Title

Effect of maturational timing on bone health in male adolescent athletes engaged in different sports: the PRO-BONE study

Authors:

Esther Ubago-Guisado[a,b], Dimitris Vlachopoulos[b], Alan R. Barker[b], Tore Christoffersen[c], Brad Metcalf[b,d], Luis Gracia-Marco[b,e,f,*]

Affiliations:

[a]IGOID Research Group, University of Castilla-La Mancha, Spain, [b]Children's Health and Exercise Research Centre, Sport and Health Sciences, University of Exeter, United Kingdom, [c]Department of Health and Care Sciences, UiT The Arctic University of Norway, Norway, [d]University of Exeter Medical School, United Kingdom Growth, Exercise, Nutrition and Development Research Group, University of Zaragoza, Spain, [f]PROFITH "PROmoting FITness and Health Through Physical Activity" Research Group, Department of Physical Education and Sport, Faculty of SportSciences, University of Granada, Spain

Abstract

Objectives: To describe differences in bone outcomes according to biological age (years from peak height velocity, PHV) in male athletes participating in osteogenic (OS, football or soccer) or non-osteogenic (NOS, swimming or cycling) sports.

Design: A 12-month longitudinal study.

Methods: 104 adolescent male athletes (12-14 years old) were measured at baseline and after one year: OS group (n=37 footballers) and NOS group (n=39 swimmers and n=28 cyclists). Years from PHV (-2 to +2) was used as a maturational landmark. Bone mineral content (BMC) was assessed using DXA. Hip structural analysis assessed cross-sectional area (CSA), cross-sectional moment of inertia (CSMI) and section modulus (Z) at the femoral neck (FN).Trabecular bone score (TBS) assessed the trabecular texture of the lumbar spine (LS). . Quantitative ultrasound measured bone stiffness.. A multilevel regression model adjusted by hours of training was fitted.

Results: Compared to the NOS group, the OS group had significantly greater TBLH BMC from PHV to +2 years from PHV (from 9.5% to 11.3%, respectively); LS BMC from -1 year from PHV to PHV (from 9.8% to 9.9%); hip BMC (from 11.6% to 22.9%), FN BMC (from 12.0% to 15.9%), TBS (from 4.2% to 4.8%) and stiffness index (from 11.9% to 23.3%) from -1 year from PHV to +2 years from PHV; and cross sectional area (CSA) (from 8.4% to 18.8%), section modulus (Z) (from 5.5% to 22.9%) and cross sectional moment of inertia (CSMI) (from 10.6% to 23.3%) from -2 years from PHV to +2 years from PHV. In addition, there was a significant trend for the differences in bone outcomes (between groups) to increase with biological age (all $p<0.05$) except for LS BMC and TBS.

Conclusions: These findings underline the differential bone response to different types of sport throughout the years surrounding PHV in male adolescent athletes.

Keywords: bone ultrasound; DXA; hip structural analysis; maturity; peak height velocity; trabecular bone score.

Clinical trial registration: ISRCTN17982776

Introduction

During puberty, there are important changes in stature, body size, proportions of muscle and fat mass [1], and also changes in bone mass as a result of increased bone size, which depend on both bone length and width [2]. It is an important period to maximize bone accrual as the skeleton suffers rapid changes due to the processes of growth, modelling, and remodelling, with about a 5 % additional bone formed by every remodelling cycle compared to resorption [3]. Also, bone mineral accrual depends on level of maturity and is site-specific (REF?). Previous longitudinal studies have concluded that the timing, pattern and magnitude of bone accrual is a highly-individualised process, and therefore, comparisons should be based on biological rather than chronological age [4].

In this regard, using peak height velocity (PHV) during growth is a useful alternative [5]. PHV is the period of time of maximum growth in stature and years from PHV is considered in terms of time before and time after the PHV [6]. In boys, age at PHV occurs approximately between 13 and 14 years old [6], and it is considered an appropriate marker of somatic maturity. During the period between -2 to 2 years from PHV, males and females accrue 39% of their adult total body bone mineral content (BMC), 43% of their adult lumbar spine (LS) BMC, 46% of their adult total hip BMC and 33% of their adult femoral neck (FN) BMC [2].

Not only bone development is site-specific, but also the type of sport affects skeleton areas in a different way [7]. Physical activity has been positively related to bone mass in adolescents [8]. Weight-bearing activities are known to increase bone mass with previous cross-sectional studies in children [9] and adolescents [10] using dual energy x-ray absorptiometry (DXA). This previous studies suggested that those engaged in osteogenic sports (OS, i.e. football, basketball or handball) had higher BMC and areal bone mineral density (aBMD) compared to those engaged in non-osteogenic sports (NOS, i.e. swimming or cycling). This is due to the fact that bone development is dependent on the mechanical load produced during the specific sport practised and the forces applied on the skeleton that trigger bone modelling and remodelling [11].

Bone strength and fracture risk depends not only on aBMD and BMC, but also on bone structure and strength [12]. In this regard, Hip Structural Analyses (HSA) provides information about bone geometry of the FN, a clinically relevant site related to fracture risk [13]. Another technique such

as quantitative ultrasound (QUS) provides useful information about the stiffness of the calcaneus, a robust indicator of bone density [14]. A cross sectional study demonstrated that adolescent athletes who participate in OS have higher CSA, CSMI, Z and bone stiffness compared to NOS [10]. Moreover, the trabecular bone score (TBS) of the LS can predict fracture risk and fragility of the LS [15]. Although most of the knowledge about TBS refers to adult population, TBS usually increases with growth and may provide very valuable information about bone quality in young populations [16]. To our knowledge, there is a lack of studies using the combination of these techniques in adolescent male athletes [17].

