Title

Bone geometry in young male and female football players; a peripheral quantitative computed tomography (pQCT) study.

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Abstract

Purpose The aim of this study was to compare bone geometry in adolescent football players and controls.
Methods A total of 107 football players (71 males/36 females; mean age 12.7±0.6/12.7±0.6 y) and 42 controls (20 males/22 females; mean age 13.1±1.4/12.7±1.3 y) participated in this study. Total and trabecular volumetric bone mineral content (Tt.BMC/Tb.BMC), cross sectional area (Tt.Ar/Tb.Ar) and bone strength index (BSI) were measured at 4% site of the non-dominant tibia by peripheral quantitative computed tomography (pQCT). Moreover, Tt.BMC, cortical BMC (Ct.BMC), Tt.Ar, cortical Ar (Ct.Ar), cortical thickness (Ct.Th), periosteal circumference (PC), endosteal circumference (EC), fracture load in X-axis and polar strength strain index (SSIp) were measured at 38% site of the tibia. Multivariate analyses of covariance were used to compare bone pQCT variables between football players and controls using the tibia length and maturity offset as covariates.
Results Female football players demonstrated 13.8-16.4% higher BSI, Ct.Th, fracture load in X-axis and SSIp than controls ($p<.0036$). Males showed no significant differences in bone strength when compared to controls ($p>.0036$). In relation to bone mineral content and area, male football players showed 8.8% higher Tt.Ar and Tb.Ar at the 4% site of the tibia when compared to controls; whereas 13.8-15.8% higher Tt.BMC, Ct.BMC and Ct.Ar at the 38% site of the tibia were found in female football players than controls ($p<.0036$).

Conclusions In this study, female adolescent football players presented better bone geometry and strength values than controls. In contrast, only bone geometry was higher in male football players than controls.

Keywords Soccer, Body composition, Bone health, Youth

Conflict of interest

Gabriel Lozano-Berges, Ángel Matute-Llorente, Alejandro Gómez-Bruton, Alejandro González-Agüero, Germán Vicente-Rodríguez, and José A. Casajús declare that they have no conflict of interest.

Mini abstract

The present study shows that football practice during growth may improve bone geometry in male and female football players. However, only females had better bone strength in comparison with controls.

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Introduction

Several studies have described the importance of environmental and genetic factors in the determination of bone mass. Genetic or hereditary factors are the major contributors (up to 80%) to the variability in peak bone mass but they are non-modifiable [1]. Environmental factors play an important role because bones adapt to the specific mechanical load [2]. Exercising is an effective strategy to attain optimal bone mass and strength during growth [3], such is it so several studies have shown that high-impact sports such as football, volleyball or racquet games have positive effects on bone mass [4-7]. Regular football training causes site-specific skeletal responses mainly because of the type of specific actions executed while playing and the biomechanical properties of the surface in which football players practice their sport [8, 9].

The majority of studies performed with children and adolescent football players evaluated bone mineral content and density via dual X-ray absorptiometry (DXA) finding positive effects on those parameters, in different moments of maturation [9-11], being more marked in pubertal than prepubertal stages [12]. Nevertheless, BMD can explain up to 60% of the variance in bone strength, but due to its intrinsic two-dimensional character, DXA cannot determine whether bone changes are due to differences in volumetric bone mineral content (BMC) or in bone geometrical parameters [13]. In addition, it is also known that physical exercise performed during growth mainly improves bone geometry rather than bone mass [14]. Further studies have measured bone geometry with peripheral quantitative computed tomography (pQCT) [15, 16] or hip structural analysis (HSA) [8, 17, 18] in young male and female football players. When compared to swimmers, cyclists and controls, higher cross sectional area, moment of inertia and stiffness index were found in male football players [17]. Also, female football players demonstrated higher strength and structure values when compared to swimmers [8, 18].

Vlachopoulos et al. [17] and Ferry et al. [8, 18] used HSA for comparing bone geometry between football players and controls; nevertheless, this technique has limitations. HSA is a calculation derived from hip scans performed by DXA and, consequently, final geometric results could be altered by the two-dimensional image obtained from DXA which is highly influenced by femur rotation, as demonstrated by Beck [19]. Furthermore, the hip is not the preferred skeletal site to measure bone mass in young populations because of the high variability of bone development during growth [20]. The use of pQCT can, at least partially, mitigate these limitations. It is a three-dimensional technique to assess bone geometry variables without the influence of bone size. Until now, only Anliker et al. [15] and Varley et al. [16] have used pQCT for measuring bone geometry within male adolescent football players; however, neither performed sex-specific bone geometry comparison between football players and controls. While no previous study has used pQCT to compare bone outcomes between adolescent football players and controls, several studies have used pQCT to compare bone outcomes between young adult football players and controls [21-23]. These authors showed that football players had better bone geometry (i.e. cortical area (Ct.Ar), periosteal circumference (PC), volumetric bone mineral density) than controls in both genders

Therefore, the main aim of this study was to examine and compare bone mass variables -at the 4% and 38% sites of the tibia length-, and geometric variables -at the 38% site of the tibia length- between adolescent football players and controls separated by gender. We hypothesized that football players will exhibit higher bone variables than controls in both genders due to the fact that loads produced by specific football actions will provoke an extra skeletal response.

Methods

Participants

Eight football clubs (all of them competed at provincial level for their age category) and two high schools of Aragon (Spain) were invited to participate in the present study. All football players agreed to participate in this study (100% of players); however, in the control group, only 45 of 114 students voluntarily decided to collaborate. An initial sample of 121 football players (81 males and 40 females) and 45 controls (23 males and 22 females) agreed to participate in the study. Nonetheless, 14 football players and 3 controls were not included because the following reasons: 11 football players did not assist to the measurement citation; and data of 3 football players and 3 controls had blurred pQCT images; Fig. 1). Consequently, the final sample for the present study consisted of 107 football players (71 males and 36 females; mean age 12.7±0.6 and 12.7±0.6 y respectively) and 42 controls (20 males and 22 females; mean age 13.1±1.4 and 12.7±1.3 y respectively). Twenty female football players (mean age of 12.9±0.6 y) and 9 female controls (mean age of 13.8±0.2 y) experienced menarche (at the mean age of 11.4±1.2 and 11.9±0.8 y respectively; Online Resource 1) before the beginning of the study. Moreover, no proportion differences between football players and controls in pre- and post-menarcheal groups was found ($\chi^2(1) = 1.172$, $p = .279$). Although controls were physically active, they were not engaged in any regular sport. Measurements took place between November and December 2013 in Zaragoza, Spain.

