Title page

Determinants of bone outcomes in adolescent athletes at baseline: the PRO-BONE study.

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Abstract

Purpose: The determinants of areal bone mineral density (aBMD) and hip geometry estimates in adolescent athletes are poorly understood. This study aimed to identify the determinants of aBMD and hip geometry estimates in adolescent male athletes. **Methods**: One hundred twenty one males (13.1±0.1 years) were measured: 41 swimmers, 37 footballers, 29 cyclists and 14 controls. Dual energy X-ray absorptiometry (DXA) measured aBMD at lumbar spine, femoral neck (FN) and total body. Hip structural analysis evaluated hip geometry estimates at the FN. Multiple linear regression examined the contribution of the sports practised, stature, lean and fat mass, serum calcium and vitamin D, moderate to vigorous physical activity (MVPA), vertical jump and cardiorespiratory fitness (CRF) with aBMD and hip geometry estimates. **Results**: Region specific lean mass was the strongest positive predictor of aBMD (β = 0.614 - 0.931) and football participation was the next strongest predictor (β = 0.304 - 0.579). Stature (β = 0.235 - 0.380), fat mass (β = 0.189), serum calcium (β = 0.103), serum vitamin D (β = 0.104 - 0.139) and vertical jump (β = 0.146 - 0.203) were associated with aBMD across various specific sites. All hip geometry estimates were associated with lean mass (β = 0.370 - 0.568) and stature (β = 0.338 - 0.430). Football participation was associated with hip cross-sectional area (β = 0.322) and MVPA (β = 0.140 - 0.142). CRF (β = 0.183 - 0.207) was associated with section modulus and cross-sectional moment of inertia. **Conclusions:** Region specific lean mass is the strongest determinant of aBMD and hip geometry estimates in adolescent male athletes. Football participation and stature were important determinants for aBMD and hip geometry estimates while the contribution of the other predictors was site specific.

Keywords BODY COMPOSITION; BONE MASS; EXERCISE, LEAN MASS, PREDICTORS, SPORT PARTICIPATION.

Trial registration ISRCTN17982776

Introduction

During growth and maturation changes in bone density and geometry occur in order to withstand the forces applied through external loading of the skeleton (9). Peak bone mass is achieved by early adulthood and is largely determined by non-modifiable genetic factors (4). However, modifiable factors, such as nutrition (24) and physical activity (11), are also known to alter peak bone mass. Exercise can significantly enhance areal bone mineral density (aBMD) and strength at loaded sites in children but not in adults (25). Optimal bone development can be achieved with adequate status of key nutrients, such as calcium and vitamin D, and may attenuate exercise-induced adaptations of aBMD (15). The type of sport practised can affect the skeletal development differently depending on training characteristics (35). Participation in weight bearing sports, such as football, is associated with greater areal bone mineral density than non-weight bearing sports, such as swimming and cycling (8, 38). However, it is poorly understood why the differences exist between different athletic groups and there is limited understanding of the determinants of aBMD and hip geometry in adolescent male athletes.

Total body lean mass has a positive association with aBMD in the growing skeleton (10) but controversy currently exists surrounding the association between fat mass and aBMD (6). There is no evidence distinguishing the site specific effects of lean mass and fat mass on aBMD in adolescent athletes and there are inconsistencies in the use of confounders to adjust bone parameters in non-athletic groups. Data on non-athletic prepubescent females indicate that leg lean mass is the most important predictor of bone mineral content at the leg and femoral neck sites (3). Although a positive association between fat mass and aBMD has been reported in non-athletic adolescents males and females (31), this is explained by an increase in lean mass (12). To date, there is no evidence explaining the effects of lean and fat mass on bone outcomes in adolescent athletes, which is of great interest due to the importance of body composition in athletic groups. Cardiorespiratory (CRF) and muscular fitness (vertical jump) have also been found to be positively associated with bone outcomes in non-athletic adolescents (1, 13), but their contribution on bone parameters in adolescent athletes is poorly understood.

Geometric properties of the hip, such as cross-sectional area (CSA), obtained by using hip structural analysis (HSA) software, can provide further insight into the determinants of bone hip geometry estimates (19). During growth, bones adapt their geometry due to increases in stature, lean and fat mass (3) and geometric parameters of the femur neck (FN) are closely adapted to lean mass (27). The primary predictor of bone hip geometry in non-athletic boys and girls is muscle CSA, accounting for 10 – 16 % of the variance (22), while other factors such as moderate to vigorous physical activity (MVPA) can have a site specific influence on bone geometry (23).

As highlighted above, numerous factors have been shown to be related to bone outcomes in non-athletic adolescents, but the determinants of aBMD and hip geometry in adolescent male athletes have yet to be comprehensively investigated. Therefore, this study aims to provide novel insight into the contribution of the independent predictors of sports participation (football, swimming and cycling), stature, region specific lean and fat mass, serum calcium and vitamin D, MVPA, muscular fitness and CRF (all adjusted by each other) on aBMD and hip geometry estimates in adolescent male athletes. It is hypothesized that football participation, lean mass and stature would be the most important determinants of aBMD and hip geometry estimates in adolescent male athletes. It is proposed that other modifiable factors (e.g. nutrition, MVPA and fitness) would have a small but significant contribution on bone outcomes.

