3), corresponding BMC (mg) and CSA (mm^2) of tibia bone section were estimated, as well as cortical thickness (CRTHK-mm), endosteal (ENDO-mm) and periosteal circumference (PERI-mm) and polar Stress Strength Index in torsion (SSIp-mm^3). Image analysis was performed using integrated software, version 5.4. Total (from the periosteum included area of the bone and bone marrow), trabecular and cortical bone density in mg/cm^3 and the cross sectional areas of the corresponding bone portions in mm^2 were calculated by the following procedure: a) voxels outside the bone (soft tissue) with lower attenuation coefficients than the selected threshold (181 mg/cm^3) are removed within the region of interest (ROI), b) the cortical and trabecular structures are separated by the areal distribution of both bone structures. By default, 55% of the outer bone area is concentrically separated and defined as cortical-subcortical region. The remaining 45% of the inner core is defined as trabecular bone. To calculate pure cortical density and area without including subcortical area, all voxels within the ROI that have an attenuation coefficient below threshold 710 mg/cm^3 density are removed. CRTHK was defined as the mean distance between inner and outer edge of the cortical shell. SSIp lies within the theory of stability of mechanical structures against bending or torsion. From CT cross-sectional images, the determination of bone strength is based on the calculation of the cross-sectional moment of inertia (CSMI). Division of CSMI by the maximum distance of any voxel from the centre of gravity (r_{max}) yields the section modulus which is directly proportional to maximum stress in bone. To take also the material properties into consideration, the section modulus is multiplied with the quotient of calculated cortical density and normal cortical density of 1200 mg/cm^3, yielding the calculation of SSIp. At the 66% slice, the muscle cross-sectional area was also calculated. The long-term *in vitro* (phantom) precision of the pQCT in 12 month daily measurements was 0.12% for total vBMD and 0.3% for trabecular vBMD. The *in vivo* precision derived from 25 postmenopausal women subjected to duplicate measurements within one month was: total vBMD: 0.2%, trabecular vBMD: 0.46%, Cortical area: 0.3%, SSIp: 1%, CRTHK: 0.8%.

Biochemical analysis

Each subject reported to the laboratory for blood sampling after an overnight fast and 72 h abstinence from exercise. Serum total calcium (corrected to a serum albumin concentration of 4 gr/liter) [26] and serum inorganic phosphate were measured by colorimetry using a Roche Hitachi 902 analyzer (Roche, Indianapolis IN). The intra- and interassay CVs for calcium and phosphate determinations were 0.9% and 1.5%, and 0.9% and 1.4%, respectively. Plasma intact PTH (iPTH) was measured by an electrochemiluminescence immunoassay (ECLIA) (Roche). The sensitivity was 1.2 pg/ml and the intra- and interassay CVs were 4% and 4.3%, respectively. Serum 25(OH) D was determined by EIA (IDS OCTEIA). The sensitivity was 5 nmol/L and the intra- and interassay CVs were 5.3% and 4.6%, respectively. Serum P1NP was measured by ECLIA (Roche). The sensitivity was 5 μg/L and the intra- and interassay CVs were 2.2% and 2.9%, respectively. Serum β-CTX (sCTX) was determined by ECLIA (Roche). The sensitivity was 0.01 ng/ml and the intra- and interassay CVs were 1.6% and 4.3%, respectively.

Statistical Analysis

Normality was tested using Kolmogorov—Smirnov test. Accordingly data are presented as mean ± SE or median (range) in case of not normally distributed variables. Between-group differences were analyzed by unpaired t-test or Mann-Whitney U test concerning continuous variables and chi-square test for categorical variables, as appropriate. Mean differences of all vBMD and geometric parameters (adjusted for weight) between gymnasts and controls were assessed by analysis of covariance (ANCOVA) [5, 27, 28]. Partial correlations were performed to estimate the association of vBMD and bone geometry indices with selected variables, independent of differences in weight.

All tests were two-tailed and $P<0.05$ was considered significant. All data analysis was performed using the Statistical Package for Social Sciences (version 10.0) software (SPSS Inc., Chicago, Illinois).