Despite not performing the same football exercises, trainings of all teams included in the present study (both males and females) lasted approximately 90 min, including 5-min warm-up consisting of low-intensity running; 5-

10 min of low-intensity games; 60 min of technical football exercises (e.g. passing, kicking, running, dribbling); and finally, 5-10 minutes of cold down performing stretching exercises.

The protocol of the study, its benefits and risks, were explained to the participants, parents and the club managers. Participants completed the written assent and their parents completed the written informed consent. This study followed the declaration of Helsinki 1961 (revision of Fortaleza 2013) and was approved by the Ethics Committee of Clinical Research from the Government of Aragon (CEICA, Spain) prior the commencement of it [C.I. PI13/0091]. This cross-sectional study is part of a larger randomized controlled trial that evaluated the effect of football surfaces and boot model on bone during growth. Football players and controls were measured three times during two football seasons. The first measurement was performed at the beginning of the first season (November-December 2013). The second measurement was performed at the end of the first season (May-July 2014) to evaluate the effect of football surfaces and boot model on bone. Finally, the third measurement took place at the end of the second season (May-July 2015) to assess the perdurability of the previously mentioned effects. Furthermore, the research project was registered in a public database Clinicaltrials.gov [NCT02399553]. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement was used as a guideline for reporting observational data [24].

Inclusion criteria

Participants must be Caucasian, with at least one year of football practice (football players) or should not be engaged in any regular sport (control group), age between 11 and 14 years old, and free of medication that could affect bone mass or development.

Anthropometric measurements

Height (stadiometer SECA 225, SECA, Hamburg, Germany;) was measured without shoes and the minimum clothes to the nearest 0.1 cm and weight to the nearest 0.1 kg (SECA 861, SECA, Hamburg, Germany). Body mass index (BMI) was calculated as weight (kilograms) divided by height (square meters).

Maturity offset

Age and height were used to estimate maturity offset in males and females using the following sex specific equations [25]:

Males: Maturity offset = -7.999994 + (0.0036124 x (age x height)

Females: Maturity offset = -7.709133 + (0.0042232 x (age x height)

Moreover, the age of peak height velocity was calculated as the subtraction of the age from maturity offset.

Calcium intake

A validated calcium food frequency questionnaire was used to calculate milligrams of daily calcium intake [26, 27].

Bone assessment by pQCT

Bone strength indexes, bone morphometry, BMC and bone area were measured at the non-dominant tibia using a Stratec XCT-2000 L pQCT scanner (Stratec Medizintechnik, Pforzheim, Germany). The device is a translate-rotate, small bore computed tomography scanner that acquires a trans-axial image. The pQCT was calibrated daily based on a quality control phantom provided by the manufacturer (Stratec Medizintechnik, Pforzheim, Germany). The coefficients of variation of the pQCT in our laboratory for each variable have been already published [28].

Dominance was determined by asking which leg would be used to kick a ball [29]. Although there is no consensus about the measurement of dominant or non-dominant leg in pQCT studies [30]; Anliker et al. [15] reported higher bone strength values in non-dominant than dominant leg in young male football players. Thus, based on their findings and protocol study, non-dominant leg was selected in the present study. Participants were seated on a chair adjustable to the body proportions of each participant. Tibia length was measured from the medial knee joint cleft to the medial malleolus of the tibia using a wooden ruler and was always measured by the same researcher. The scanner was positioned on the distal tibia, and a scout view was performed to manually set the reference line on the midpoint of the distal tibia endplate. Then, the measurements were performed at 4% and 38% sites of the tibia length to assess trabecular and cortical bone. Following the International Society for Clinical Densitometry (ISCD) recommendations [30], the measured variables at the 4% site of the tibia were total BMC (Tt.BMC, mg/mm), trabecular BMC (Tb.BMC, mg/mm), total area (Tt.Ar, mm^2), trabecular area (Tb.Ar, mm^2) and bone strength index (BSI was calculated as Tt.Ar multiplied by squared total density; mg/mm). Moreover, the parameters examined at the 38% site of the tibia were total BMC (Tt.BMC, mg/mm), cortical BMC (Ct.BMC, mg/mm), total area (Tt.Ar, mm^2), Ct.Ar (mm^2), cortical thickness (Ct.Th, mm), PC (mm), endosteal circumference (EC, mm), fracture load in X-axis (N) and polar strength strain index (SSIp, mm^3). Muscle and fat cross sectional areas (mm^2) were measured at the 66% site of the length of the tibia.

Images were analysed with version 6.20 of the manufacturer's software. Contour mode 1 with a threshold of 180 mg/cm^3 for the 4% site of the tibia and 280 mg/cm^3 for the 38% site of the tibia was used to determine the periosteal surface of the bone. At 4% site of the tibia, trabecular bone was determined from a central area covering 45% of the total bone cross sectional area. At 38% site of the tibia, cortical bone was obtained using cortical mode 1 with a threshold of 710 mg/cm^3. Additionally, cortical mode 1 with a threshold of 280 mg/cm^3 was used to obtain bone strength variables (SSIp and fracture load in X-axis). After that, bone mineralization of 1200 mg/cm^3 was assumed.

Statistical analyses

As no previous studies had measured bone geometry and strength by pQCT in young football players and controls, HSA data from the Vlachopoulos et al. [17] and Ferry et al. [8, 18] studies evaluating cross sectional area at the femoral shaft section in football players and controls (males: 140.9±20.4 vs 109.8±21.0 mm^2; females: 4.66±0.54 vs 3.97±0.27 cm^2 respectively) were used to calculate sample size.