Methods

Study design and participants

Participants comprised 121 adolescent males (41 swimmers, 37 footballers, 29 cyclists and 14 controls) participating in the PRO-BONE (effect of a PROgram of short bouts of exercise on BONE health in adolescents involved in different sports) longitudinal study (39). The data in the current study are taken from the baseline data of the PRO-BONE study and was completed between autumn and winter 2014/15. The inclusion and exclusion criteria were: 1) males 12–14 years old, engaged (≥3 h/week) in osteogenic (football) and/or non-osteogenic (swimming and cycling) sports for the last 3 years or more; 2) active males 12–14 years old who were not engaged in football, cycling and swimming (≥3 h/week) in the last 3 or more years but who were physically active (control group); 3) not taking part in another clinical trial; 4) not having an acute infection lasting until < 1 week before inclusion; 5) to be free of any medical history of diseases or medications affecting bone metabolism; 6) to be white Caucasian.

Participants were recruited from athletic clubs and schools across the South West of England. Written informed consent and assent forms were signed from parents and participants accordingly and all participants completed the first visit at the research centre as part of the study. The methods and procedures of the study have been checked and approved by: 1) the Ethics Review Sector of Directorate-General of Research (European Commission, ref. number 618496); 2) the Sport and Health Sciences Ethics Committee (University of Exeter, ref. number 2014/766) and 3) the National Research Ethics Service Committee (NRES Committee South West – Cornwall & Plymouth, ref. number 14/SW/0060).

Dual energy x-ray absorptiometry

A dual energy X-ray absorptiometry (DXA) scanner (GE Lunar Prodigy Healthcare Corp., Madison, WI, USA) was used to measure aBMD (g/cm^2), fat mass (g) and lean mass

(g) at specific regions of the body. Four scans were performed to obtain data for the lumbar spine (LS, L1-L4), bilateral proximal femora (the mean of both was used for the current analysis) and the total body. The total body scan was then used to obtain data for specific regions such as: arms, legs and total body less head (TBLH). All DXA scans and subsequent in-software analyses were completed by the same researcher, using the same DXA scanner and the GE encore software (2006, version 14.10.022).

Hip structural analysis

Hip geometry estimates at the FN were determined using HSA software which analyses the distribution of bone mineral mass in a line of pixels across the bone axis. The hip geometry estimates of the bone were obtained and the following variables used: 1) the cross sectional area (CSA, mm^2), which is the total bone surface area of the hip excluding the soft tissue area and the trabecular bone; 2) the cross-sectional moment of inertia (CSMI, mm^4), which is an index of structural rigidity and reflects the distribution of mass in the centre of a structural element; and 3) section modulus (Z, mm^3), which is an indicator of maximum bending strength in a cross section. The short term precision percentage coefficient of variation of these variables has been reported to be between 2.4 % and 10.1 % (19).

Anthropometry, physical activity and nutritional markers

Stature (cm) and body mass (kg) were measured using a stadiometer (Harpenden, Holtain Ltd, Crymych, UK) and an electronic scale (Seca 877, Seca Ltd, Birmingham, UK), respectively. Body mass index was calculated as body mass (kg) divided by the stature (m) squared. Sexual maturation was self-reported using adapted drawings of the five stages (Tanner) of pubic hair (34).

Physical activity was measured for seven consecutive days using validated wrist accelerometers (GENEActiv, GENEA, UK) (7). Participants were instructed to place the accelerometer on their non-dominant wrist and data was collected at 100 Hz. Data were analysed using 1 s epoch to establish time spent in MVPA using a cut-off point of ≥ 1140 counts per minute previously validated in youth (28).

Total serum levels of calcium and 25 hydroxyvitamin D [25(OH)D] were analysed. Serum samples were analysed by using ELISA kits (Abbexa Ltd., Cambridge, UK) for 25(OH)D and had a test range of 3 - 80 $\mu g \cdot mL^{-1}$ and a sensitivity of 1.2 $\mu g \cdot mL^{-1}$ (inter and intra-assay CVs: 5.7 % and 9.5 % respectively). Total serum levels of calcium was measured using direct colorimetric assay (Cayman Chemical Company, MI, U.S.A.) and had linear assay range of 0.25-10 $mg \cdot mL^{-1}$ (inter and intra-assay CVs: 8.1 % and 12.8 % respectively).

Physical fitness

The fitness tests used in the present investigation have been shown to be reliable and valid in youth (26). A counter movement vertical jump test was used to provide an estimate of lower limb muscular power. The jumps were performed on a jump mat (Probotics Inc., Huntsville, USA) which calculates jump height based on flight time. Each participant performed three maximal vertical jumps and the highest jump was used for the analysis.

Cardiorespiratory fitness was evaluated using the 20 m shuttle run test (21). The test ended when the participants failed to reach the line on two consecutive occasions. The last completed shuttle determined the score of the test and the number of shuttles completed was taken as an indicator of CRF.

Statistical analyses

Data were analysed using SPSS IBM statistics (version 21.0 for Windows, Chicago, IL, USA) and descriptive data are reported as mean and standard deviation (SD). The normal distribution of the raw variables and of the regression model residuals was checked and verified using Shapiro-Wilk's test, skewness and kurtosis values, visual check of histograms, Q-Q and box plots. Collinearity was checked for the variables using the variance inflation factor (VIF) and tolerance levels. One way analysis of variance (ANOVA) with Bonferroni post hoc comparisons and Chi-Square tests were used to detect between-group mean differences for the descriptive variables (table 1).

