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A 9-month Jumping Intervention to Improve Bone Geometry in Adolescent Male Athletes

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Trial registration number: ISRCTN17982776 http://www.isrctn.com/ISRCTN17982776

Keywords: ADOLESCENCE, BONE CHANGES, EXERCISE, INTERVENTION, JUMPING, SPORTS.

ABSTRACT

Purpose: Weight-bearing and non weight-bearing sports have different effects on bone geometry during growth and there is need to identify effective interventions to improve bone geometry of adolescent athletes. The purpose of this study was to investigate the effect of a 9-month jumping intervention on bone geometry and metabolism in adolescent male athletes **Methods:** Nighty three adolescent (14.1 years old) male swimmers (SWI), footballers (FOO) and cyclists (CYC) were randomized to an intervention and sport (INT-SWI=19, INT-FOO=15, INT-CYC=14) or sport only control (CON-SWI =18, CON-FOO =15, CON-CYC =12) groups. Cross-sectional area (CSA), cross-sectional moment of inertia (CSMI) and section modulus (Z) at the femoral neck were assessed using hip structural analysis, and trabecular texture of the lumbar spine using trabecular bone score (TBS). Bone mineral content (BMC) at femoral neck and lumbar spine was assessed using dual-energy x-ray absorptiometry. Serum N-terminal propeptide of procollagen type I (PINP), isomer of the Carboxi-terminal telopeptide of type 1 collagen (CTX-I), total serum calcium and 25 hydroxyvitamin D [25(OH)D] were analysed. **Results:** INT-CYC gained significantly higher lumbar spine BMC (4.6 %) and femoral neck BMC (9.8 %) than CON-CYC. INT-CYC gained significantly higher CSA (11.0 %), CSMI (10.1 %) and TBS (4.4 %) than CON-CYC. INT-SWI gained significantly higher femoral neck BMC (6.0 %) and CSMI (10.9 %) than CON-SWI. There were no significant differences between INT-FOO and CON-FOO in any bone outcomes. PINP significantly decreased in CON-SWI, INT-FOO, CON-FOO and CON-CYC. CTX-I significantly decreased in CON-SWI and CON-CYC. 25(OH)D significantly increased in INT-CYC, CON-CYC, INT-FOO and CON-FOO. **Conclusions:** A 9-month jumping intervention improved bone outcomes in adolescent swimmers and cyclists, but not in footballers. This intervention might be used by sports clubs to improve bone health of adolescent athletes.

INTRODUCTION

Exercise during childhood and adolescence can improve bone mineral content (BMC) and areal bone mineral density (aBMD) with benefits maintained into adulthood (1). Low bone mass during adolescence is associated with increased fracture risk and osteoporosis later in life (2, 3). The adolescent years are critical for bone development with up to 43 % of peak bone mass acquired during the 5-year period surrounding peak height velocity (PHV) (4). Bone acquisition depends on the ground reaction forces applied to the skeleton and the muscular contractions produced during exercise (5), therefore not all the types of sport can improve bone geometry and structure (6, 7).

Previous evidence indicates that weight-bearing sports, such us football, have higher aBMD and BMC at the loaded sites of the skeleton compared to non-weight bearing sports, such as cycling and swimming (8-10). Prolonged participation in non-weight-bearing sports, such as swimming and cycling, may have a negative or no impact on bone status compared to controls (11, 12), which may compromise the achievement of a higher peak bone mass (13). As part of the cross-sectional analysis of the PRO-BONE study we have recently shown that footballers had significantly higher bone outcomes compared to swimming and cycling participation in adolescent males at baseline (10). Additionally, we followed the adolescent athletes for 12 months and found that cyclists and swimmers had lower bone BMC (14) and bone geometry (15) compared to footballers after controlling for baseline bone outcomes, lean mass, age, height and moderate to vigorous physical activity. Collectively, these studies highlight the need to improve bone development of athletes involved in non-weight-bearing sports, such as swimming and cycling.