Despite the established importance of the years surrounding PHV for the accrual of bone mass, there is limited evidence evaluating the effects of osteogenic and non-osteogenic sports on bone outcomes in male adolescent athletes, and combining DXA, HSA, TBS and QUS outcomes. Therefore, the aim of the current investigation was to investigate differences in bone outcomes according to years from PHV in young male athletes participating in OS (football) or NOS (swimming or cycling). We hypothesised that adolescent athletes engaged in OS will not only present greater bone outcomes when aligned against years from PHV compared to those in NOS, but also that the magnitude of the difference will increase with the level of maturity.

Methods

The present study shows a 12-month longitudinal analysis of sport participation as part of the longitudinal PRO-BONE (effect of a PROgram of short bouts of exercise on BONE health in adolescents involved in different sports) study, whose purpose, methodology and inclusion/exclusion criteria have been fully described elsewhere [18]. The inclusion and exclusion criteria were: 1) male adolescents 12–14 years old, engaged (≥3 h/week) in osteogenic (football or soccer) or non-osteogenic (swimming or cycling) sports for the last 3 years or more; 2) not taking part in another clinical trial; 3) not having an acute infection lasting until < 1 week before inclusion; 4) to be free of any medical history of diseases or medications affecting bone metabolism; 5) to be white Caucasian.

For the present study, data were obtained at baseline (T0) during autumn/winter 2014/15 and at follow-up (T1) during autumn/winter 2015/2016 (mean difference of visits = 372 days). After exclusion of three participants who dropped out from the study before T1, the study sample was

composed by one hundred and four 12-14 year old adolescent male athletes. Baseline anthropometry and bone outcomes did not differ between those who withdrew and those who continued in the study (data not shown).

Participants and parents/guardians were contacted through athletic clubs in the South West of England to participate in the study. Informative meetings were organized to explain the project and answer questions that could arise. At the end of these meetings, consent forms and information letters were given for consideration and reminders calls were performed to those that did not send the consent form to check whether they wished or not to participate.

Written informed consent and assent was signed from parents and participants, respectively. The methods of the study have been approved by: 1) the European Commission (nº. 618496); 2) the University of Exeter (nº. 2014/766) and 3) the National Research Ethics Service Committee (nº. 14/SW/0060).

Body mass (kg) and stature (cm) were measured following standard procedures and body mass index (BMI, kg/m^2) was calculated.

Years from PHV was used as a maturational landmark due to its relevance in longitudinal studies [4, 5], and was predicted using age and height in a validated algorithm in healthy children [19], as follows: -7.999994 + (0.0036124 x (age x stature in cm)); R^2 = 0.90; standard error = 0.5 years. Each participant had a chronological age and biological age (calculated as years from PHV) associated with each testing occasion. Biological age categories were constructed using 1-year intervals such that the -1 year from PHV group included observations between -0.49 and -1.50 years from (ie, before) PHV, as performed in previous studies [2, 4]. According to the participants' characteristics, five groups were created (at -2 years from PHV, at -1 year from PHV, at PHV, at +1 year from PHV and at +2 years from PHV).

A Lunar Prodigy DXA scanner (GE Healthcare Inc., Wisconsin, USA) was used to assess BMC (g), and whole body lean mass (g). The whole body (total body less head, TBLH), LS (L1-L4) and the mean of right and left hip scans (total hip, and femoral neck, FN) were used to measure BMC. All DXA scans and subsequent in-software analyses were completed by the same researcher and the

GE encore software (2006, version 14.10.022). The coefficients of variation have been reported in previous studies as 0.81% for TBLH BMC and 0.89% for LS BMC in 14-16 year olds [20].

HSA software was used to estimate the hip geometry of the FN (the mean of right and left hip scans) and the following variables were used: 1) CSA (mm^2), which is the total bone surface area of the hip excluding the soft tissue area and the trabecular bone; 2) Z (mm^3), which is an indicator of maximum bending strength in a cross section; and 3) CSMI (mm^4), which is an index of structural rigidity and reflects the distribution of mass in the centre of a structural element. The coefficients of variation of these variables have been reported in previous studies and range from 7.9 % to 11.7% [21].

TBS is a DXA based technological tool that provides an index of bone microarchitectural texture in the LS that predicts fracture risk independently of aBMD [15]. All TBS analyses were performed by the same trained researcher using the TBS iNsight Software (Medimaps, research version 3.0, Pessac, France). The coefficients of variation of TBS in relation to BMC are between 1.1 to 1.9% [15].

QUS measurements to measure bone stiffness were carried out by Lunar Achilles Insight (TM Insight GE Healthcare, Milwaukee, WI, USA). The real-time image of the calcaneus and the region of interest assure the reliability and validity of the measures in paediatric studies [22]. Both feet were measured twice and the mean was calculated. Then, the mean of both means was used for statistical analyses. The precision data for QUS in children has been reported as 1.8% for stiffness [23].

Statistical analyses were performed using SPSS version 22.0 for Windows (IBM Corp, New York, USA) and the significance level was set at $p<0.05$. Data were expressed as mean (standard deviation, SD) unless otherwise stated. Normal distribution of variables was checked and verified using Shapiro-Wilk's test and visual check of histograms. Independent sample t-tests (table 1 and supplementary tables 1 and 2) were performed to assess: descriptive differences between groups (OS and NOS) at PHV; differences in chronological age by years from PHV (from -2 to +2) and; raw differences in bone outcomes between OS and NOS groups by years from PHV (from -2 to +2), respectively. Hierarchical linear models (Figures 1 and 2) were constructed using a multilevel modelling technique commonly used in the analysis of the repeated measures/longitudinal data. Multi-level modelling accounts for between-child variation by modelling within-child trajectories. This is

achieved by entering 'years from PHV' into the model as a random effect, thus allowing the 'years from PHV'-related trajectories to vary for each individual child. In addition, analysis of covariance (ANCOVA) was used to assess mean-adjusted differences in bone outcomes between OS and NOS groups at each category of years from PHV (Figures 1 and 2). Hours of training was used as a covariate due to the significant differences observed between OS and NOS at PHV (see table 1).