Results

Baseline characteristics

Baseline characteristics are presented in table 1. Of the 60 children initially evaluated (30 athletes and 30 controls), 4 athletes and 7 controls were excluded due to either unsatisfactory pQCT scan (movement artefacts, 2 athletes and 4 controls) or recent trauma that could affect BTM levels (2 athletes and 3 controls). The two groups were comparable according to chronological age, height, $\text{height}_{\text{sds}}$, calcium, vitamin D daily intake, and recreational daily physical activity. There was a non-significant trend for lower bone age in RG. Athletes had significantly lower weight, BMI, BMI_{SDS} and skinfold sum ($p<0.001$). Thus differences in bone density and geometry parameters were adjusted for weight.

Biochemical Characteristics

Biochemical analysis revealed that RG had higher total calcium (9.85 ± 0.08 mg/dl vs. 9.56 ± 0.09 mg/dl, p=0.025), while phosphate was comparable (table 2). iPTH was lower in RG compared with controls (p=0.012). RG had higher 25(OH) D (102.22 ± 4.20 nmol/L vs. 87.46 ± 3.74 nmol/L, p=0.013). However, all subjects had levels higher than 50 nmol/L, with 5 controls and 2 RG having levels between 50.16-74.64 nmol/L. P1NP and sCTX were comparable.

Comparison of vBMD and bone geometry between groups by pQCT

Trabecular site (10mm proximal to the distal surface of the distal metaphysis of the tibia). At this site trabecular BMC, area and vBMD were comparable between groups (table 3). Total BMC was higher in RG (p=0.022), while total area and vBMD were comparable.

Cortical site (38% of the tibia length). At 38% site, total and cortical BMC and area were significantly higher in RG compared with controls. (All p≤0.001). Indeed cortBMC was 30.26% higher, while cortical area was increased by 30.07%. Cortical vBMD was comparable. Concerning geometric indices, RG demonstrated higher CRTHK (25.83%, p<0.001), which was due to increase in periosteosteal circumference (7.70%, p=0.001), while endosteal circumference was comparable, indicating that intensive RG is associated with increased periosteal apposition. Moreover SSIp, an index of bone strength was increased (31.53%, p<0.001), indicating a positive response to applied loads.

Transition zone (14% of the tibia length). At the 14% site, total, trabecular and cortical BMC and area were increased in RG compared with controls (p<0.05-0.001), while the corresponding vBMD's were comparable. SSIp was increased in RG (18.98%, p=0.002). Muscle area was 11.13% higher in RG compared with controls (p=0.002).

Correlation analyses

Partial correlation analysis, adjusted for weight, revealed modest positive association of age, height, and muscle area with cortical area ($r_{age}=0.51$, $r_{height}=0.63$, $r_{muscle\ area}=0.46$, p<0.001-0.01), trabecular area ($r_{age}=0.33$, $r_{height}=0.41$, $r_{muscle\ area}=0.36$, p<0.01-0.05), periosteal circumference ($r_{age}=0.51$, $r_{height}=0.59$, $r_{muscle\ area}=0.45$, p<0.001-0.01) and SSIp ($r_{age}=0.47$, $r_{height}=0.57$, $r_{muscle\ area}=0.48$, p<0.001-0.01), while skinfold sum, an index of adiposity, showed modest negative association. Furthermore at cortical sites training age, assessed only in RG, had positive association with both area (r=0.58, p<0.01), BMC (r=0.53, p<0.01) and CRTHK (r=0.48, p<0.05) which remained significant even after adjustment for chronological age or bone age ($r_{area}=0.51$, $r_{BMC}=0.44$, $r_{CRTHK}=0.47$, p<0.05-0.01 and $r_{area}=0.59$, $r_{BMC}=0.56$, $r_{CRTHK}=0.52$, p<0.05-0.01, respectively).

Correlation of bone turnover markers with bone strength indices gives an insight of the underlying mechanism of bone adaptation to applied loads. Thus sCTX, an index of bone resorption, had modest negative association with CRTHK (r=-0.48, P<0.001) and positive with endocortical circumference (r=0.43, p<0.01). On the contrary, P1NP levels did not show any particular association with bone strength indices, apart from a marginal positive association with trabecular CSA (r=0.33, p<0.05).