The sample size for MANCOVA analysis was calculated for the cross sectional area at the femoral shaft to get a power of 95% at the 5% alpha power and to observe differences in comparison to a null hypothesis H_0:$\mu1=\mu2$. In males, assuming that the means of football players and controls are 140.9 and 109.8 mm^2 respectively and the standard deviation (SD) of both groups is 20.7 mm^2, at least 32 participants (a minimum of 16 participants per group) would be needed. In females, assuming that the means of football players and controls are 4.66 and 3.97 cm^2 respectively and the SD of both groups is 0.40 cm^2, at least 24 participants (a minimum of 12 participants per group) would be needed.

The Statistical Package for the Social Sciences (SPSS) version 22.0 for Mac OS X (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. Continuous data were presented as mean ± SD. All variables showed normal distribution by the Kolmogorov-Smirnov test.

Two-way analysis of variance (ANOVA) was used to test for an interaction of football practice and gender on participant characteristics. A multivariate analysis of covariance (MANCOVA) was performed to analyze differences at bone pQCT variables within football players and controls, using the length of the tibia and maturity offset as covariates (Model 1). After that, these analyses were repeated adding other two covariates as follows: Model 1 + weight (Model 2); and Model 1 + muscle area (Model 3). Bonferroni corrections were applied to control the overall Type I error rate of multiple comparison, and therefore, the *p* value of .05 was divided by 14 (the number of comparisons conducted). Effect sizes calculated by SPSS were reported as omega squared (ω^2) for ANOVAs and partial eta squared (η^2_p) for MANCOVAs. The effect size for ω^2 and η^2_p can be small (0.01 – 0.06), medium (0.06 – 0.14) or large (>0.14).

Results

Table 1 presents descriptive characteristics of the football players and controls by sex. There were significant interaction effects between the practice of football and gender on weight, BMI and muscle cross sectional area, ($p < .05$, ω^2 ranged from 0.03 to 0.04). Muscle cross sectional area was higher in female football players than the control group (mean difference was 14.3%; $p < .05$; Cohen's *d* 0.73). Male football players demonstrated lower fat area than controls (mean difference was -18.3%; $p < .05$; Cohen's *d* 0.56).

There was a significant effect of football practice (in both males and females separately) on bone geometry and strength, Wilk´s $\Lambda = 0.71$, $F(13/75) = 2.39$, $p = .010$, $\eta^2_p = 0.29$ (males); and Wilk´s $\Lambda = 0.52$, $F(13/42) = 2.97$, $p = .004$, $\eta^2_p = 0.48$ (females).

Data of BMC and bone area at the 4% and the 38% sites of the length of the tibia are shown in Table 2. Male football players demonstrated higher Tt.Ar and Tb.Ar at the 4% site of the tibia in comparison to male controls (both mean differences were 8.8%; both $p = .001$; both η^2_p 0.11; Table 2). Female football players showed higher Tt.BMC at the distal tibia and also Tt.BMC, Ct.BMC and Ct.Ar at diaphyseal tibia than controls (mean differences ranged from 14.9 to 15.8%; $p < .001$; η^2_p ranged from 0.23 to 0.28; Table 2).

Geometric variables measured at the 38% diaphyseal tibia and strength indexes at the 4% and the 38% sites of the tibia are also summarized in Table 2. Only female football players exhibited higher BSI, Ct.Th, fracture load in X-axis and SSIp than controls (mean differences ranged from 13.8 to 26.8%; $p < .001$; η^2_p ranged from 0.18 to 0.26; Table 2). Similar results were obtained when weight (Model 2) or muscle area (Model 3) were added as covariates (Online Resource 2).

Discussion

The main finding of the present study was that female adolescent football players showed better bone geometry and higher bone strength indexes than controls. When comparing male groups, football players exhibited better bone geometry at 4% site of the tibia than controls; nevertheless, no bone strength differences were found between these groups.

The lack of differences between male groups could be explained by the fact that cortical bone parameters and bone strength values (all of these variables measured at 38% site of the tibia) abruptly increase after 14 years old in males [31] and participants included in this study were younger. Moreover, trabecular bone is more sensitive and has more remodeling activity than cortical bone due to trabecular bone having a higher surface-to-volume ratio in comparison with cortical one [32]. Thus, bone increments caused by football practice before maturation may be more marked on trabecular than on cortical bone.

Previous studies have reported higher bone mineral content and bone mineral density at most weight-bearing sites in young male and female football players than controls [6, 11, 33], these differences being more marked in pubertal than prepubertal players. The previously commented studies used DXA for evaluating bone mass; which is known to explain 60% of the variance of bone strength [13]; bone geometry (via pQCT) explains the remaining percentage. Physical exercise during growth improves more bone geometry than bone mass parameters [14]. The present study found better bone geometry (Tt.BMC, Ct.BMC and Ct.Ar) and higher bone strength (except PC and EC) in female football players compared to controls. In males, football players demonstrated better bone geometry at 4% site of the tibia (Tt.Ar and Tb.Ar) compared to controls. The effects of football actions and their inherent loads cause microdamages in bone and an increase of bone remodeling activity [34]. Due to this bone adaptation, football players could attain wider and stronger bones during adolescence, and more importantly, they could reduce future bone diseases in adulthood. Thus, football practice could be a good choice to improve bone health in those children and adolescent who have weak bones.

It has been demonstrated that bones adapt to the loads modifying their shape, size, architecture and mass [35]. To the best of our knowledge, this is the first study to evaluate tibia with pQCT in female adolescent football players and controls. A cross-sectional study by Ferry et al. [18] assessed bone mass and geometry measured by DXA and HSA in late adolescent female football players and swimmers. These authors reported better bone geometry values in football players than swimmers. Another longitudinal study [8] with the same participants, reported improvements on cross sectional area and subperiosteal width after 8 months of football training in female players. According to the present study, female football players demonstrated higher BSI, Ct.Th, fracture load in X-axis, and SSIp than controls. These results could be justified as periosteal expansion is the main response of the bones to exercise loading during prepubertal stage [36, 37], increasing, at the same time, cortical thickness and the resistance of the tibia to bending and torsional forces [38].