Multiple linear regression analyses were used to examine the contribution of sport participation, stature, lean mass, fat mass, total calcium, 25(OH)D, MVPA, vertical jump and 20 m shuttle run test to bone outcomes. The selection of the predictors was based on their relationship with bone outcomes (22, 27, 32). To account for the differences between the sports groups a dummy variable was computed (footballers, swimmers, cyclists and controls) and controls were selected as the reference group. In a preliminary analysis we found that Tanner stage was not a significant predictor after adjusting for stature and age and consequently was not included in the model. All remaining predictors were entered into the regression models simultaneously. For the multiple linear regressions, the standardised regression coefficients (β) are reported and significance was set at alpha level of 0.05. The squared semi-partial correlation coefficients (sr^2) were used to determine the contribution of each predictor in the overall variance of the model after removing shared contributions with other predictors.

Results

(Table 1 here)

Characteristics of the participants

The raw descriptive characteristics of the participants and the differences between sports groups are presented in Table 1. Swimmers were significantly older, taller, heavier and had more lean mass than the footballers and controls, and cyclists were significantly older than controls. All groups were similar for total serum calcium and 25(OH)D. Swimmers had significantly higher muscular and CRF than the controls. Footballers spent significantly more time in MVPA compared to swimmers and controls and had a significantly higher CRF compared to all the other groups. Cyclists had significantly higher MVPA than swimmers and significantly higher CRF than controls.

The raw unadjusted data showed that swimmers had significantly higher aBMD at the arms compared to footballers and higher aBMD at all sites except for the legs compared to controls. Footballers had significantly higher aBMD at TBLH, FN compared to controls and higher aBMD at TH compared to all groups. Cyclists had significantly higher aBMD at all sites expect LS and legs compared to controls. Swimmers, footballers and cyclists had significantly enhanced hip geometry estimates compared to controls.

(Table 2 here)

Determinants of bone density and hip geometry estimates

Multivariate regression models significantly explained 49.0% - 76.4% (on average, 60.0%) of the variance in the aBMD outcomes (Table 2). Region specific lean mass and football participation were consistently the strongest significant predictors of aBMD at TBLH, LS, TH, legs and arms (β = 0.614 - 0.931, sr^2 = 0.031 - 0.161, $P < 0.01$). Football participation (compared to the control group) was positively associated with aBMD at TBLH, FN, TH and legs (β = 0.304 - 0.579, sr^2 = 0.031 - 0.068, $P < 0.01$). Stature was positively associated with aBMD at FN and arms (β = 0.235 - 0.380, sr^2 = 0.021, $P < 0.05$). Region specific fat mass was positively associated with aBMD at TBLH (β = 0.189, sr^2 = 0.015, P <

0.05). Serum calcium was positively associated with aBMD at the arms ($\beta = 0.103$, $sr^2 = 0.009$, $P < 0.05$). In addition, serum 25(OH)D was positively associated with aBMD at the arms and LS ($\beta = 0.104 - 0.139$, $sr^2 = 0.009$, $P < 0.05$). Muscular fitness was positively associated with aBMD at TBLH and LS ($\beta = 0.146 - 0.203$, $sr^2 = 0.010 - 0.019$, $P < 0.05$). CRF was not associated with aBMD outcomes at any skeletal site after accounting for the other predictors.

(Table 3 here)

In the multivariate regression analysis of the hip geometry estimates (Table 3) the predictors explained 71.7% - 77.8% (on average, 75.7%) of the variance. Region specific lean mass was the strongest significant predictor and was positively associated with CSA, CSMI and Z ($\beta = 0.370 - 0.568$, $sr^2 = 0.017 - 0.039$, $P < 0.05$). Football participation (compared to the control group) was positively associated with CSA ($\beta = 0.322$, $sr^2 = 0.023$, $P < 0.01$). Stature was positively associated CSA, CSMI and Z ($\beta = 0.338 - 0.430$, $sr^2 = 0.017 - 0.025$, $P < 0.001$). MVPA was positively associated with CSMI and Z ($\beta = 0.140 – 0.142$, $sr^2 = 0.014$, $P < 0.05$). CRF was positively associated with CSMI and Z ($\beta = 0.183 – 0.207$, $sr^2 = 0.011 – 0.014$, $P < 0.05$).

Discussion

The present study aimed to identify, for the first time, the determinants of aBMD and hip geometry estimates in adolescent male athletes involved in football, swimming and cycling. It has recently been shown that football has a beneficial impact on bone outcomes in comparison to cycling and swimming in adolescent males (38). However the determinants responsible for these differences are not known for this population. In support with our hypothesis, region specific lean mass was the primary explanatory variable on aBMD and hip geometry estimates at most sites of the skeleton. In addition, we found that only participation in football was a significant predictor of aBMD and hip geometry estimates when contrasted to the control group. Finally, it was observed that modifiable factors such as nutrition status (calcium and vitamin D), MVPA and physical fitness (vertical jump and CRF) had a small but significant contribution to bone density and hip geometry estimates across specific sites of the skeleton.

Determinants of bone mineral density

The determinants explained an important significant variance of aBMD at different skeletal sites (49.0 % - 76.4 %, average 64.5 %) with previous findings in non-athletic population reporting that a similar model of determinants explained 40 % - 83 % of the variance in BMC in prepubertal girls (3). Region specific lean mass was consistently the strongest determinant of aBMD at TBLH, LS, legs and arms. A previous study in non-athletic boys and girls reported that total lean mass was the best predictor of total body and lumber spine aBMD, but they did not report the relationship with other sites of the skeleton (6). Another study in non-athletic children found that total lean mass was the strongest predictor of the aBMD at total body, and LS (16). However the study did not distinguished the site specific relationship of lean and bone mass which was considered in the present study. It is of great interest to understand the region specific relationship of lean mass and aBMD due to the site specific adaptation of the skeleton during external loading, specifically in athletic populations (2). It is still not clear to what extent fat mass is associated with aBMD in adolescents and especially after adjusting for confounding factors. In our study we found that region specific fat mass only had a positive association with TBLH aBMD, suggesting that after accounting for other covariates its influence on bone development in athletic male adolescents is negligible, perhaps due to the strong effect of region specific lean mass. In addition, an increase in fat mass in adolescent athletes can have a negative effect on their performance (36).