Discussion

In this study we evaluated the effect of elite RG on vBMD and bone geometry assessed by pQCT at the tibia in premenarcheal athletes. The major findings were: 1) RG was associated with a positive bone adaptation especially at cortical sites, characterized by an increase in the degree of mineralization and area, while vBMD remained unchanged. 2) Geometric parameters such as cortical thickness, periosteal circumference and ultimately bone strength were increased, providing evidence that intensive long-term RG in the growing years might result in bone health benefits. 3) Muscle mass was associated with a positive response of both quantitative and qualitative bone parameters, while training age showed similar association, possibly independent of chronological age.

The effect of weight-bearing exercise on vBMD and bone geometry has been evaluated in a relatively small number of studies, which differ in terms of methodology (e.g. DXA with or without hip structural analysis, QCT, MRI or pQCT), region of evaluation (humerus, radius, femur, tibia or spine), type (cross-sectional using inactive subjects as controls, cross-sectional examining side-to-side differences and prospective) and more importantly study population differing in terms of age, sex, race, pubertal maturation, type and intensity of exercise[29]. Limited data to date suggest that in prepubertal years the shafts of the long bones respond to increased loading by increasing periosteal apposition, while distal sites increase tissue density rather than bone size[29]. However, to our knowledge, no study has examined the effect of long-term elite RG on bone geometry using pQCT at the tibia, along with detailed evaluation of maturational stages, dietary habits and bone turnover.

In the present study we observed the most prominent effects of RG at cortical sites, subject to bending and torsional forces. At the respective 38% of the tibia, both area and BMC were higher by about 30% compared with controls, while cortical vBMD was comparable. This difference was due to the deposition of bone on the periosteal surface, leading to significant increase in bone strength assessed by SSIp. Post-hoc power analysis indicated that our study (n=49) had at least 90% power to detect a 30% between group difference in SSIp, with a significance level of 0.05.

In contrast the data concerning the effect of RG at trabecular sites subject to axial and compressive forces indicate that bone appears to increase BMC rather than its size, leading to a relatively higher tissue density. Indeed, at the distal tibia and at 14%, trabecular BMC was higher by 26.80% and 39.80% respectively, while the corresponding area was higher by 12.70% and 10.90%, leading to a trend for higher trabecular vBMD. These findings are in agreement with Ward et al[9], who reported a trend for higher trabecular vBMD at this site. Taking into account the inherent limitation of pQCT for estimating trabecular architecture due to low resolution, it is possible that more sensitive methods such as magnetic resonance would provide more information concerning the effect of weight-bearing exercise on trabecular bone. Nevertheless, it appears that at distal skeletal sites weight-bearing exercise increases tissue density to more efficiently transmit loads through the joint surface.

The effect of duration and intensity of weight- bearing exercise on the osteogenic response in prepubertal children has been assessed in a number of prospective controlled trials[3]. Given the limitations of the cross-sectional design, the positive association of training age with bone geometry and BMC at cortical sites, independent of chronological or bone age, indicate that elite rhythmic gymnastics is associated with a continuous positive response at least during premenarcheal years. Furthermore, Frost's "mechanostat" theory[30] suggests that bone can become accustomed to constant loading of similar magnitude and no osteogenic response will be elicited until a higher magnitude load is applied. Given that older gymnasts had higher weight, foot contacts per year based on training logs increased with years of training, while type of exercise and thus generated ground reaction forces expressed in times body weight remained the same, the association of training years with the positive bone adaptation is in accordance with Frost's theory.

Several studies have assessed the association of muscle mass, a surrogate of muscle force, with bone strength indices at the tibia and radius[31, 32, 33]. Our data are in almost complete accordance with Macdonald[31] et al, who found strong association of muscle area with bone area and strength at the distal tibia and the midshaft, in a large group of early pubertal and pre-pubertal children. Furthermore Daly[33] et al who examined side-to-side differences in the playing and non-playing arm in female tennis players using MRI found that percent differences in muscle area were positively associated with differences in all bone traits, except in the medullary area. However, as reported by Macdonald et al, the variance in bone traits explained by differences in muscle area was less than 16%, indicating that other factors associated with loading, contribute to the skeletal adaptive response.