Although no differences in pQCT variables at the 38% site of the tibia were found in male football players, they exhibited higher BMC and cross sectional area at the 4% site of the length of the tibia. Up to now, only Vlachopoulos et al. [17] compared bone mass and geometry measured by DXA and HSA between male football players, swimmers, cyclist and controls. They reported better bone geometry and higher stiffness index and BMD in football players than the other groups. These bone geometry differences between studies could be explained by different techniques used (pQCT vs. HSA) and the different bone site measured (tibia vs. proximal femur). Moreover, HSA could be more imprecise in measuring geometric variables because it uses a two-dimensional image obtained from DXA, and the rotation of femur may fundamentally affect bone geometry [19]. Thus, future longitudinal studies using pQCT are in need to clarify if football practice causes an adaptation in bone geometry and strength also in males.

As it is known that peak bone mineral accretion rate occurs approximately 2 years earlier in girls (12.5 years old) than boys (14.1 years old) [39]. Male players in this study were 12.7±0.6 years old, and females were 12.7±0.6 years old; therefore, it is most likely that the peak bone accretion rate was reached by a higher percentage of females than males. Almost half of females included in this study had experienced menarche, suggesting a higher biological development than their male counterparts. Therefore due to such reasons only female football players showed higher geometric variables and strength indexes at 38% site of the tibia than controls, and not males. On the other hand, taking into account the effects of high-impact sports on bone geometry during growth, the principal response during prepubertal years in males and females is periosteal apposition. Nevertheless, during pubertal years is periosteal apposition in males and is endocortical apposition in females [37]. Following this statement, either male or female football players should have better bone geometry and higher bone strength than controls; nevertheless, males only demonstrated higher but not significant bone values. As explained above, male football players in the present study were all under 14 years old, which is determined as the point of higher increase of cortical bone [31].

The main limitation of this study is that due to the cross-sectional design, causal conclusions cannot be attained. Bailey et al. [39] demonstrated that age of peak bone mineral accretion was different between genders (14.1 years old in males and 12.5 years old in females). Thus, males and females of this study who had similar chronological ages (12.7 and 12.7 years old respectively) might have presented different bone maturation age. On the other hand, the main strength is that this is the first study comparing bone geometry between young football players and controls with pQCT. Moreover, the analyses have been divided by genders in order to clarify if differences in bone parameters in males and females were separately present. Another strength was the sample size of 107 football players (71 males and 36 females) and 42 controls (20 males and 22 females). A large sample size compared to certain studies that evaluated bone geometry during growth (37 or 32 football players vs. 14 or 15 controls [8, 17])

Conclusions

Overall, football practice during growth could potentially be a useful strategy for improving bone geometry and strength in females and, consequently, for reducing future osteoporotic problems during adulthood and elderly life. On the other hand, despite male football players showed higher bone geometry values in comparison with controls, there were no bone strength differences between them. Therefore, male football players should continue practicing this sport to get improvements in bone geometry as females did.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent

Informed consent was obtained from all individual participants included in the study.

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Fig. 1 Flow diagram of football players and controls who participated in this study.

Table 1 Descriptive values of football players and controls.

	Males			Females			Interaction gender*group	
	Football players (n = 71)	Controls (n = 20)	*d*	Football players (n = 36)	Controls (n = 22)	*d*	*p*	ω^2
Age (y)	12.7 ± 0.6	13.1 ± 1.4	0.39	12.7 ± 0.6	12.7 ± 1.3	0.05	0.150	0.007
Weight (kg)	45.4 ± 10.1	49.9 ± 10.8	0.42	49.3 ± 8.2	44.9 ± 11.0	045	0.017‡	0.038
Height (cm)	154.5 ± 8.8	156.7 ± 10.9	0.22	155.4 ± 7.0	153.0 ± 9.1	0.29	0.164	0.006
BMI (kg·m^{-2})	18.9 ± 2.9	20.1 ± 2.8	0.44	20.4 ± 2.6	19.0 ± 3.2	0.48	0.013‡	0.035
Tibia length (mm)	350 ± 24	357 ± 29	0.25	347 ± 21	345 ± 23	0.12	0.300	0.001
Muscle CSA (mm^2)	5300 ± 1037	5575 ± 1106	0.26	5449 ± 922*	4767 ± 952	0.73	0.011‡	0.036
Fat CSA (mm^2)	1984 ± 785*	2430 ± 803	0.56	2373 ± 689	2380 ± 765	0.01	0.123	0.010
Daily calcium intake (mg)	862.4 ± 401.1	785.5 ± 288.7	0.22	765.7 ± 486.4	759.4 ± 294.3	0.02	0.633	0.005
Maturity offset (y)	-0.9 ± 0.6	-0.5 ± 1.3	0.40	0.6 ± 0.7	0.5 ± 1.2	0.10	0.107	0.011
Age PHV (y)	13.6 ± 0.4	13.7 ± 0.4	0.25	12.1 ± 0.3	12.2 ± 0.4	0.28	0.793	0.006
Training years (y)	5 ± 2	-	-	3 ± 3	-	-	-	-
Training hours (h/week)	3.2 ± 1.3	-	-	2.9 ± 0.6	-	-	-	-

Values are mean ± SD.

BMI: body mass index; CSA: cross sectional area; PHV: peak height velocity.

* significant differences between football players and controls; ‡: significant interaction. Cohen's *d* can be small (0.2 – 0.5), medium (0.5 – 0.8) or large (>0.8); ω^2_p can be small (0.01 – 0.06). medium (0.06 – 0.14) or large (>0.14).

Table 2 Adjusted pQCT values of football players and controls.