Football, cycling and swimming are among the most popular sports worldwide for adolescents, however there is currently no evidence on the effectiveness of interventions to improve bone mineralization in athletes during this period of life. Previous intervention studies were

conducted in the school environment (16, 17) and have shown that jumping can improve bone outcomes in non-athletic prepubertal and pubertal children (17, 18). Adolescent footballers have been found to obtain the weight-bearing stimulus needed to optimise their bone health through the sport specific weight-bearing training (10), but there is no evidence whether a jumping intervention can improve further their bone health. In contrast, adolescent swimmers and cyclists, despite having muscle contractions during sport specific practise, may not obtain the optimal bone mineralisation during this critical period due to the lack of weight-bearing stimulus in the unloaded sites of the skeleton, such as lower limbs for swimmers (11, 12). However, it is not known whether a jumping intervention can counteract the lack of weight-bearing stimulus in non-weight bearing adolescent athletes, such as swimmers and cyclists.

Changes in BMC and aBMD and bone area (19) due to external mechanical loading can be measured by dual-energy x-ray absorptiometry (DXA), but adaptations in strength, structure and geometry during growth or following an intervention such as jumping, may not be detected due to the two dimensional nature of DXA (20). However, there are studies using techniques such as hip structural analysis (HSA) to assess bone geometry estimates, such as cross-sectional area (CSA), cross-sectional moment of inertia (CSMI) and section modulus at the femoral neck in adolescents (8). In addition, the recently developed trabecular bone score (TBS), which can predict fracture risk and fragility of the lumbar spine, can provide an indirect textural index of trabecular microarchitecture in the lumbar spine (21). Moreover, the assessment of bone turnover and nutrition markers, such as N-terminal propeptide of procollagen type I (PINP), isomer of the Carboxi-terminal telopeptide of type 1 collagen (CTX-I), total serum calcium and 25 hydroxyvitamin D [25(OH)D] can provide important information about bone formation and resorption in relation to the sports practised during adolescence (22). We recently found that footballers gained significantly higher HSA and TBS outcomes compared to swimmers

and cyclists after one year of sport specific training, and footballers had significantly higher bone formation compared to both non weight-bearing sports (15).

The scope of the study was to examine the effects of a 9-month progressive jumping intervention programme on BMC, hip geometry estimates, TBS at the clinically relevant skeletal sites of lumbar spine and femoral neck, and bone turnover markers in adolescent male swimmers (SWI), footballers (FOO) and cyclists (CYC). It was hypothesised that the intervention will induce significantly positive changes on bone outcomes in swimmers and cyclists but not in footballers.

METHODS

Cohort and study design

The present 9-month randomized controlled trial intervention is the last part of the 21-month longitudinal PRO-BONE study that consisted of three measurement points (baseline, 12-months and 21-months), and the methodology has been described previously (23). We have published the findings from: i) the cross-sectional differences in bone outcomes between the studies groups at baseline (10), ii) the longitudinally after 12 months of sport specific practise in bone mass (14) and geometry and metabolism (15). The present novel 9-month randomized controlled trial jumping intervention includes bone mass and geometry outcomes at the lumbar spine and femoral neck and metabolism markers of the athletic groups.

The initial inclusion criteria of the study were adolescent males 12-14 years old, engaged (≥ 3 h/week) in weight-bearing (football) and/or non weight-bearing (swimming and cycling) sports in the last 3 years or more. The exclusion criteria were participation in another clinical trial, any acute infection lasting until < 1 week before inclusion, medical history of diseases or medications affecting bone metabolism or the presence of an injury (before inclusion) that may

affect participation in their respective sports and/or any variable considered in the present study and non-Caucasian participants. Informed consent was obtained from all parents and participants included in the study. 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. 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). For the present study, data obtained at pre (autumn/winter 2015/16) and post (summer/autumn 2016) the intervention programme (mean difference of visits = 289 days) are used. The Consolidated Standards of Reporting Trials (CONSORT) flow diagram is presented in Figure 1. A total of 93 adolescent males (14.1±1.0 years at PRE-intervention) completed all pre and post measurements. The sports groups were simply randomized by an independent researcher into two different groups: INT and sport (INT-SWI=19, INT-FOO=15, INT-CYC=14) and sport only (without any additional intervention) (CON-SWI=18, CON-FOO=15, CON-CYC=12).