Football participation was positively associated with aBMD at TBLH, FN, TH and legs. There was no significant contribution of swimming and cycling in contrast to the control group on aBMD at any sites of the skeleton. The contribution of football on aBMD was independent of lean mass and is likely to be explained by the intermittent and high-intensity characteristics of football that can produce high strains on the skeleton and stimulate bone mineral acquisition (35). The concentric contractions during football generate greater forces compared to cycling and swimming and this might explain the increased skeletal loading in this group (33). In addition, our findings show that stature was positively significant associated with aBMD at the arms and FN. Similarly with our results, previously it was found that stature had a weak and site specific relationship with BMC at different skeletal sites in non-athletic children (16). The movement characteristics of the sports practised seems to be important for bone acquisition and the present study found that football participation is one of the most important determinants, possibly because it includes high intensity concentric contractions that can enhance aBMD in adolescents.

Both MVPA and nutrition (calcium and vitamin D) are considered to be essential for optimal bone growth, but their contribution was diminished once other factors (e.g. stature, lean mass, sports participation) were considered. In the present analysis we found that blood serum calcium and 25(OH)D had a small contribution on aBMD at the arms and only 25(OH)D contributed to aBMD at LS. Previous findings indicated that dietary calcium and 25(OH)D can have a weak, but significant contribution on specific sites of the skeleton in adolescents (22). The sites of the skeleton, such as arms, are less loaded through sport and nutritional factors may have a potential influence. The site specific relationship between nutrition and bone outcomes can be attributed to the interactions of nutrients in relation to bone health (17). In relation to the contribution of MVPA and fitness on aBMD, we found that vertical jump height was the only significant predictor of aBMD at TBLH and LS. These findings show that overall MVPA does not appear to be important once participation in a particular sport is considered in the regression model. This suggests the characteristics of the sport practised and the contribution of lean mass mediates the relationship between fitness and bone outcomes (37).

Determinants of hip geometry estimates

Using the HSA method we showed that the multiple regression model can explain a large proportion of the variance (71.7 % - 77.8 %, average 75.7 %) in geometrical parameters (CSA, CSMI, Z) at the narrow neck site of the hip. To the best of our knowledge, this is the first study examining the determinants of HSA outcomes in adolescent athletes. The strongest predictor for the geometrical parameters was the region specific lean mass followed by stature. The contribution of region specific lean mass was consistent for all the geometrical parameters. The findings of the present study highlight the influence of region specific lean mass on hip geometry estimates during adolescence which is linked with bone outcomes in young adulthood (2). Despite the lack of significant association between region specific lean mass and aBMD at the FN and TH, all the geometrical parameters of the narrow neck of the femur were significantly associated with region specific lean mass. This may reflect previous work in children and adolescents showing that HSA can provide more in depth geometrical evaluation at the hip site compared to BMD outcomes (27). In addition, studies using peripheral quantitative computed tomography (pQCT) found that muscle cross sectional area was the strongest predictor of bone strength parameters in early pubertal boys and girls (22). The latter study highlighted the importance of using site specific lean mass to understand its contribution to hip geometry estimates. On the other hand, region specific fat mass was not associated with any geometrical parameters and this is in agreement with findings in non-athletic adolescent females indicating that fat mass was not associated with CSA (29).

Stature was associated with all hip geometry estimates showing that the size of an adolescent athlete plays an important role in modifying hip geometry estimates. A previous study reported that femoral length is one of the most important predictors of CSA and Z in female adolescents (3) highlighting the importance of bone length at the hip. In addition, football participation was associated with CSA in hip geometry estimates of female footballers (5). There was no contribution of swimming and cycling on geometrical parameters which is similar with the findings on aBMD outcomes. The different contribution of stature and football in geometrical parameters compared to aBMD parameters might be due to fact that we used stature and not femoral length to control for the size in geometrical parameters. Also, the estimated geometrical parameters might not be affected from the external loading that football applies at the narrow neck site and higher forces might be needed (20).

All groups of the present study had similar serum levels of calcium and vitamin D and there was no association found between serum levels of calcium and vitamin D and geometrical bone outcomes, which is consistent with no contribution of hip related aBMD outcomes in the present study. In contrast, MVPA was a significant predictor of CSMI and Z independent of the sport participation suggesting that MVPA might induce changes in geometrical parameters and not aBMD due to mechanical stimuli applied at the hip site (23). The association between MVPA and bone outcomes was evident for the geometrical parameters but not for the aBMD parameters. This may be explained by previous findings showing that geometrical adaptations can occur before the adaptation of aBMD outcomes due to the initial respond inside the bone to the change in external strains (14, 40). CRF was a significant predictor of CSMI and Z after accounting for all the other predicting determinants, but there was no association with vertical jump. The different associations between fitness parameters and MVPA with aBMD and the geometrical parameters might be attributed to the sensitivity of the geometrical parameters of the hip to detect changes (19). The bone structure at the hip and specifically CSA site might be associated with CRF due to the use lower leg muscle units during the sport specific movements. The training characteristics are dominant in the present study and our population was at the 75th percentile for CRF compared to same age and ethnicity matched population (30).