Results

Descriptive characteristics of the participants at PHV by type of sport are shown in table 1. The OS group trained more hours per week compared to NOS group ($p<0.001$) but there were not significant differences in age, stature, body mass, BMI and lean mass between the OS and NOS groups. In addition, OS and NOS athletes did not differ in chronological age at any PHV (supplementary table 1).

Results of unadjusted bone outcomes by years from PHV and type of sport are presented in supplementary table 2. Overall, all bone outcomes increased during growth both in the OS and NOS group. The OS group had higher values on all bone outcomes compared to the NOS. More specifically, CSA was higher from -2 to +2 years from PHV; hip BMC, FN BMC, Z, CSMI and stiffness index from -1 to +2 years from PHV; TBS from -1 to +1 years from PHV; LS from -1 year from PHV to PHV and; TBLH at -1, +1 and +2 years from PHV.

Figure 1 presents BMC-adjusted data by years from PHV and type of sport. Compared to the NOS group, the OS group had significantly greater TBLH BMC from PHV to +2 years from PHV, LS BMC from -1 year from PHV to PHV and, hip and FN BMC from -1 to +2 years from PHV (all $p<0.05$). In addition, for TBLH, the interaction coefficient was 47.5g ($p=0.012$), so for every 1 unit increase in years from PHV, the BMC of those in the OS group goes up 47.5g more than those in the NOS group. For example, -2 years from PHV, the BMC of the OS group was 56.7g greater than the NOS group, yet +2 years from PHV, the BMC of the OS group was 246.7g greater than the NOS group. The interaction coefficient for hip was 1.9g ($p=0.014$) and for FN 0.1g ($p=0.016$). However, no interaction was found for LS ($p=0.253$).

Figure 2 presents HSA, TBS and stiffness index-adjusted data by years from PHV and type of sport. The OS group showed significantly greater values in CSA, Z and CSMI from -2 to +2 years from PHV compared to the NOS group. The OS group had significantly greater scores in TBS and stiffness index from -1 to +2 years from PHV compared to the NOS group. Moreover, for CSA, the interaction coefficient was 5.7mm^2 (p=0.013), so for every 1 unit increase in years from PHV, the CSA of those in the OS group goes up 5.7mm^2 more than those in the NOS group. The interaction coefficient for Z was 38.0mm^3 (p=0.006), for CSMI was 642.0mm^4 (p=0.014) and for stiffness index was 4.0 units (p=0.023). However, no interaction was found for TBS (p=0.712).

Discussion

The present study describes bone outcomes from -2 years before and +2 years after PHV, which represents a crucial period of bone development [4, 24]. The main findings of this study are: 1) OS athletes had greater BMC, HSA estimates, TBS and stiffness index at a given years from PHV compared to NOS athletes; 2) the differences in bone outcomes between OS and NOS groups increase with biological age.

In the present study, the OS and NOS groups showed a linear increase in all bone outcomes from -2 to +2 years from PHV, supporting the idea that bone accrual occurs because the remodelling activity is greater than the resorption activity during puberty [3]. For BMC-adjusted outcomes, differences between groups favouring the OS group became evident from -1 year from PHV at hip and FN, and from PHV at TBLH. In this regard, the lack of significant differences at -2 years from PHV might be affected the small sample size of each group at this PHV. The percentage of difference between groups from -2 to +2 years from PHV ranged from 5.7 to 11.3% for TBLH, from 4.8 to 22.9% for hip and from 9.7 to 15.9% for FN. Our results did not show an interaction effect for LS BMC, and significant differences between groups were only observed from -1 year from PHV to PHV, favouring the OS group. In addition, we observed an almost significant trend in the differences between OS and NOS groups at +1 years from PHV (p=0.052). This can be due to the fact that the differences in bone tissue at each bone site are influenced by the environment and the type of specific actions of each sport [25]. In our study, the type of sport practiced by the OS group is football, in which

208 the lower limbs suffer an important mechanical load, creating high strains that may be a powerful
209 stimuli to increase bone mass [26]. Besides, a previous study reported that the maximum speed in LS
210 BMC occurs slightly later (approximately +0.7 years from PHV) compared to other sites, such as FN
211 BMC that occurs at +0.5 years from PHV [24]. Based on this, it could be that the bone accrual at LS
212 may not have occurred at the same speed as in other regions shown in this manuscript.

213 For comparison and discussion purposes, years from PHV from other investigations has been
214 estimated using validated algorithms for boys and girls (both R=0.90) [19]. In a previous cross-sectional
215 study with adolescent athletes from this cohort we showed that the footballers at -1 year from PHV
216 had 5 to 7% more TBLH aBMD and 10 - 12% more hip aBMD compared with swimmers and cyclists
217 at PHV [10]. Another cross-sectional study in adolescent athletes at +2 years from PHV concluded that a
218 NOS group (swimmers) had lower BMC in the total body, lower limbs and LS compared to OS
219 (gymnastics, basketball, and handball) [27]. Moreover, adolescent male cyclists at +3 years from PHV
220 showed a 10% lower BMC in the lower limbs compared to an active control group [28]. A cross-
221 sectional study conducted in female swimmers at -1 year from PHV showed 5-17% lower aBMD at
222 FN, pelvis and hip compared to footballers at -1 year from PHV [9]. Similarly, an 8-month longitudinal
223 study [29] comparing female swimmers at +1 year from PHV but footballers at +2 years from PHV
224 showed swimmers had 25.3% lower aBMD at the hip than footballers. These results in NOS groups
225 mostly agree with ours in swimmers and cyclists, who had lower BMC values not only at the hip and
226 FN but also at TBLH compared to the OS group (footballers).