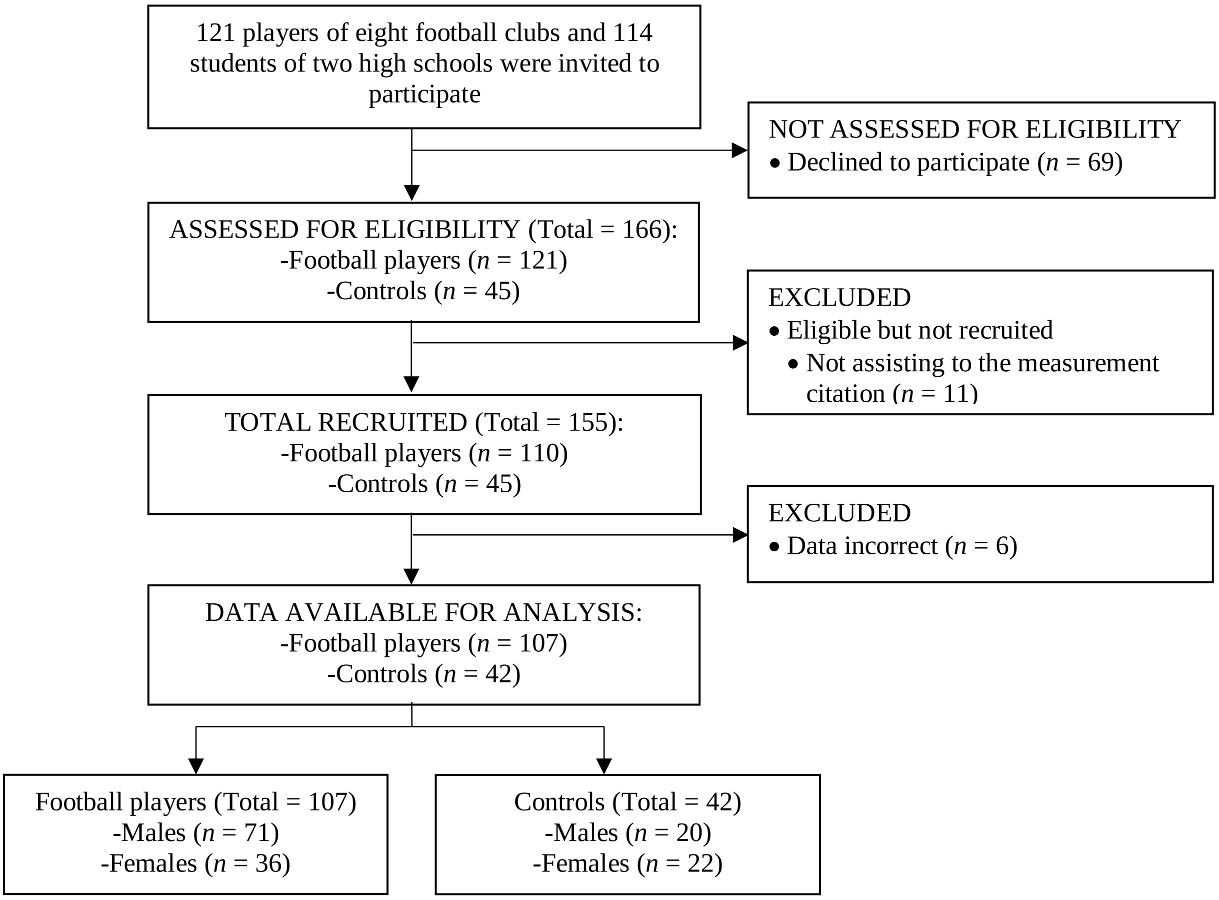
	Males				Females			
	Football players (n = 71)	Controls (n = 20)	MD (95% CI)	Test statistic	Football players (n = 36)	Controls (n = 22)	MD (95% CI)	Test statistic
4% site								
Tt.BMC (g)	3.88 ± 0.62	3.53 ± 0.62	0.35 (0.04, 0.66)	$F(1,87) = 4.98, p = .028, \eta^2_p = 0.05$	3.11 ± 0.38	2.69 ± 0.38	0.42 (0.21, 0.62)*	$F(1,54) = 16.30, p < .001, \eta^2_p = 0.23$
Tb.BMC (g)	1.62 ± 0.31	1.41 ± 0.32	0.21 (0.04, 0.37)	$F(1,87) = 6.43, p = .013, \eta^2_p = 0.07$	1.19 ± 0.19	1.05 ± 0.19	0.15 (0.04, 0.25)	$F(1,54) = 7.75, p = .007, \eta^2_p = 0.13$
Tt.Ar (mm^2)	1192 ± 114	1095 ± 116	97 (39, 155)*	$F(1,87) = 10.87, p = .001, \eta^2_p =0.11$	1001 ± 90	956 ± 90	45 (-4, 94)	$F(1,54) = 3.42, p = .070, \eta^2_p = 0.06$
Tb.Ar (mm^2)	536 ± 51	493 ± 52	44 (17, 70)*	$F(1,87) = 10.85, p = .001, \eta^2_p = 0.11$	450 ± 40	430 ± 40	20 (-2, 42)	$F(1,54) = 3.42, p = .070, \eta^2_p = 0.06$
BSI (mg/mm)	127.9 ± 32.0	116.1 ± 32.3	11.8 (-4.6, 28.2)	$F(1,87) = 2.06, p = .155, \eta^2_p = 0.02$	97.4 ± 19.9	76.8 ± 19.9	20.6 (9.7, 31.4)*	$F(1,54) = 14.50, p < .001, \eta^2_p = 0.21$
38% site								
Tt.BMC (g)	2.96 ± 0.32	2.92 ± 0.32	0.04 (-0.12, 0.20)	$F(1,87) = 0.23, p = .634, \eta^2_p < 0.01$	2.91 ± 0.29	2.56 ± 0.30	0.35 (0.19, 0.51)*	$F(1,54) = 19.65, p < .001, \eta^2_p = 0.27$
Ct.BMC (g)	2.68 ± 0.30	2.62 ± 0.31	0.07 (-0.09, 0.23)	$F(1,87) = 0.74, p = .391, \eta^2_p = 0.01$	2.65 ± 0.29	2.31 ± 0.29	0.34 (0.18, 0.50)*	$F(1,54) = 18.83, p < .001, \eta^2_p = 0.26$
Tt.Ar (mm^2)	378 ± 43	369 ± 43	9 (-14, 30)	$F(1,87) = 0.59, p = .445, \eta^2_p = 0.01$	348 ± 34	322 ± 34	27 (8, 45)	$F(1,54) = 8.52, p = .005, \eta^2_p = 0.14$
Ct.Ar (mm^2)	255 ± 29	246 ± 30	9 (-6, 24)	$F(1,87) = 1.39, p = .242, \eta^2_p = 0.02$	243 ± 27	209 ± 27	33 (19, 48)*	$F(1,54) = 21.11, p < .001, \eta^2_p = 0.28$
Ct.Th (mm)	4.73 ± 0.44	4.59 ± 0.45	0.13 (-0.09, 0.36)	$F(1,87) = 1.37, p = .245, \eta^2_p = 0.02$	4.74 ± 0.49	4.16 ± 0.49	0.58 (0.31, 0.84)*	$F(1,54) = 19.07, p < .001, \eta^2_p = 0.26$
PC (mm)	68.7 ± 3.9	67.8 ± 4.0	0.9 (-1.1, 2.9)	$F(1,87) = 0.78, p = .379, \eta^2_p = 0.01$	66.0 ± 3.3	63.4 ± 3.3	2.6 (0.9, 4.4)	$F(1,54) = 8.86, p = .004, \eta^2_p = 0.14$
EC (mm)	39.0 ± 3.9	39.0 ± 3.9	0.1 (-1.9, 2.0)	$F(1,87) = 0.00, p = .952, \eta^2_p < 0.01$	36.3 ± 3.8	37.3 ± 3.8	-1.0 (-3.1, 1.1)	$F(1,54) = 0.94, p = .338, \eta^2_p = 0.02$
Frc.LdX (N)	2964.3 ± 516.8	2927.8 ± 523.0	36.5 (-227.9, 300.9)	$F(1,87) = 0.08, p = .784, \eta^2_p < 0.01$	2811.8 ± 417.4	2415.9 ± 417.7	395.9 (169.1, 622.8)*	$F(1,54) = 12.24, p = .001, \eta^2_p = 0.19$
SSIp (mm^3)	1323.2 ± 228.8	1233.9 ± 231.5	89.3 (-27.7, 206.3)	$F(1,87) = 2.30, p = .133, \eta^2_p = 0.03$	1202.7 ± 159.4	1056.5 ± 159.5	146.3 (59.6, 232.9)*	$F(1,54) = 11.46, p = .001, \eta^2_p =0.18$