(Figure 1 here)

PRO-BONE study jumping intervention programme

The 9-month progressive jump intervention programme (~10 min/day) consisted of counter movement jumps (CMJ) and was performed by participants in the INT groups. The intervention consisted of 3 levels (12 weeks each) using adjustable weight vests (The Sports HQ, UK) and was performed on a hard surface. The intensity and the volume increased progressively by modifying the weight in the vests and the number of sets performed at each level (Level 1= 20

jumps, 0 kg, 3 sets/day, 3 times/week; Level 2= 20 jumps, 2 kg, 4 sets/day, 3 times/week; Level 3= 20 jumps, 5 kg, 4 sets/day, 4 times/week). A jump diary was used to record the number of jumps performed at each level and was returned to the research group every 3 months. Before the intervention, trained research assistants explained and demonstrated the CMJ only to INT groups, and participants executed the CMJ to ensure proper technique. The CMJ was chosen for the intervention as it has a high rate of change in force (493 times body weight/s) and ground reaction forces (5 times body weight) in 8.3 - 11.7 years old boys and girls (24). The reliability and validity of the CMJ has been previously reported (25).

(Table 1 here)

Bone outcomes: DXA, HSA, TBS and biochemical markers

A Lunar Prodigy DXA scanner (GE Healthcare Inc., Wisconsin, USA) was used to measure BMC (g), fat mass (g) and lean mass (g). The lumbar spine (LS, L1-L4) and bilateral proximal femora scans were used to assess BMC. All DXA scans and subsequent in-software analyses were completed by the same researcher using the same Lunar Prodigy DXA scanner and the enCORE software version 14.10.022 (GE Healthcare Inc, Wisconsin, USA) and following the International Society of Clinical Densitometry guidelines (26). The coefficient of variation (CV) was not determined in the present study, but previous paediatric studies have shown that the DXA percentage CV was between 0.64 % and 1.16 % at femoral neck and lumbar spine regions (27).

The HSA software analysed the hip scans at the narrow neck region across the narrowest point of the femoral neck. The HSA software uses the distribution of bone mineral mass in line of pixels across the bone axis to measure the structural dimensions of bone cross sections (28). The hip geometry estimates of the femoral neck 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

167 excluding the soft tissue area and the trabecular bone; 2) the cross-sectional moment of inertia
168 (CSMI, mm^4), which is an index of structural rigidity and reflects the distribution of mass in
169 the centre of a structural element; and 3) section modulus (Z, mm^3), which is an indicator of
170 maximum bending strength in a cross section. The CVs of these variables has been reported to
171 be between 7.9 % and 11.7 % (29).

172 TBS is a DXA based technological tool that provides an indirect textural index of trabecular
173 microarchitecture in the lumbar spine and has been shown to significantly predict fracture risk
174 independent of BMC (21). TBS assesses DXA images of the lumbar spine scans using a grey-
175 level analysis as the slope at the origin of the log-log representation of the experimental
176 variogram (30). All TBS analyses were performed by the same trained researcher using the
177 TBS iNsight Software (Medimaps, research version 3.0, Pessac, France). The calculation was
178 performed at the lumbar spine region of interest as in the BMC measurement. The CVs of TBS
179 in relation to BMC has been reported to be 1.1 % to 1.9 % (31).

180 Capillary blood samples were collected in the morning of non-training weekends using heparin
181 fluoride coated microvettes (CB 300 tubes, Sarstedt Ltd, Leicester, UK) and centrifuged at
182 3000 rpm for 15 minutes at 4°C. Serum samples were stored at -80°C until later analysis. Total
183 serum levels of PINP, CTX-I, 25(OH)D and total calcium were analysed. ELISA kits (Abbexa
184 Ltd., Cambridge, UK) for PINP (test range: 6-400 $pg \cdot mL^{-1}$, sensitivity: 1.2 $pg \cdot mL^{-1}$, inter and
185 intra-assay CVs: 8.6 % and 9.1 % respectively), CTX-I (test range: 0.1-7.0 $ng \cdot mL^{-1}$, sensitivity:
186 0.03 $ng \cdot mL^{-1}$, inter and intra-assay CVs: 8.3 % and 9.2 % respectively), and 25(OH)D (test
187 range: 3-80 $ng \cdot mL^{-1}$, sensitivity: 1.2 $ng \cdot mL^{-1}$, inter and intra-assay CVs: 6.4 % and 8.0 %
188 respectively) were used. Total calcium serum was measured using direct colorimetric assay
189 (Cayman Chemical Company, MI, U.S.A.) and had a sensitivity of 0.25 $mg \cdot dL^{-1}$ and the
190 absorbance was read at 570-590 nm (inter and intra-assay CVs: 7.9 % and 9.0 % respectively).