227 In relation to bone geometry and bone quality, our results are in line with those of a cross-
228 sectional study with this cohort [10], in which footballers at -1 year from PHV had higher CSA, CSMI,
229 Z and stiffness index (8-21%) compared with swimmers and cyclists at PHV. In the present study, the
230 percentage of difference between groups from -2 to +2 years from PHV ranged from 8.4 to 18.8% for
231 CSA, from 5.6 to 22.9% for Z, from 10.6 to 23.3% for CSMI and from 7.5 to 23.3% for SI. According
232 to a previous review in 10 to 30 year-old athletes, the adaptations observed in bone geometry
233 outcomes consequence of sports practice are different depending on the type of sport. This is due to
234 the fact that the skeleton is adapted to the load resulting from sport-specific actions [7]. As for the LS
235 BMC, our results did not show an interaction effect for TBS which can be explained by the reasons

mentioned above but significant differences between groups were observed from -1 year from PHV. Since TBS assesses DXA images of the LS scans the same reasons as highlighted for LS BMC may explain the lack of interaction. TBS is a novel bone score parameter [30] of bone microarchitectural texture in the LS and little is known about its use in children. In this regard, a recent cross-sectional study, though in female adults, showed that footballers, squash players and power lifters had about 2%, 3% and 4% higher TBS, respectively, compared with a NOS (swimmers) [30]. Similar to our findings, a longitudinal study in girls at +4 years from PHV found that CSA in the FN increased more in footballers (3.2% vs. 2.3%) than swimmers after 8 months of sport participation [29]. In other sports, Maimoun et al. showed that young girls at +1 year from PHV engaged in artistic gymnastics (OS) had greater CSA and Z (20.3% and 21.8%, respectively) compared to swimmers (NOS) at +2 years from PHV [26]. These findings could be extrapolated to our study, in which the practice of OS promotes a higher CSA, CSMI, Z, TBS and stiffness index compared to that of NOS before and after PHV.

A recent meta-analysis found that the differences between swimmers and the athletes of osteogenic sports increased with age [31]. Similarly, our findings show that the difference in BMC outcomes, geometry outcomes and stiffness index between OS and NOS groups increase with biological age, from -2 to +2 years from PHV. This suggests that participation in NOS may affect the acquisition of a high peak bone mass (compared to that of OS) during adolescence. On average, 26% of adult total body BMC is accrued during the 2 years around peak BMC velocity [24] and achieving a high peak bone mass is essential to protect against future bone fractures and diseases [32]. It has also been suggested that sport stimuli during childhood and adolescence may provoke a permanent change on bone metabolism that promotes enhanced accrual throughout growth [33]. Therefore, we suggest the practice of OS during the years surrounding PHV (from -2 to +2), since it is an important period characterized by significant linear growth and BMC accrual [24] in order to contribute to the prevention of osteopenia and/or osteoporosis later in life.

This is the first longitudinal study in male adolescent athletes to investigate the differences in bone quantity (BMC), bone geometry (HSA estimates), bone texture (TBS) and bone quality (stiffness index) between OS (football) and NOS (swimming or cycling) according to biological age. The combination of these techniques provides a thorough insight of bone health during adolescence. To

date, the number of studies using TBS in adolescent male population is very limited and further research is needed to better understand its use in young populations [17, 34]. The number of scans in the -2 years from PHV is relatively small and results should be treated with caution. Despite the present study covers the range of -2 to +2 years from PHV (33% to 46% of adult BMC is accrued in this period [2], future studies with longer follow-up periods will help to better understand bone changes in response to sport participation throughout the adolescence period. Our findings allow results to be compared between sport groups (OS vs NOS) but cannot be compared against non-athletic population due to the lack of a control group (we only had 14 control participants and therefore not enough for a study on biological maturation)Other factors, such as changes in weight and/or nutritional habits may also contribute to the differences between groups. Future studies in girls are needed as the timing of peak BMC accrual occurs at different periods between sexes [16, 35].

Conclusion

These findings suggest that participation in OS during adolescence promotes a greater improvement in bone quantity (BMC), bone geometry (HSA estimates), bone texture (TBS) and bone quality (stiffness index) compared to the practice of NOS. These findings underline the differential bone response to different types of sport throughout the years surrounding PHV in male adolescent athletes.

Practical implications

- This study provides evidence that osteogenic sport athletes (football) had better bone health compared to non-osteogenic sport athletes (swimming and cycling) at a given year from PHV.
- Interestingly, the differences increase with biological age, which may have important implications for the achievement of a high peak bone mass in those engaged in non-osteogenic sport athletes.
- This has been explored by measuring bone quantity, geometry, texture and quality, which adds novelty to this research question.
- From a public health and sport medicine perspective, this is especially important as football,

swimming and cycling are among the most practiced sports worldwide.

Acknowledgments

The authors gratefully acknowledge the sport coaches, school teachers and the CHERC research team for their help to run the study. This work was supported by the European Union Seventh Framework Programme [FP7/2007-2013] under grant agreement n°. PCIG13-GA-2013-618496.

EUG has been funded by University of Castilla-La Mancha within "Contratos postdoctorales para la formación de personal investigador en el marco del Plan Propio de I+D+i, cofinanciados por el Fondo Social Europeo" (2014/10340), and LGM acknowledges "Programa de Captación de Talento – UGR Fellows" as part of "Plan Propio" of the University of Granada (Spain).