Limitations

To our knowledge, this is the first study conducted in adolescent male athletes to examine the determinants of aBMD and hip geometry estimates. A large list of predictors has been included and their effects have been adjusted by each other. In addition, the present study uses region specific lean mass as predictor of aBMD and hip geometry estimates due to the site specific adaptations of the skeleton during exercise and growth (18). The cross-sectional analysis of the present study is a limitation and cannot prove cause and effect between the determinants and bone outcomes studies. In spite of using DXA as a surrogate estimate of lean mass due to the 2 component model, DXA-derived lean mass has been found to be highly correlated (r = 0.82) with muscle cross sectional area measured by pQCT (27).

Conclusions

The present study has shown, for the first time, the determinants of aBMD and hip geometry estimates in adolescent male athletes. Region specific lean mass was consistently the most important determinant of aBMD and hip geometry estimates parameters in adolescent male athletes. Football participation and stature were found to be important determinants for the aBMD and HSA parameters, respectively. Calcium and 25(OH)D had a small site specific contribution only on aBMD. MVPA and CRF positively influenced only the geometrical parameters and vertical jump was associated with aBMD parameters. Studies focusing on bone outcomes of young athletes should account for the region specific lean mass differences due to the site-specific adaptations of the skeleton to external loading. Future practical approaches of sports clubs should include weight-bearing and muscle strengthening exercises, such as jumps, which can optimise bone outcomes during the important period of adolescence.

ACKNOWLEDGEMENTS

Funding sources

The research leading to these results has received funding from the European Union Seventh Framework Programme ([FP7/2007-2013] under grant agreement n°. PCIG13-GA-2013-618496.

Authors' Contributions

DV obtained and analysed the data and drafted the manuscript under the supervision of LGM (principal investigator), ARB and CAW. BSM, KMK, AA, IGF, EUG and LAM reviewed the draft. All authors have read and approved this work.

The authors gratefully acknowledge the adolescents, parents and sport coaches and schools who helped and participated in this study. The authors also gratefully acknowledge the CHERC researchers for their continuous support and help when needed.

Ethics approval and consent to participate

Ethics approval received from the following committees: 1) the Ethics Review Sector of Directorate-General of Research (European Commission, ref. number 618496); 2) the Sport and Health Sciences Ethics Committee (University of Exeter, ref. number 2014/766) and 3) the National Research Ethics Service Committee (NRES Committee South West – Cornwall & Plymouth, ref. number 14/SW/0060).

List of abbreviations

BMC: Bone mineral content; aBMD: Areal bone mineral density; BMI: Body mass index; CSA: Cross-sectional area; CSMI: Cross-sectional moment of inertia; CRF: Cardiorespiratory fitness; DXA: Dual Energy X-Ray Absorptiometry; HSA: Hip structural Analysis; MVPA: Moderate to vigorous physical activity; TBLH: Total body less head, Z: section modulus, 25(OH)D: 25-hydroxyvitamin D.

Conflicts of interest

The authors declare that they have no competing interests.
The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation, and the present study do not constitute endorsement by ACSM.

Refernces

1. Baptista F, Mil-Homens P, Carita AI, Janz K, Sardinha LB. Peak Vertical Jump Power as a Marker of Bone Health in Children. *Int J Sports Med*. 2016;37(8):653-8.
2. Baxter-Jones AD, Kontulainen SA, Faulkner RA, Bailey DA. A longitudinal study of the relationship of physical activity to bone mineral accrual from adolescence to young adulthood. *Bone*. 2008;43(6):1101-7.
3. Daly RM, Stenevi-Lundgren S, Linden C, Karlsson MK. Muscle determinants of bone mass, geometry and strength in prepubertal girls. *Med Sci Sports Exerc*. 2008;40(6):1135-41.
4. Duren DL, Sherwood RJ, Choh AC et al. Quantitative genetics of cortical bone mass in healthy 10-year-old children from the Fels Longitudinal Study. *Bone*. 2007;40(2):464-70.
5. El Hage R. Geometric indices of hip bone strength in young female football players. *Journal of Musculoskeletal & Neuronal Interactions*. 2013;13(2):206-12.
6. El Hage RP, Courteix D, Benhamou CL, Jacob C, Jaffre C. Relative importance of lean and fat mass on bone mineral density in a group of adolescent girls and boys. *Eur J Appl Physiol*. 2009;105(5):759-64.
7. Esliger DW, Rowlands AV, Hurst TL, Catt M, Murray P, Eston RG. Validation of the GENEA Accelerometer. *Med Sci Sports Exerc*. 2011;43(6):1085-93.
8. Ferry B, Duclos M, Burt L et al. Bone geometry and strength adaptations to physical constraints inherent in different sports: comparison between elite female soccer players and swimmers. *J Bone Miner Metab*. 2011;29(3):342-51.
9. Frost HM. Bone's mechanostat: a 2003 update. *Anat Rec A Discov Mol Cell Evol Biol*. 2003;275(2):1081-101.
10. Gracia-Marco L. Physical activity, bone mass and muscle strength in children. *Acta Paediatr*. 2016;105(10):1127-8.
11. Gracia-Marco L, Moreno LA, Ortega FB et al. Levels of physical activity that predict optimal bone mass in adolescents: the HELENA study. *Am J Prev Med*. 2011;40(6):599-607.
12. Gracia-Marco L, Ortega FB, Jimenez-Pavon D et al. Adiposity and bone health in Spanish adolescents. The HELENA study. *Osteoporos Int*. 2012;23(3):937-47.
13. Gracia-Marco L, Vicente-Rodriguez G, Casajus JA, Molnar D, Castillo MJ, Moreno LA. Effect of fitness and physical activity on bone mass in adolescents: the HELENA Study. *Eur J Appl Physiol*. 2011;111(11):2671-80.
14. Haapasalo H, Kontulainen S, Sievanen H, Kannus P, Jarvinen M, Vuori I. Exercise-induced bone gain is due to enlargement in bone size without a change in volumetric bone density: A peripheral quantitative computed tomography study of the upper arms of male tennis players. *Bone*. 2000;27(3):351-7.
15. Harvey NC, Cole ZA, Crozier SR et al. Physical activity, calcium intake and childhood bone mineral: a population-based cross-sectional study. *Osteoporos Int*. 2012;23(1):121-30.
16. Hrafnkelsson H, Sigrudsson G, Magnusson KT, Johannsson E, Sigurdsson EL. Factors associated with bone mineral density and content in 7-year-old children. *Bone*. 2010;46(4):1058-62.
17. Ilich JZ, Kerstetter JE. Nutrition in bone health revisited: a story beyond calcium. *J Am Coll Nutr*. 2000;19(6):715-37.
18. Ireland A, Maden-Wilkinson T, McPhee J et al. Upper limb muscle-bone asymmetries and bone adaptation in elite youth tennis players. *Med Sci Sports Exerc*. 2013;45(9):1749-58.
19. Khoo BC, Beck TJ, Qiao QH et al. In vivo short-term precision of hip structure analysis variables in comparison with bone mineral density using paired dual-energy X-ray absorptiometry scans from multi-center clinical trials. *Bone*. 2005;37(1):112-21.
20. Korhonen MT, Heinonen A, Siekkinen J et al. Bone Density, Structure and Strength, and Their Determinants in Aging Sprint Athletes. *Med Sci Sport Exer*. 2012;44(12):2340-9.