Values are mean ± SD. pQCT variables adjusted by tibia length and maturity offset.

pQCT: peripheral quantitative computed tomography; MD: mean difference; CI: confidence interval; Tt.BMC: total volumetric bone mineral content; Tb.BMC: trabecular volumetric bone mineral content; Tt.Ar: total cross sectional area; Tb.Ar: trabecular cross sectional area; BSI: bone strength index; Ct.BMC: cortical volumetric bone mineral content; Ct.Ar: cortical cross sectional area; Ct.Th: cortical thickness; PC: periosteal circumference; EC: endosteal circumference; Frc.LdX: fracture load in axe X; SSIp: strength strain index in polar; η^2_p: partial eta squared.

Bonferroni correction *: $p < .0036$ differences between football players and controls.

Fig. 1


121 players of eight football clubs and 114 students of two high schools were invited to participate
NOT ASSESSED FOR ELIGIBILITY
• Declined to participate (*n* = 69)
ASSESSED FOR ELIGIBILITY (Total = 166):
-Football players (*n* = 121)
-Controls (*n* = 45)
EXCLUDED
• Eligible but not recruited
• Not assisting to the measurement citation (*n* = 11)
TOTAL RECRUITED (Total = 155):
-Football players (*n* = 110)
-Controls (*n* = 45)
EXCLUDED
• Data incorrect (*n* = 6)
DATA AVAILABLE FOR ANALYSIS:
-Football players (*n* = 107)
-Controls (*n* = 42)
Football players (Total = 107)
-Males (*n* = 71)
-Females (*n* = 36)
Controls (Total = 42)
-Males (*n* = 20)
-Females (*n* = 22)

Online Source 1 Descriptive values of female football players and controls.

	Males			Females			Menarche*Group	
	Football players (n = 16)	Controls (n = 13)	*d*	Football players (n = 20)	Controls (n = 9)	*d*	*p*	ω^2
Age (y)	12.4 ± 0.6	11.9 ± 0.7	0.86	12.9 ± 0.6	13.8 ± 1.2	0.92	0.150	0.014
Weight (kg)	45.8 ± 6.5	39.7 ± 10.0	0.73	52.1 ± 8.5	52.4 ± 7.8	0.04	0.017‡	0.038
Height (cm)	150.5 ± 5.6	148.9 ± 8.8	0.21	159.2 ± 5.4	158.9 ± 6.0	0.06	0.164	0.013
BMI (kg·m^{-2})	20.2 ± 2.7	17.7 ± 3.2	0.84	20.5 ± 2.6	20.7 ± 2.4	0.09	0.013‡	0.042
Tibia length (mm)	337 ± 20	338 ± 23	0.05	356 ± 18	355 ± 21	0.06	0.300	0.007
Muscle CSA (mm^2)	5150 ± 821	4343 ± 838	0.97	5688 ± 948	5379 ± 782	0.36	0.011‡	0.043
Fat CSA (mm^2)	2364 ± 886	2149 ± 796	0.25	2381 ± 505	2712 ± 614	0.59	0.123	0.016
Daily calcium intake (mg)	905.0 ± 654.5	724.1 ± 354.3	0.34	765.7 ± 486.4	810.5 ± 184.6	0.69	0.633	0.002
Maturity offset (y)	0.2 ± 0.6	-0.2 ± 0.8	0.62	1.0 ± 0.5	1.5 ± 1.0	0.74	0.107	0.018
Age PHV (y)	12.2 ± 0.3	12.1 ± 0.4	0.38	11.9 ± 0.4	12.2 ± 0.4	0.44	0.793	<0.001
Age of menarche (y)	-	-	-	12 ± 1	12 ± 1	0.71	-	-
Training years (y)	3 ± 2	-	-	3 ± 3	-	-	-	-
Training hours (h/week)	3.1 ± 0.6	-	-	2.8 ± 0.6	-	-	-	-

Values are mean ± SD.