EUG analysed the data and drafted the manuscript under the supervision of LGM (principal investigator). DV obtained the data. All authors have critically reviewed and approved this work. The authors gratefully acknowledge the adolescents, parents and sport coaches and schools who helped and participated in this study.

List of abbreviations

aBMD: areal bone mineral density; BMC: bone mineral content; BMI: body mass index; CSA: cross-sectional area; CSMI: cross-sectional moment of inertia; DXA: dual energy x-ray absorptiometry; FN: femoral neck; OS: osteogenic sport; HSA: hip structural analysis; NOS: non-osteogenic sports; LS: lumbar spine; PHV: peak height velocity; TBLH: total body less head; QUS: quantitative ultrasound; TBS: trabecular bone score; Z: section modulus.

315 **References**

316

317 1. Baxter-Jones AD, Maffulli N. Intensive training in elite young female athletes. Effects of
318 intensive training on growth and maturation are not established. *Br J Sports Med* 2002;
319 36(1):13-15.

320 2. Baxter- Jones ADG, Faulkner RA, Forwood MR, Mirwald RL, Bailey DA. Bone mineral
321 accrual from 8 to 30 years of age: an estimation of peak bone mass. *J Bone Miner Metab*
322 2011; 26(8):1729-1739.

323 3. Parfitt AM, Travers R, Rauch F, Glorieux FH. Structural and cellular changes during bone
324 growth in healthy children. *Bone* 2000; 27(4):487-494.

325 4. Nurmi-Lawton JA, Baxter-Jones AD, Mirwald RL, et al. Evidence of sustained skeletal
326 benefits from impact-loading exercise in young females: a 3-year longitudinal study. *J Bone*
327 *Miner Metab* 2004; 19(2):314-322.

328 5. Weeks BK, Beck BR. The relationship between physical activity and bone during
329 adolescence differs according to sex and biological maturity. *J Osteoporos* 2010; 546593.

330 6. Mirwald RL, Baxter-Jones AD, Bailey DA, Beunen GP. An assessment of maturity from
331 anthropometric measurements. *Med Sci Sports Exerc* 2002; 34(4):689-694.

332 7. Tenforde AS, Fredericson M. Influence of sports participation on bone health in the young
333 athlete: a review of the literature. *PM R* 2011; 3(9):861-867.

334 8. Gracia-Marco L, Vicente-Rodríguez G, Casajús JA, Molnar D, Castillo MJ, Moreno LA.
335 Effect of fitness and physical activity on bone mass in adolescents: the HELENA Study. *Eur*
336 *J Appl Physiol* 2011; 111(11):2671-2680.

337 9. Ubago-Guisado E, Gómez-Cabello A, Sánchez-Sánchez J, García-Unanue J, Gallardo L.
338 Influence of different sports on bone mass in growing girls. *J Sports Sci Med* 2015;
339 33(16):1710-1718.

340 10. Vlachopoulos D, Barker AR, Williams CA, et al. The impact of sport participation on bone
341 mass and geometry in adolescent males. *Medicine & Science in Sports & Exercise* 2016;
342 49(2):317-326.

11. Wolff I, Van Croonenborg JJ, Kemper HCG, Kostense PJ, Twisk JWR. The effect of exercise training programs on bone mass: a meta-analysis of published controlled trials in pre- and postmenopausal women. *Osteoporos Int* 1999; 9(1):1-12.
12. Bailey CA, Brooke-Wavell K. Optimum frequency of exercise for bone health: randomised controlled trial of a high-impact unilateral intervention. *Bone* 2010; 46(4):1043-1049.
13. Kaptoge S, Beck TJ, Reeve J, et al. Prediction of incident hip fracture risk by femur geometry variables measured by hip structural analysis in the study of osteoporotic fractures. *J Bone Miner Res* 2008; 23(12):1892-1904.
14. Krieg MA, Barkmann R, Gonnelli S, et al. Quantitative ultrasound in the management of osteoporosis: the 2007 ISCD Official Positions. *J Clin Densitom* 2008; 11(1):163-187.
15. Silva BC, Leslie WD, Resch H, et al. Trabecular Bone Score: A Noninvasive Analytical Method Based Upon the DXA Image. *J Bone Miner Metab* 2014; 29(3):518-530.
16. Messina C, Lastella G, Sorce S, et al. Pediatric dual-energy X-ray absorptiometry in clinical practice: What the clinicians need to know. *European journal of radiology* 2018; 105:153-161.
17. Vlachopoulos D, Barker AR, Ubago-Guisado E, et al. Longitudinal Adaptations of Bone Mass, Geometry and Metabolism in Adolescent Male Athletes. The PRO-BONE Study. *J Bone Miner Res* 2017; 32(11):2269-2277.
18. 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):1-10.
19. Moore SA, McKay HA, Macdonald H, et al. Enhancing a Somatic Maturity Prediction Model. *Med Sci Sports Exerc* 2015; 47(8):1755-1764.
20. Shepherd JA, Wang L, Fan B, et al. Optimal Monitoring Time Interval Between DXA Measures in Children. *Journal of Bone and Mineral Research* 2011; 26(11):2745-2752.
21. 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):112-121.

22. Sioen I, Lust E, De Henauw S, Moreno LA, Jimenez-Pavon D. Associations Between Body Composition and Bone Health in Children and Adolescents: A Systematic Review. *Calcif Tissue Int* 2016; 99(6):557-577.

23. Jaworski M, Lebiedowski M, Lorenc RS, Trempe J. Ultrasound bone measurement in pediatric subjects. *Calcif Tissue Int* 1995; 56(5):368-371.