21. Leger LA, Mercier D, Gadoury C, Lambert J. The multistage 20 metre shuttle run test for aerobic fitness. *J Sports Sci*. 1988;6(2):93-101.
22. Macdonald H, Kontulainen S, Petit M, Janssen P, McKay H. Bone strength and its determinants in pre- and early pubertal boys and girls. *Bone*. 2006;39(3):598-608.
23. Michalopoulou M, Kambas A, Leontsini D et al. Physical activity is associated with bone geometry of premenarcheal girls in a dose-dependent manner. *Metabolism-Clinical and Experimental*. 2013;62(12):1811-8.
24. Mouratidou T, Vicente-Rodriguez G, Gracia-Marco L et al. Associations of dietary calcium, vitamin D, milk intakes, and 25-hydroxyvitamin D with bone mass in Spanish adolescents: the HELENA study. *J Clin Densitom*. 2013;16(1):110-7.
25. Nikander R, Sievanen H, Heinonen A, Daly RM, Uusi-Rasi K, Kannus P. Targeted exercise against osteoporosis: A systematic review and meta-analysis for optimising bone strength throughout life. *BMC Med*. 2010;8:47.
26. Ortega FB, Artero EG, Ruiz JR et al. Reliability of health-related physical fitness tests in European adolescents. The HELENA Study. *Int J Obes (Lond)*. 2008;32 Suppl 5:S49-57.
27. Petit MA, Beck TJ, Shults J, Zemel BS, Foster BJ, Leonard MB. Proximal femur bone geometry is appropriately adapted to lean mass in overweight children and adolescents. *Bone*. 2005;36(3):568-76.
28. Phillips LR, Parfitt G, Rowlands AV. Calibration of the GENEA accelerometer for assessment of physical activity intensity in children. *J Sci Med Sport*. 2013;16(2):124-8.
29. Pollock NK, Laing EM, Baile CA, Hamrick MW, Hall DB, Lewis RD. Is adiposity advantageous for bone strength? A peripheral quantitative computed tomography study in late adolescent females. *Am J Clin Nutr*. 2007;86(5):1530-8.
30. Sandercock G, Voss C, Cohen D, Taylor M, Stasinopoulos DM. Centile curves and normative values for the twenty metre shuttle-run test in English schoolchildren. *J Sports Sci*. 2012;30(7):679-87.
31. Sayers A, Tobias JH. Fat mass exerts a greater effect on cortical bone mass in girls than boys. *J Clin Endocrinol Metab*. 2010;95(2):699-706.
32. Sioen I, Mouratidou T, Kaufman JM et al. Determinants of vitamin D status in young children: results from the Belgian arm of the IDEFICS (Identification and Prevention of Dietary- and Lifestyle-Induced Health Effects in Children and Infants) Study. *Public Health Nutr*. 2012;15(6):1093-9.
33. Soderman K, Bergstrom E, Lorentzon R, Alfredson H. Bone mass and muscle strength in young female soccer players. *Calcif Tissue Int*. 2000;67(4):297-303.
34. Tanner JM, Whitehouse RH. Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. *Arch Dis Child*. 1976;51(3):170-9.
35. Ubago-Guisado E, Gomez-Cabello A, Sanchez-Sanchez J, Garcia-Unanue J, Gallardo L. Influence of different sports on bone mass in growing girls. *J Sports Sci*. 2015;33(16):1710-8.
36. Ubago-Guisado E, Mata E, Sánchez-Sánchez J, Plaza-Carmona M, Martín-García M, Gallardo L. Influence of different sports on fat mass and lean mass in growing girls. *Journal of Sport and Health Science*. 2015.
37. Vicente-Rodriguez G, Urzanqui A, Mesana MI et al. Physical fitness effect on bone mass is mediated by the independent association between lean mass and bone mass through adolescence: a cross-sectional study. *Journal of Bone and Mineral Metabolism*. 2008;26(3):288-94.
38. Vlachopoulos D, Barker AR, Williams CA et al. The Impact of Sport Participation on Bone Mass and Geometry in Adolescent Males. *Med Sci Sports Exerc*. 2016;Publish Ahead of Print.
39. Vlachopoulos D, Barker AR, Williams CA, Knapp KM, Metcalf BS, Gracia-Marco L. Effect of a program of short bouts of exercise on bone health in adolescents involved in different sports: the PRO-BONE study protocol. *BMC Public Health*. 2015;15(1):361.