To our knowledge this is the first study examining bone strength indices in athletes along with a detailed evaluation of calcium homeostasis and bone turnover markers. Our data are consistent with previous studies in children that obesity is associated with lower 25(OH)D levels[34]. Although all subjects had vitamin D levels higher than 50 nmol/L, this difference was associated with higher iPTH and lower calcium levels in controls, completely abolished after adjustment for weight. Considering the fact that between group vitamin D intake and daytime physical activity were comparable, the only possible explanation might be related to less sunlight exposure due to reduced mobility or clothing habits, reduced production of vitamin D following sun exposure and reduced mobilization of vitamin D from fat depots. Concerning bone markers, our data indicate that long-term elite training in premenarcheal females does not significantly modify BTM. This is in line with studies[7, 35] in this age group, which did not find differences in BTM between female gymnasts and controls. However given the relatively small number of subjects studied, additional work is needed to settle this issue.

The long-term significance of intensive exercise-induced skeletal adaptations for fracture reduction remains uncertain, since several studies indicate a higher rate of aBMD loss following detraining[1, 36]. Nevertheless, structural changes, such as increased cortical thickness might be retained even after detraining. Indeed a recent study[37] found greater BMC, CSA, cortical thickness and SSIp at the tibia in female gymnasts 6 years after retirement. However given that bone response to exercise is site-specific, the results cannot be applied to other skeletal sites, including typical fracture sites (hips, vertebrae, forearms). Furthermore, since waning of the positive effects of exercise on bone might be expected over time, the long term effect on fracture risk is difficult to be assessed, even if the observed bone response could be generalized to typical fracture sites.

There are limitations to this study. First, the cross-sectional design is limiting, and long-term longitudinal studies are needed to assess whether the observed positive effects are maintained or further improved with or without sustained activity. Furthermore, we did not perform power analysis; however post hoc power analysis indicated that our study had at least 80% power to detect adjusted differences between groups concerning important pQCT variables. Moreover, although athletes and controls were comparable according to chronological age, height and bone age, we failed to match our subjects by weight and BMI; thus we adjusted bone strength indices according to weight. Finally, our data concern only elite RG and should not be extrapolated to other sports or to normally active children.

In conclusion our findings indicate that intensive rhythmic gymnastics in premenarcheal girls is associated with positive effects on the skeleton, especially in cortical bone, characterized by increased bone mass and improved geometric properties. Increased duration of exercise is associated with a positive response of bone geometry.

Acknowledgments

We would like to thank George Kiniklis for performing the pQCT, Dr Vasiliki Antoniou M.D. from the Radiology Department, Childrens Pentelis Hospital, Athens, Greece and Dr Manolis Mavromatis M.D., Radiology Department, Aglaia Kyriakou Childrens Hospital, Athens, Greece, for skeletal age estimation from left hand and wrist radiographs, and Dr Spyros Stavropoulos and Dr. Antonios Xydakis M.D. for editorial assistance.

References

1. Seeman E 2002 An exercise in geometry. J Bone Miner Research 17:373-380.
2. Heaney RP, Abrams S, Dawson-Hughes B, Looker A, Marcus R, Matkovic V, Weaver C. 2000 Peak bone mass. Osteoporos Int 11: 985-1009.
3. Hind K, Burrows M 2007 Weight-bearing exercise and bone mineral accrual in children and adolescents: A review of controlled trials. Bone 40:14-27.
4. Nurmi-Lawton JA, Baxter-Jones AD, Mirwald RL, Bishop JA, Taylor P, Cooper C, New SA. 2004 Evidence of sustained skeletal benefits from impact-loading exercise in young females: A 3-year longitudinal study. J Bone Miner Res 19:314-322.
5. Dowthwaite JN, DiStefano JG, Ploutz-Snyder RJ, Kanaley JA, Scerpella TA. 2006 Maturity and activity-related differences in bone mineral density: Tanner I vs. II and gymnasts vs. non-gymnasts. Bone 39: 895-900.
6. Zanker Cl, Gannon L, Cooke CB, Gee KL, Oldroyd B, Truscott JG. 2003 Differences in bone density, body composition, physical activity and diet between child gymnasts and untrained children 7-8 years of age. J Bone Miner Res 18:1043-1050.
7. Nickols-Richardson SM, O'Connor PJ, Shapes SA, Lewis RD. 1999 Longitudinal bone mineral density changes in female child artistic gymnasts. J Bone Miner Res 14:994-1002.
8. Laing EM, Wilson AR, Modlesky CM, O'Connor PJ, Hall DB, Lewis RD. 2005 Initial years of recreational gymnastic training improves lumbar spine bone mineral accrual in 4-to 8-year old females. J Bone Miner Res 20:509-519.
9. Ward KA, Roberts SA, Adams JE, Mughal MZ. 2005 Bone geometry and density in the skeleton of pre-pubertal gymnasts and school children. Bone 36: 1012-1018.
10. Faulkner RA, Forwood MR, Beck TJ, Mafukidze JC, Russell K, Wallace W. 2003 Strength indices of the proximal femur and shaft in prepubertal female gymnasts. Med Sci Sports Exerc 35: 513-518.