24. Bailey DA, McKay HA, Mirwald RL, Crocker PRE, Faulkner RA. A six-year longitudinal study of the relationship of physical activity to bone mineral accrual in growing children: the University of Saskatchewan bone mineral accrual study. *J Bone Miner Res* 1999; 14(10):1672-1679.

25. Iuliano-Burns S, Stone J, Hopper JL, Seeman E. Diet and exercise during growth have site-specific skeletal effects: a co-twin control study. *Osteoporos Int* 2005; 16(10):1225-1232.

26. Maïmoun L, Coste O, Philibert P, et al. Peripubertal female athletes in high-impact sports show improved bone mass acquisition and bone geometry. *Metabolism* 2013; 62(8):1088-1098.

27. Dias Quiterio AL, Carnero EA, Baptista FM, Sardinha LB. Skeletal mass in adolescent male athletes and nonathletes: relationships with high-impact sports. *J Strength Cond Res* 2011; 25(12):3439-3447.

28. Olmedillas H, Gonzalez-Aguero A, Moreno LA, Casajus JA, Vicente-Rodriguez G. Bone related health status in adolescent cyclists. *PLoS One* 2011; 6(9):e24841.

29. Ferry B, Lespessailles E, Rochcongar P, Duclos M, Courteix D. Bone health during late adolescence: Effects of an 8-month training program on bone geometry in female athletes. *Joint Bone Spine* 2013; 80(1):57-63.

30. Heinio L, Nikander R, Sievanen H. Association between long-term exercise loading and lumbar spine trabecular bone score (TBS) in different exercise loading groups. *J Musculoskelet Neuronal Interact* 2015; 15(3):279-285.

31. Gomez-Bruton A, Montero-Marín J, González-Agüero A, et al. The Effect of Swimming During Childhood and Adolescence on Bone Mineral Density: A Systematic Review and Meta-Analysis. *Sports Med* 2015; 46(3):365-379.

399 32. Rizzoli R, Bianchi ML, Garabédian M, McKay HA, Moreno LA. Maximizing bone mineral
400 mass gain during growth for the prevention of fractures in the adolescents and the elderly.
401 *Bone* 2010; 46(2):294-305.
402 33. Gunter K, Baxter-Jones AD, Mirwald RL, et al. Impact exercise increases BMC during
403 growth: an 8-year longitudinal study. *J Bone Miner Res* 2008; 23(7):986-993.
404 34. Shawwa K, Arabi A, Nabulsi M, et al. Predictors of trabecular bone score in school children.
405 *Osteoporos Int* 2016; 27(2):703-710.
406 35. Baxter-Jones AD, Mirwald RL, McKay HA, Bailey DA. A longitudinal analysis of sex
407 differences in bone mineral accrual in healthy 8-19-year-old boys and girls. *Ann Hum Biol*
408 2003; 30(2):160-175.

409

Figure 1. Bone mineral content (BMC) according to type of sport (osteogenic vs. non-osteogenic) aligned by years from peak high velocity (PHV), where 0 is the PHV. Results (mean and SEM) are adjusted by hours of training. TBLH, total body less head; LS, lumbar spine; FN, femoral neck. Asterisk shows significant differences between type of sports at each biological age category ($p<0.05$).

Figure 2. Hip structural analysis (HSA) of the femoral neck (FN), trabecular bone score (TBS) of the lumbar spine (LS) and stiffness index of the calcaneus according to type of sport (osteogenic vs. non-osteogenic) aligned by years from peak high velocity (PHV), where 0 is the PHV. Results (mean and SEM) are adjusted by hours of training. CSA, cross sectional area; Z, section modulus; CSMI, cross sectional moment of inertia. Asterisk shows significant differences between type of sports at each biological age category ($p<0.05$).