40. Wang QJ, Suominen H, Nicholson PH et al. Influence of physical activity and maturation status on bone mass and geometry in early pubertal girls. *Scand J Med Sci Sports*. 2005;15(2):100-6.

TABLES

Table 1. Descriptive characteristics of the participants

Characteristics	**All (n=121)**	**Swimmers (n=41)**	**Footballers (n=37)**	**Cyclists (n=29)**	**Controls (n=14)**
Age (yrs)	13.1 (1.0)	13.4 (1.0)^{b,dd}	12.8 (0.9)	13.2 (1.0)^{d}	12.3 (0.5)
Stature (cm)	159.9 (10.6)	165.5 (9.7)^{bb,d}	155.2 (9.3)	160.8 (9.9)	154.5 (9.9)
Pubertal maturation (I/II/III/IV/V) (%)	(18/29/21/30/2)	(13/25/13/46/3)	(24/35/25/16)	(13/28/28/28/3)	(29/21/21/29)
Body composition					
Body mass (kg)	48.7 (10.4)	52.4 (9.0)^{bb}	44.3 (7.9)	49.5 (12.3)	48.3 (13.0)
BMI (kg/m^2)	18.9 (2.3)	19.0 (1.7)	18.3 (1.4)	18.9 (3.3)	20.0 (3.4)
Lean mass (kg)	37.6 (8.4)	41.6 (9.1)^{b,dd}	35.4 (7.2)	37.7 (7.5)	31.7 (5.5)
Fat mass (kg)	8.5 (5.5)	8.3 (3.2)	6.6 (2.4)	8.6 (7.2)	14.1 (8.5)^{a,bb,c}
Micronutrient status					
Total Calcium (mg/dl)	9.98 (0.41)	10.01 (0.46)	9.97 (0.4)	9.94 (0.41)	10.0 (0.35)
25 (OH)D (µg/l)	14.13 (1.25)	13.75 (1.19)	14.44 (1.63)	14.38 (0.58)	13.92 (0.94)
Physical activity and fitness					
MVPA (min/day)	101.3 (33.8)	85.9 (30.4)	119.8 (29.7)^{aa,d}	107.2 (33.3)^{a}	83.2 (26.8)
Vertical jump height (cm)	41.0 (6.7)	42.3 (6.9)^{d}	41.4 (6.0)^{d}	41.0 (6.8)	35.9 (5.8)
CRF (No of shuttles)	69.3 (24.2)	69.6 (20.3)^{dd}	82.9 (17.6)^{a,c,dd}	69.6 (21.2)^{dd}	31.8 (16.1)
Weekly training hours (h)	7.5 (4.8)	9.5 (5.1)^{cc}	10.0 (2.3)^{cc}	5.1 (2.1)	-
Bone mineral density (DXA)					
TBLH BMD (g/c m^2)	0.908 (0.079)	0.918 (0.067)^{d}	0.931 (0.071)^{dd}	0.905 (0.086)^{d}	0.828 (0.071)
Lumbar Spine BMD (g/cm^2)	0.872 (0.112)	0.892 (0.114)^{d}	0.883 (0.095)	0.867 (0.122)	0.791 (0.101)
Femoral Neck BMD (g/c m^2)	0.9516 (0.110)	0.948 (0.098)^{d}	1.001 (0.081)^{dd}	0.975 (0.192)^{d}	0.832 (0.118)
Total Hip BMD (g/c m^2)	0.968 (0.119)	0.962 (0.107)^{dd}	1.034 (0.085)^{a,c,dd}	0.959 (0.116)^{dd}	0.830 (0.116)
Legs BMD (g/c m^2)	1.084 (0.113)	1.091 (0.010)	1.124 (0.106)	1.077 (0.116)	0.975 (0.103)
Arms BMD (g/c m^2)	0.750 (0.068)	0.784 (0.071)^{b,dd}	0.736 (0.047)	0.747 (0.069)^{d}	0.690 (0.049)
Bone geometry (HSA)					
CSA (mm^2)	134.9 (22.7)	137.2 (20.2)^{dd}	140.9 (20.4)^{dd}	135.9 (22.7)^{d}	109.8 (21.0)
Z (mm^3)	530.9 (126.5)	558.3 (121.4)^{dd}	548.1 (116.7)^{dd}	530.8 (123.3)^{dd}	395.0 (123.4)
CSMI (mm^4)	8331.5 (2644)	8943.5 (2574)^{d}	8471.6 (2607)^{d}	8403.1 (2552)^{d}	6020.7 (2673)

Values presented as mean ± SD. BMD: Bone mineral density, BMI: Body mass index, CRF: Cardiorespiratory fitness, CSMI: Cross sectional moment of inertia, CSA: Cross sectional area, DXA: Dual-energy X-ray absorptiometry, MVPA: Moderate to vigorous physical activity, Z: section modulus, 25(OH)D: 25-hydroxyvitamin D.
Superscript letters denote a higher significant difference with: a (swimmers), b (footballers), c (cyclists), d (controls), ^{a,b,c,d} $p<0.05$, ^{aa,bb,cc,dd} $p<0.001$.