BMI: body mass index; CSA: cross sectional area; PHV: peak height velocity.

* significant differences between football players and controls; ‡: significant interaction. Cohen's *d* can be small (0.2 – 0.5), medium (0.5 – 0.8) or large (>0.8); ω^2 can be small (0.01 – 0.06). medium (0.06 – 0.14) or large (>0.14).

Online Resource 2 Adjusted pQCT values of football players and controls.

	Males				Females			
	Football players (n = 71)	Controls (n = 20)	MD (95% CI)	Test statistic	Football players (n = 36)	Controls (n = 22)	MD (95% CI)	Test statistic
Model 2								
4% site								
Tt.BMC (g)	3.89 ± 0.60	3.50 ± 0.61	0.39 (0.08, 0.70)	$F(1,86) = 6.23, p = .014, \eta^2_p = 0.07$	3.06 ± 0.31	2.76 ± 0.31	0.30 (0.13, 0.47)*	$F(1,53) = 12.16, p = .001, \eta^2_p = 0.19$
Tb.BMC (g)	1.62 ± 0.31	1.40 ± 0.31	0.22 (0.06, 0.38)	$F(1,86) = 7.75, p = .007, \eta^2_p = 0.08$	1.17 ± 0.16	1.08 ± 0.16	0.09 (0.00, 0.18)	$F(1,53) = 3.97, p = .051, \eta^2_p = 0.07$
Tt.Ar (mm^2)	1195 ± 108	1088 ± 110	107 (51, 162)*	$F(1,86) = 14.50, p < .001, \eta^2_p = 0.14$	997 ± 88	964 ± 89	33 (-16, 82)	$F(1,53) = 1.81, p = .185, \eta^2_p = 0.03$
Tb.Ar (mm^2)	537 ± 49	489 ± 50	48 (23, 73)*	$F(1,86) = 14.48, p < .001, \eta^2_p = 0.14$	448 ± 40	434 ± 40	15 (-7, 37)	$F(1,53) = 1.80, p = .185, \eta^2_p = 0.03$
BSI (mg/mm)	128.1 ± 31.8	115.1 ± 32.3	13.0 (-3.3, 29.4)	$F(1,86) = 2.50, p = .117, \eta^2_p = 0.03$	95.1 ± 16.6	80.5 ± 16.8	14.7 (5.4, 23.9)*	$F(1,53) = 10.16, p = .002, \eta^2_p = 0.16$
38% site								
Tt.BMC (g)	2.97 ± 0.28	2.89 ± 0.28	0.08 (-0.06, 0.22)	$F(1,86) = 1.26, p = .264, \eta^2_p = 0.01$	2.87 ± 0.22	2.62 ± 0.22	0.25 (0.13, 0.37)*	$F(1,53) = 17.04, p < .001, \eta^2_p = 024$
Ct.BMC (g)	2.69 ± 0.26	2.58 ± 0.27	0.11 (-0.03, 0.25)	$F(1,86) = 2.50, p = .117, \eta^2_p = 0.03$	2.61 ± 0.22	2.37 ± 0.23	0.24 (0.12, 0.37)*	$F(1,53) = 15.59, p < .001, \eta^2_p = 0.23$
Tt.Ar (mm^2)	379 ± 38	365 ± 39	14 (-6, 33)	$F(1,86) = 1.88, p = .174, \eta^2_p = 0.02$	345 ± 29	327 ± 29	17 (1, 33)	$F(1,53) = 4.66, p = .035, \eta^2_p = 0.08$
Ct.Ar (mm^2)	256 ± 26	243 ± 26	13 (-1, 26)	$F(1,86) = 3.51, p = .064, \eta^2_p = 0.04$	239 ± 21	215 ± 21	25 (13, 36)*	$F(1,53) = 17.69, p < .001, \eta^2_p = 0.25$
Ct.Th (mm)	4.74 ± 0.42	4.57 ± 0.43	0.17 (-0.05, 0.39)	$F(1,86) = 2.38, p = .126, \eta^2_p = 0.03$	4.69 ± 0.44	4.23 ± 0.44	0.46 (0.22, 0.70)*	$F(1,53) = 14.29, p < .001, \eta^2_p = 0.21$
PC (mm)	68.8 ± 3.5	67.5 ± 3.6	1.4 (-0.5, 3.2)	$F(1,86) = 2.22, p = .140, \eta^2_p = 0.03$	65.7 ± 2.8	64.0 ± 2.8	1.7 (0.2, 3.3)	$F(1,53) = 4.95, p = .030, \eta^2_p = 0.09$
EC (mm)	39.1 ± 3.8	38.8 ± 3.8	0.3 (-1.6, 2.2)	$F(1,86) = 0.10, p = .759, \eta^2_p = 0.00$	36.2 ± 3.9	37.4 ± 3.9	-1.2 (-3.3, 1.0)	$F(1,53) = 1.15, p = .288, \eta^2_p = 0.02$
Frc.LdX (N)	2979.5 ± 443.6	2873.6 ± 450.3	106.0 (-122.0, 333.9)	$F(1,86) = 0.85, p = .358, \eta^2_p = 0.01$	2765.9 ± 352.0	2491.1 ± 354.9	274.7 (79.5, 469.9)	$F(1,53) = 7.97, p = .007, \eta^2_p = 0.13$
SSIp (mm^3)	1329.7 ± 199.1	1210.8 ± 202.1	118.9 (16.6, 221.2)	$F(1,86) = 5.34, p = .023, \eta^2_p = 0.06$	1184.0 ± 130.0	1087.1 ± 131.1	96.9 (24.8, 169.0)	$F(1,53) = 7.27, p = .009, \eta^2_p = 0.12$
Model 3								
4% site								