11. Courteix D, Rieth N, Thomas T, van Praagh E, Benhamou CL, Collomp K, Lespessailles E, Jaffre C. 2007 Preserved bone health in adolescent elite rhythmic gymnasts despite hypoleptinemia. Horm Res 68:20-27.
12. Munoz TM, de la Piedra C, Barrios V, Garrido V, Argente J.2004 Changes in bone density and bone markers in rhythmic gymnasts and ballet dancers: implications for puberty and leptin levels. Eur J Endocrinol 151:491-496.
13. Georgopoulos N, Markou K, Theodoropoulou A, Denardot D, Leglise M, Vagenakis AG. 2002 Growth Retardation in Artistic Compared with Rhythmic Elite Female Gymnasts. J Clin Endocrinol Metab 87: 3169–3173.
14. Markou KB, Mylonas P, Theodoropoulou A, Kontogiannis A, Leglise M, Vagenakis AG, Georgopoulos NA. 2004 The influence of intensive physical exercise on bone acquisition in adolescent elite female and male artistic gymnasts. J Clin Endocrinol Metab 89:4383–4387.
15. Vicente-Rodriguez G, Dorado C, Ara I, Perez-Gomez J, Olmedillas H, Delgado-Guerra S, Calbet JAL. 2007 Artistic versus rhythmic gymnastics: Effects on bone and muscle mass in young girls. Int J Sports Med 28:386-393.
16. Kalkwarf HJ, Zemel BS, Gilsanz V, Lappe JM, Horlick M, Oberfield S, Mahboubi S, Fan B, Frederick MM, Winer K, Shepherd JA 2007 The Bone Mineral Density in Childhood Study: Bone Mineral Content and Density According to Age, Sex, and Race. J Clin Endocrinol Metab 92: 2087-2099.
17. Seeman E 2002 Pathogenesis of bone fragility in women and men. Lancet 359: 1841–50
18. Rico H, Gonzales –Riola J, Revilla M, Villa LF, Gomez-Casresana F, Escribano J. 1994 Cortical versus trabecular bone mass: Influence of activity on both bone components. Calcif Tissue Int 54:470-72.
19. Kontulainen SA, Hughes JM, Macdonald HM, Johnston JD 2007 The Biomechanical Basis of Bone Strength Development during Growth Med Sport Sci. 51: 13–32.

20. Macdonald HM, Kontulainen S, Petit M, Janssen P, McKay H 2006 Bone strength and its determinants in pre- and early pubertal boys and girls. Bone 39: 598–608.
21. Greulich W, Pyle S. Radiographic atlas of skeletal development of the hand and wrist. Stanford, CA: Stanford Univ. Press 1959.
22. Tanner JM. Foetus into man. Cambridge: Harvard Press; 1978.
23. Argiropoulou E.C., Michalopoulou M., Aggeloussis N., Avgerinos A. 2004. Validity and reliability of physical activity measures in Greek high school age children. Journal of Sports Science and Medicine 3: 147-159
24. Fatouros IG, Tournis S., Leontsini D, Jamurtas AZ, Sxina M, Thomakos P, Manousaki M, Douroudos I, Taxildaris K, Mitrakou A. 2005 Leptin and Adiponectin Responses in Overweight Inactive Elderly Following Resistance Training and Detraining are Intensity-Related. J Clin Endocrinol Metab 90: 5970-5977.
25. Charopoulos I, Tournis S, Trovas G, Raptou P, Kaldrymides P, Skarandavos G, Katsalira K, Lyritis GP. 2006 Effect of Primary Hyperparathyroidism on Volumetric Bone Mineral Density and Bone Geometry Assessed by Peripheral Quantitative Computed Tomography in Postmenopausal Women. J Clin Endocrinol Metab 91:1748-1753.
26. Shaw N. 2009 A practical approach to hypocalcemia in children. Endocr Dev. 16:73-92.
27. Nikander R, Sievänen H, Uusi-Rasi K, Heinonen A, Kannus P. 2006 Loading modalities and bone structures at nonweight-bearing upper extremity and weight-bearing lower extremity: A pQCT study of adult female athletes. Bone 39:886-894.
28. Nurmi-Lawton JA, Baxter-Jones AD, Mirwald RL, Bishop JA, Taylor P, Cyrus Cooper C, New SA 2004 Evidence of Sustained Skeletal Benefits From Impact-Loading Exercise in Young Females: A 3-Year Longitudinal Study. J Bone Miner Resear 19:314–322
29. Daly RM. 2007 The effect of exercise on bone mass and structural geometry during growth. Med Sport Sci 51:33-49.