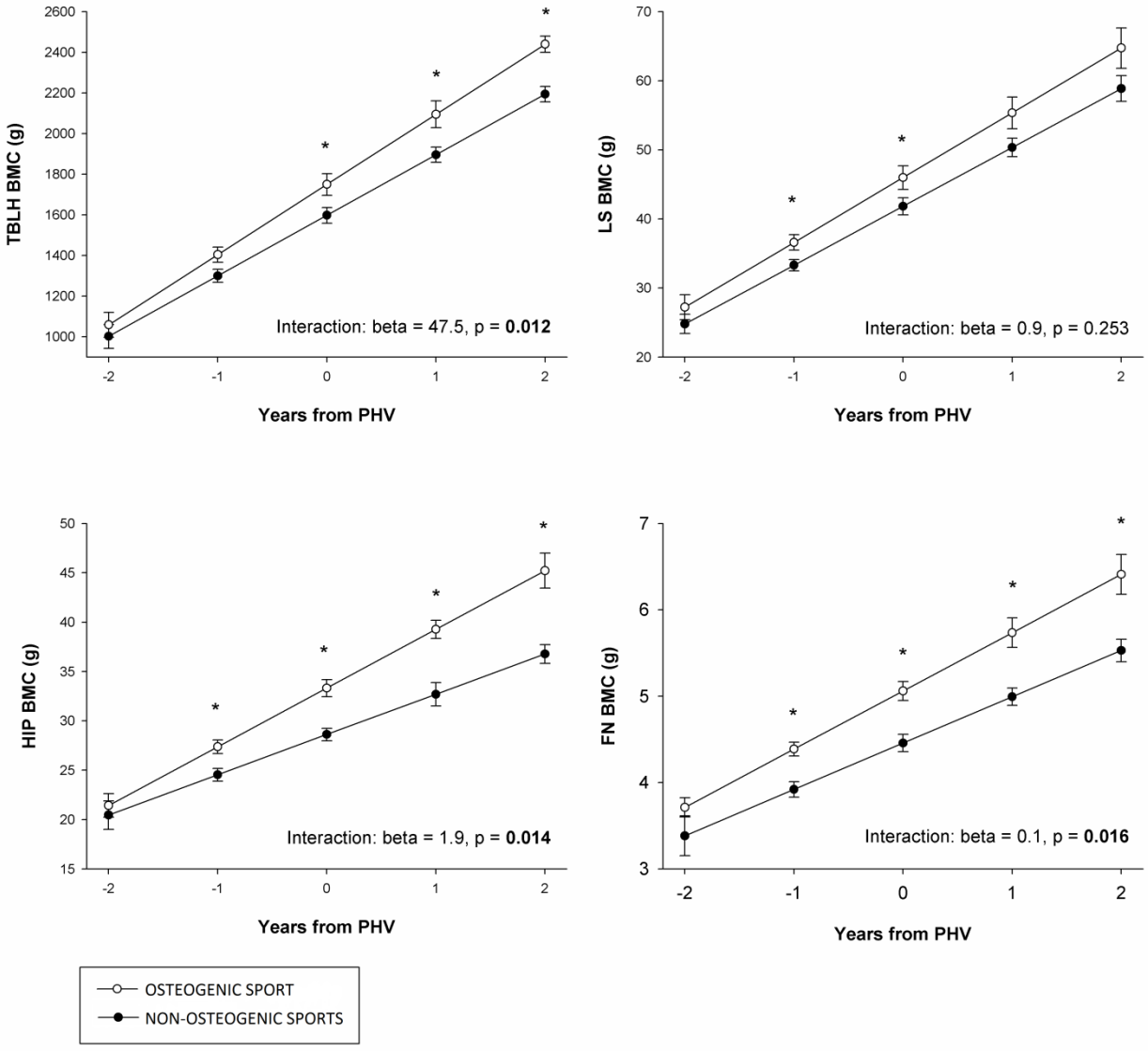
Table 1. Descriptive data at peak height velocity (PHV).

	Osteogenic sport (N=23)	Non-osteogenic sports (N= 38)
Age (years)	13.7 (0.4)	13.6 (0.4)
Stature (cm)	161.9 (5.7)	163.7 (5.8)
Body mass (kg)	49.4 (5.9)	51.7 (8.5)
BMI (kg/m^2)	18.8 (1.4)	19.2 (2.5)
Lean mass (kg)	40.10 (5.32)	39.51 (4.85)
Hours of training	9.4 (1.6)*	6.4 (2.9)

Values presented as mean (SD).

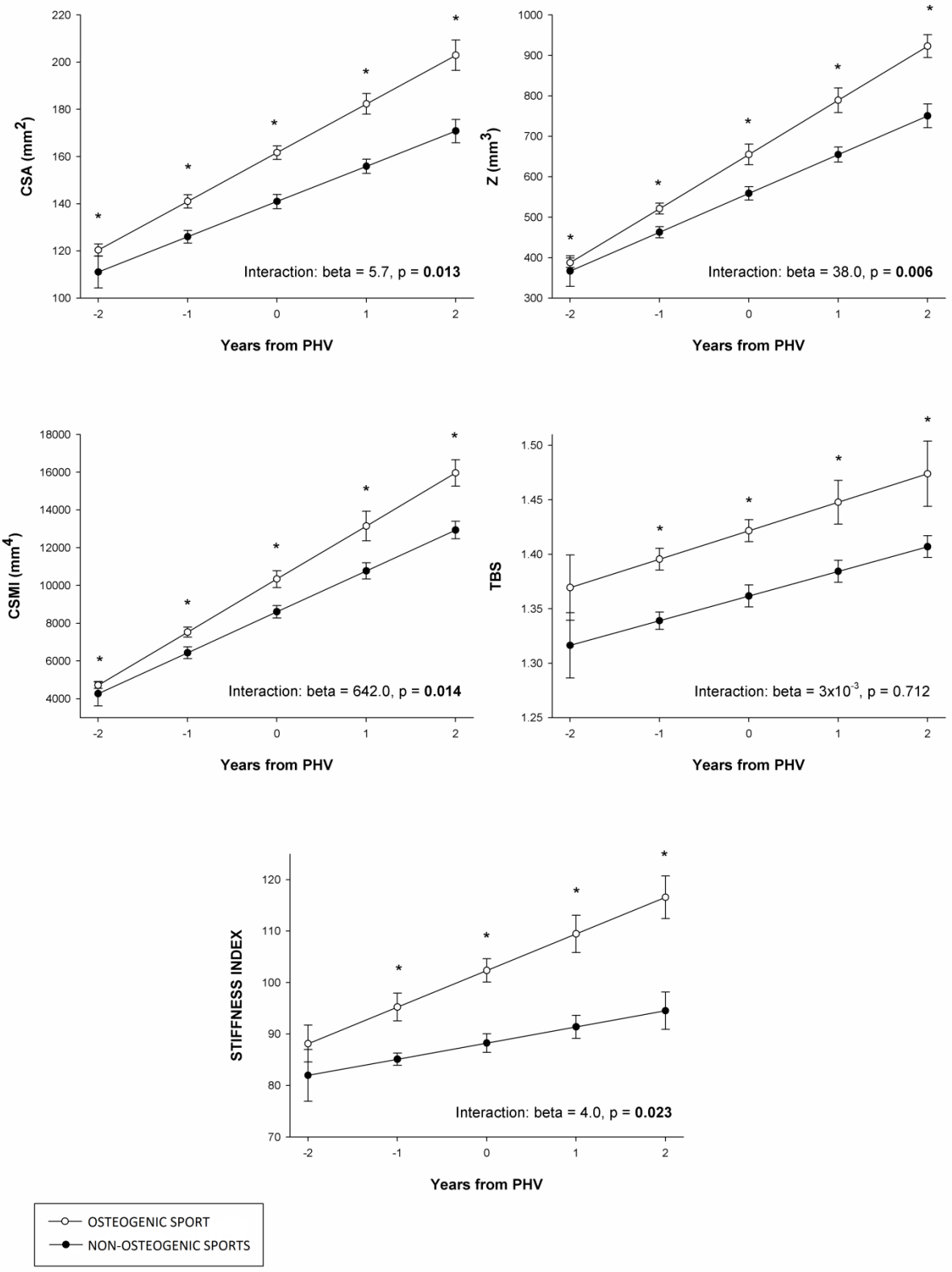
Differences between osteogenic and non-osteogenic sports at PHV * $p<0.001$