Tt.BMC (g)	3.88 ± 0.55	3.53 ± 0.55	0.35 (0.07, 0.63)	$F(1,86) = 6.24, p = .014, \eta^2_p = 0.07$	3.04 ± 0.33	2.79 ± 0.34	0.26 (0.07, 0.44)	$F(1,53) = 7.45, p = .009, \eta^2_p = 0.12$
Tb.BMC (g)	1.62 ± 0.28	1.41 ± 0.28	0.21 (0.06, 0.35)	$F(1,86) = 8.09, p = .006, \eta^2_p = 0.09$	1.16 ± 0.17	1.09 ± 0.18	0.07 (-0.03, 0.17)	$F(1,53) = 2.20, p = .144, \eta^2_p = 0.04$
Tt.Ar (mm^2)	1192 ± 103	1095 ± 104	97 (44, 150)*	$F(1,86) = 13.43, p < .001, \eta^2_p = 0.14$	993 ± 88	970 ± 90	23 (-27, 73)	$F(1,53) = 0.86, p = .359, \eta^2_p = 0.02$
Tb.Ar (mm^2)	536 ± 46	493 ± 47	44 (20, 67)*	$F(1,86) = 13.41, p < .001, \eta^2_p = 0.14$	447 ± 40	436 ± 41	10 (-12, 33)	$F(1,53) = 0.86, p = .359, \eta^2_p = 0.02$
BSI (mg/mm)	127.9 ± 29.4	116.0 ± 29.7	11.8 (-3.2, 26.9)	$F(1,86) = 2.45, p = .121, \eta^2_p = 0.03$	94.5 ± 18.0	81.5 ± 18.3	13.0 (2.8, 23.2)*	$F(1,53) = 6.48, p = .014, \eta^2_p = 0.11$
38% site								
Tt.BMC (g)	2.96 ± 0.24	2.92 ± 0.25	0.04 (-0.09, 0.17)	$F(1,86) = 0.38, p = .537, \eta^2_p = 0.00$	2.85 ± 0.22	2.66 ± 0.23	0.20 (0.07, 0.32)*	$F(1,53) = 9.81, p = .003, \eta^2_p = 0.16$
Ct.BMC (g)	2.68 ± 0.25	2.62 ± 0.26	0.07 (-0.06, 0.20)	$F(1,86) = 1.09, p = .299, \eta^2_p = 0.01$	2.60 ± 0.24	2.39 ± 0.24	0.20 (0.07, 0.34)*	$F(1,53) = 9.11, p = .004, \eta^2_p = 0.15$
Tt.Ar (mm^2)	378 ± 31	369 ± 32	9 (-7, 25)	$F(1,86) = 1.14, p = .290, \eta^2_p = 0.01$	342 ± 26	332 ± 27	9 (-6, 24)	$F(1,53) = 1.57, p = .216, \eta^2_p = 0.03$
Ct.Ar (mm^2)	255 ± 23	246 ± 24	9 (-3, 21)	$F(1,86) = 2.21, p = .141, \eta^2_p = 0.03$	237 ± 21	218 ± 21	20 (8, 32)*	$F(1,53) = 11.03, p = .002, \eta^2_p = 0.17$
Ct.Th (mm)	4.73 ± 0.42	4.59 ± 0.42	0.13 (-0.08, 0.35)	$F(1,86) = 1.51, p = .222, \eta^2_p = 0.02$	4.68 ± 0.46	4.25 ± 0.47	0.43 (0.16, 0.69)*	$F(1,53) = 10.52, p = .002, \eta^2_p = 0.17$
PC (mm)	68.7 ± 2.9	67.8 ± 2.9	0.9 (-0.6, 2.4)	$F(1,86) = 1.50, p = .224, \eta^2_p = 0.02$	65.4 ± 2.6	64.4 ± 2.6	1.0 (-0.5, 2.4)	$F(1,53) = 1.81, p = .184, \eta^2_p = 0.03$
EC (mm)	39.0 ± 3.4	39.0 ± 3.5	0.1 (-1.7, 1.8)	$F(1,86) = 0.01, p = .940, \eta^2_p = 0.00$	36.0 ± 3.9	37.7 ± 3.9	-1.7 (-3.9, 0.5)	$F(1,53) = 2.43, p = .125, \eta^2_p = 0.04$
Frc.LdX (N)	2964.5 ± 406.4	2927.0 ± 411.3	37.6 (-170.4, 245.5)	$F(1,86) = 0.13, p = .720, \eta^2_p = 0.00$	2736.1 ± 341.2	2539.8 ± 347.3	196.3 (2.7, 389.9)	$F(1,53) = 4.14, p = .047, \eta^2_p = 0.07$
SSIp (mm^3)	1323.3 ± 181.1	1233.5 ± 183.3	89.8 (-2.9, 182.4)	$F(1,86) = 3.71, p = .057, \eta^2_p = 0.04$	1174.7 ± 132.6	1102.4 ± 134.9	72.2 (-3.0, 147.5)	$F(1,53) = 3.71, p = .059, \eta^2_p = 0.07$

Values are mean ± SD. Model 2: pQCT variables adjusted by tibia length, maturity offset and weight; Model 3: pQCT variables adjusted by tibia length, maturity offset and muscle cross sectional area.

pQCT: peripheral quantitative computed tomography; MD: mean difference; CI: confidence interval; Tt.BMC: total volumetric bone mineral content; Tb.BMC: trabecular volumetric bone mineral content; Tt.Ar: total cross sectional area; Tb.Ar: trabecular cross sectional area; Ct.BMC: cortical volumetric bone mineral content; Ct.Ar: cortical cross sectional area; Ct.Th: cortical thickness; PC: periosteal circumference; EC: endosteal circumference; Frc.LdX: fracture load in axe X; SSIp: strength strain index in polar.

*: $p < 0.0036$ differences between football players and controls.