30. Frost HM. 1990 Structural adaptation to mechanical usage (SATMU).1. Redefining Wolff's law: the bone modelling problem. Anat Rec 226: 403-13.
31. Macdonald H, Kontulainen S, Petit M, Janssen P, McKay H. 2006 Bone strength and its determinants in pre- and early pubertal boys and girls. Bone 39: 598-608.
32. Heinonen A, McKay HA, Whittall KP, Fortser BB, Khan KM. 2001 Muscle cross-sectional area is associated with specific site of bone in prepubertal girls: A quantitative magnetic resonance imaging study. Bone 29: 388-392.
33. Daly RM, Saxon L, Turner CH, Robling AG, Bass SL. 2004 The relationship between muscle size and bone geometry during growth and in response to exercise. Bone 34: 281-287.
34. Reinehr T, de Sousa G, Alexy U, Kersting M, Andler W. 2007 Vitamin D status and parathyroid hormone in obese children before and after weight loss. Eur J Endocrinol 157:225-232.
35. Lehtonen-Veromaa M, Mottonen T, Irjala K, Nuotio I, Leino A, Viikari J. 2000 A 1-Year Prospective Study on the Relationship between Physical Activity, Markers of Bone Metabolism, and Bone Acquisition in Peripubertal Girls. J Clin Endocrinol Metab 85:3726-3732.
36. Karlsson MK. 2007 Does exercise during growth prevent fractures in later life? Med Sport Sci. 51: 121-136.
37. Eser P, Hill B, Ducher G, Bass S. 2009 Skeletal benefits after long-term retirement in former elite female gymnasts. J Bone Miner Resear 24:1981-1988.

Table 1: Physical and dietary characteristics of the two groups.

	RG (n=26)	Controls (n=23)	P
Age (years)	11.26 ± 0.17	10.87 ± 0.13	0.096
Weight (Kg)	31.07 ± 0.69	40.34 ± 1.71	<0.001
Height (cm)	142.75 ± 1.60	145.76 ± 1.10	0.13
$\text{Height}_{\text{SDS}}$	-0.14 (2.2)	0.30 (2.49)	0.085
Sitting height (cm)	75.80 ± 0.60	76.81 ± 0.80	0.33
BMI (Kg/m^2)	15.20 ± 0.18	18.85 ± 0.58	<0.001
BMI_{SDS}	-1.10 (2.44)	0.23 (3.02)	<0.001
Skinfold sum (mm)	42.63 (32.55)	81.00 (183.00)	<0.001
Bone age (years)	10.61 ± 0.25	11.24 ± 0.23	0.079
Tanner stage[a]	I:10, II:13, III:3	I:7, II:12 III:4	0.767
Tibial length (cm)	32.84 ± 0.34	33.65 ± 0.56	0.22
Training age (years)	4.34 ± 0.25	NA	
Dietary calcium intake (mg/day)	1004.16 ± 62.97	916.83 ± 60.72	0.32
Dietary vitamin D intake (IU/day)	147.48 ± 7.92	146.17 ± 12.14	0.92
Protein Intake (mg/day)	60.26 ± 3.16	64.77 ± 3.52	0.34
Energy Intake (KJ/day)	5985.40 ± 377.99	6943.75 ± 439.15	0.103
Physical activity (METs/day)	46.8 ± 1.36	34.4 ± 1.20	0.002