BMI, body mass index.


TBLH BMC (g)
Interaction: beta = 47.5, p = 0.012
Years from PHV
LS BMC (g)
Interaction: beta = 0.9, p = 0.253
Years from PHV
HIP BMC (g)
Interaction: beta = 1.9, p = 0.014
Years from PHV
FN BMC (g)
Interaction: beta = 0.1, p = 0.016
Years from PHV
OSTEOGENIC SPORT
NON-OSTEOGENIC SPORTS

437

438


CSA (mm2)
Interaction: beta = 5.7, p = 0.013
Years from PHV
Z (mm3)
Interaction: beta = 38.0, p = 0.006
Years from PHV
CSMI (mm4)
Interaction: beta = 642.0, p = 0.014
Years from PHV
TBS
Interaction: beta = 3x10-3, p = 0.712
Years from PHV
STIFFNESS INDEX
Interaction: beta = 4.0, p = 0.023
Years from PHV
OSTEOGENIC SPORT
NON-OSTEOGENIC SPORTS

439

440

Supplementary table 1. Number of scans and chronological age by years from PHV (PHV=0).

Years from PHV	Number of scans		Chronological age (years)	
	Osteogenic sport	Non-osteogenic sports	Osteogenic sport	Non-osteogenic sports
-2	9	4	12.1 (0.4)	11.7 (0.5)
-1	27	28	12.7 (0.5)	12.5 (0.5)
0	23	38	13.7 (0.4)	13.6 (0.4)
1	10	42	14.7 (0.6)	14.5 (0.4)
2	5	21	15.2 (0.2)	15.5 (0.4)

Values presented as mean (SD).

No significant differences in chronological age between osteogenic and non-osteogenic sports.

441

Supplementary table 2. Bone parameters reported by years from PHV across sport groups (PHV=0).

					Osteogenic sport					
Years from PHV	**-2**		**-1**		**0**		**1**		**2**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
TBS	1.37	0.08	1.41**	0.06	1.41**	0.06	1.46*	0.07	1.51	0.07
Stiffness index	99.33	10.74	100.37**	14.04	105.78**	10.94	111.85*	11.52	114.00*	9.22
BMC (g)										
TBLH	1089.03	183.71	1403.04*	194.61	1708.44	254.34	2159.73*	207.52	2444.20**	87.16
LS	29.54	5.40	37.27*	5.82	44.57*	8.14	56.28	7.21	63.80	6.53
Hip	21.24	3.56	27.72**	3.61	33.51**	4.17	40.89**	2.89	44.89*	3.95
FN	3.75	0.33	4.41**	0.40	4.97*	0.52	6.03**	0.53	6.33*	0.51
HSA										
CSA	124.33*	7.81	138.48**	14.82	152.78*	13.97	183.80**	13.81	197.40*	14.43
Z	448.60	34.90	523.86*	68.86	613.21*	121.34	821.85**	96.75	938.58*	62.67
CSMI	6512.56	511.91	7900.37*	1375.62	9844.43*	2152.04	14271.90*	2471.94	17268.00**	1552.79
					Non-osteogenic sports					
Years from PHV	**-2**		**-1**		**0**		**1**		**2**	

	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
TBS	1.32	0.05	1.35	0.05	1.35	0.07	1.40	0.07	1.42	0.06
Stiffness index	94.75	10.04	85.20	6.30	90.86	11.22	97.43	14.51	98.21	16.60
BMC (g)										
TBLH	1006.08	118.02	1298.94	172.21	1589.11	239.45	1892.63	239.99	2116.27	172.10
LS	26.63	2.79	33.50	4.32	40.37	7.58	50.72	8.66	59.53	8.63
Hip	19.20	2.88	24.10	3.34	28.89	3.85	32.09	7.67	36.61	4.36
FN	3.30	0.45	3.98	0.47	4.46	0.59	5.01	0.64	5.37	0.58
HSA										
CSA	105.00	13.64	123.57	13.97	137.61	18.61	153.45	19.33	162.29	22.65
Z	370.80	76.13	467.44	72.62	542.09	100.81	645.93	120.73	731.15	135.54
CSMI	5204.50	1283.06	7089.68	1632.23	8530.32	2009.44	10831.07	2809.81	12921.67	2136.71

Raw data presented as mean and SD.

Differences between osteogenic and non-osteogenic sports in the same year from PHV (-2 vs -2; -1 vs -1; 0 vs 0; 1 vs 1; 2 vs 2) * $p<0.05$, ** $p<0.001$.

TBS, trabecular bone score; BMC, bone mineral content; TBLH, total body less head; LS, lumbar spine; FN, femoral neck; CSA, cross sectional area; Z, section modulus; CSMI, cross sectional moment of inertia.

442