Table 2. Multiple regression models for aBMD variables in adolescent male athletes

	Predictors	β STD	sr^2 values	P values		Predictors	β STD	sr^2 values	P values
TBLH aBMD (R^2=0.75)	**Footballers**	**.374**	**.031**	**<.001**	**Total Hip aBMD (R^2=0.53)**	**Footballers**	**.549**	**.068**	**<.001**
	Swimmers	.077	.002	.404		Swimmers	.161	.007	.211
	Cyclists	.139	.006	.114		Cyclists	.212	.014	.080
	Stature	.056	.000	.662		Stature	.216	.007	.215
	Lean mass	**.617**	**.045**	**<.001**		Lean mass	.226	.006	.238
	Fat mass	**.189**	**.015**	**.013**		Fat mass	-.020	.000	.857
	Calcium	.082	.006	.125		Calcium	.109	.010	.137
	25(OH)D	.083	.006	.120		25(OH)D	.035	.001	.628
	MVPA	-.003	.000	.955		MVPA	.026	.000	.741
	Vertical jump	**.146**	**.010**	**.043**		Vertical jump	.133	.008	.192
	CRF	.136	.006	.115		CRF	.102	.003	.382
Lumbar Spine aBMD (R^2=0.59)	Footballers	.094	.002	.475	**Legs aBMD (R^2=0.75)**	**Footballers**	**.304**	**.034**	**<.001**
	Swimmers	-.061	.001	.603		Swimmers	.024	.002	.689
	Cyclists	-.008	.000	.945		Cyclists	-.061	.003	.347
	Stature	-.090	.001	.582		Stature	.068	.001	.596
	Lean mass	**.703**	**.058**	**<.001**		**Lean mass**	**.614**	**.046**	**<.001**
	Fat mass	-.014	.000	.885		Fat mass	.147	.008	.067
	Calcium	.084	.006	.222		Calcium	.055	.003	.305
	25(OH)D	**.139**	**.016**	**.043**		25(OH)D	.067	.004	.212
	MVPA	.007	.000	.927		MVPA	-.021	.000	.711
	Vertical jump	**.203**	**.019**	**.028**		Vertical jump	.107	.005	.153
	CRF	.000	.000	1.000		CRF	.153	.008	.076
Femur Neck aBMD (R^2=0.49)	**Footballers**	**.486**	**.053**	**.001**	**Arms aBMD (R^2=0.76)**	Footballers	.140	.004	.161
	Swimmers	.131	.005	.326		Swimmers	.170	.008	.058
	Cyclists	.208	.013	.099		Cyclists	.158	.008	.066
	Stature	**.380**	**.021**	**.038**		**Stature**	**.235**	**.013**	**.016**
	Lean mass	.052	.000	.792		**Lean mass**	**.931**	**.161**	**<.001**
	Fat mass	.074	.002	.515		Fat mass	.132	.007	.069
	Calcium	.077	.005	.311		**Calcium**	**.103**	**.009**	**.049**
	25(OH)D	.015	.000	.847		**25(OH)D**	**.104**	**.009**	**.045**
	MVPA	.039	.001	.635		MVPA	-.037	.001	.503
	Vertical jump	.184	.015	.083		Vertical jump	.058	.002	.404
	CRF	.154	.008	.208		CRF	.066	.002	.406

β: standardised regression coefficient, aBMD: Areal bone mineral density, CRF: Cardiorespiratory fitness, MVPA: Moderate to vigorous physical activity, sr^2: Squared semi-partial correlation coefficients, 25(OH)D: 25-hydroxyvitamin D.

Table 3. Multiple regression models for bone geometry estimates in adolescent male athletes

	Predictors	β STD	sr^2 values	P values		Predictors	β STD	sr^2 values	P values
CSA	**Footballers**	**.322**	**.023**	**.004**	**Z**	Footballers	.157	.005	.109
(R^2=0.72)	Swimmers	.068	.001	.495	**(R^2=0.78)**	Swimmers	.019	.000	.831
	Cyclists	.123	.005	.190		Cyclists	.005	.000	.951
	Stature	**.394**	**.023**	**.004**		**Stature**	**.417**	**.025**	**.001**
	Lean mass	**.370**	**.017**	**.014**		**Lean mass**	**.430**	**.023**	**.001**
	Fat mass	-.010	.000	.905		Fat mass	-.040	.001	.592
	Calcium	.025	.001	.657		Calcium	.016	.000	.755
	25(OH)D	-.038	.001	.498		25(OH)D	-.054	.003	.281
	MVPA	.103	.008	.093		**MVPA**	**.142**	**.014**	**.010**
	Vertical jump	.029	.000	.713		Vertical jump	-.024	.000	.734
	CRF	.178	.010	.051		**CRF**	**.207**	**.014**	**.012**
CSMI	Footballers	.064	.001	.506					
(R^2=0.78)	Swimmers	-.030	.000	.732					
	Cyclists	-.038	.000	.645					
	Stature	**.338**	**.017**	**.005**					
	Lean mass	**.568**	**.039**	**<.001**					
	Fat mass	-.087	.003	.245					
	Calcium	-.011	.000	.820					
	25(OH)D	-.048	.002	.334					
	MVPA	**.140**	**.014**	**.011**					
	Vertical jump	-.090	.003	.199					
	CRF	**.183**	**.011**	**.024**					

β: standardised regression coefficient, aBMD: Areal bone mineral density, CRF: Cardiorespiratory fitness, MVPA: Moderate to vigorous physical activity, sr^2: Squared semi-partial correlation coefficients, 25(OH)D: 25-hydroxyvitamin D.