Data are presented as means ± SE or median (range) as appropriate. SDS: Standard Deviation Score, a: Tanner stage of breast development. Training age: number of years each participant participated in RG training and competition. NA: not applicable

Table 2: Biochemical characteristics of the study group

	RG	**Control**	**P**
Calcium (mg/dl) NR: 8.2-10.2	9.85 ± 0.08	9.56 ± 0.09	0.025
Phosphate (mg/dl) NR: 3.6-5.8	5.41 ± 0.11	5.19 ± 0.10	0.17
iPTH (pg/ml) NR:15-65	36.01 ± 1.85	45.34 ± 3.14	0.012
25 (OH) D (nmol/L) NR: 37.5-190	102.22 ± 4.2	87.46 ± 3.74	0.013
P1NP (µg/L)	684.62 ± 49.49	785.88 ± 43.1	0.133
sCTX (ng/ml)	1.65 ± 0.07	1.77 ± 0.08	0.320

Data are presented as means ± SE, NR: Normal range

Table 3: vBMD, BMC, and bone geometric characteristics assessed by pQCT

	RG	Control	p
10mm proximal to the distal surface of the distal metaphysis			
Total BMC (mg)	251.10 ± 10.43	210.96 ± 11.24	0.022
Total vBMD (mg/cm^3)	301.45 ± 6.58	283.75 ± 7.09	0.105
Total CSA (mm^2)	836.53 ± 31.18	741.97 ± 33.61	0.069
Trabecular BMC (mg)	92.92 ± 6.21	73.24 ± 6.70	0.058
Trabecular vBMD (mg/cm^3)	243.61 ± 9.7	216.05 ± 10.46	0.088
Trabecular CSA (mm^2)	376.31 ± 14.03	333.75 ± 15.13	0.069
38% site			
Total BMC (mg)	272.73 ± 5.47	218.19 ± 5.90	<0.001
Total vBMD (mg/cm^3)	789.76 ± 12.47	732.55 ± 13.45	0.007
Total CSA (mm^2)	346.87 ± 8.04	300.34 ± 8.67	0.001
Cortical BMC (mg)	243.82 ± 5.45	187.17 ± 5.88	<0.001
Cortical vBMD (mg/cm^3)	1043.70 ± 8.28	1042.31 ± 8.93	0.919
Cortical CSA (mm^2)	233.85 ± 5.28	179.78 ± 5.70	<0.001
CRTHK (mm)	4.53 ± 0.09	3.60 ± 0.10	<0.001
PERI (mm)	65.96 ± 0.80	61.22 ± 0.86	0.001
ENDO (mm)	37.48 ± 0.91	38.59 ± 0.98	0.461
SSIp (mm^3)	1129.74 ± 38.01	858.92 ± 40.98	<0.001
14% site			
Total BMC (mg)	187.77 ± 3.58	162.27 ± 3.86	<0.001
Total vBMD (mg/cm^3)	471.99 ± 10.71	449.81 ± 11.55	0.210
Total CSA (mm^2)	402.70 ± 11.01	362.94 ± 11.87	0.032
Trabecular BMC (mg)	29.30 ± 2.13	20.96 ± 2.30	0.021
Trabecular vBMD (mg/cm^3)	164.88 ± 12.75	129.12 ± 13.75	0.092

Trabecular CSA (mm^2)	181.07 ± 4.95	163.17 ± 5.34	0.032
Cortical BMC (mg)	123.64 ± 2.98	104.79 ± 3.21	<0.001
Cortical vBMD (mg/cm^3)	984.79 ± 7.69	971.57 ± 8.29	0.296
Cortical CSA (mm^2)	125.63 ± 2.77	107.71 ± 2.99	<0.001
SSIp (mm^3)	964.57 ± 31.70	810.68 ± 34.18	0.005
66% site			
Muscle area (mm^2)	4551.06 ± 85.76	4094.89 ± 92.46	0.002

Data are presented as means ± SE. All data are adjusted for differences in weight. CRTHK: Cortical thickness; PERI: periosteal circumference; ENDO: endocortical circumference.