Anthropometry and maturity status

Stature (cm) and body mass (kg) were measured by using a stadiometer (Harpenden, Holtain Ltd, Crymych, UK) and an electronic scale (Seca 877, Seca Ltd, Birmingham, UK), respectively. Somatic maturity status was assessed using predicted age at peak height velocity (PHV) which is a somatic biological maturity indicator and reflects the maximum growth velocity during adolescence. The age at PHV was predicted using age and height in validated algorithm showing how far an individual is from this maturity milestone (years from age at PHV). The coefficient of determination has been reported ($R^2 = 0.90$; standard error = 0.5) (32).

Physical activity and training characteristics

Physical activity was measured for seven consecutive days at PRE- and POST-intervention using wrist accelerometers (GENEA, Cambridgeshire, UK). The validity and reliability of the accelerometer has been established previously in children and adolescents (33). Data were collected at 100 Hz and analysed at 1 s epoch intervals to establish time spent in MVPA using a validated cut-point (33). Weekly training hours were obtained by face to face interviews at PRE- and POST-intervention.

Statistical analyses

Statistical analyses were performed using the SPSS version 21.0 for Windows (IBM Corp, New York, USA). The sample size was calculated according to achieve at least 90 % of statistical power as previously described (23). Data were checked for normality and presented as mean and standard deviation (SD). Data were analysed for each sport group separately using: 1) paired t-tests to detect mean differences in descriptive characteristics and blood marker outcomes between PRE- and POST-intervention visits, 2) one-way analysis of variance (ANOVA) with Bonferroni post hoc to detect differences in the bone outcomes and blood

markers between the intervention and the non-intervention groups of each specific sport at PRE- and POST-intervention, and 3) one-way analysis of covariance (ANCOVA) with Bonferroni post hoc was used after controlling for PRE bone status, change in lean mass and POST maturity status (years from PHV) to detect differences between the intervention and the non-intervention groups in 9-month adjusted gains (Δ BMC, Δ HSA and Δ TBS). The selection of the covariates was based on relevant predictors of bone outcomes in adolescents (34-36). Percentages of difference between the intervention and non-intervention groups were used to quantify the magnitude of the differences in adjusted bone outcome gains. Significance was set at $p<0.05$.

RESULTS

Cohort characteristics

Table 1 shows the mean total compliance of the intervention. There were no differences between the groups on the number of jumps performed. Table 2 presents the descriptive characteristics of the participants PRE- and POST-intervention. No differences were observed in the descriptive characteristics presented at table 2 between INT and CON groups at PRE- and POST-intervention for each specific sport, $p>0.05$. The footballers reported significantly higher levels of participation in plyometric training (INT-FOO= 57%, CON-FOO= 55%) compared to cyclists (INT-CYC= 29%, CON-CYC= 26%) and swimmers (INT-SWI= 43%, CON-SWI= 41%). In all INT groups, all variables significantly increased from PRE- and POST-intervention, except fat mass in INT-CYC and MVPA in all groups. Similarly, all variables significantly increased from pre to post in CON-SWI, CON-FOO and CON-CYC except MVPA and fat mass.

(Table 2 here)

Bone quantity, geometry and texture

Figure 2 shows the percentage of difference on 9-month adjusted bone change in BMC between the sport specific intervention and control groups. INT-CYC group gained significantly higher lumbar spine BMC (4.6 %) and femoral neck BMC (9.8 %) than CON-CYC. Figure 3 shows the percentage of difference on 9-month adjusted bone change in HSA and TBS outcomes between the sport specific intervention and control groups. INT-CYC gained significantly higher CSA (11.0 %), CSMI (10.1 %) and TBS (4.4 %) than CON-CYC. INT-SWI gained significantly higher femoral neck BMC (6.0 %) and CSMI (10.9 %) than CON-SWI. There were no significant differences between INT-FOO and CON-FOO for any of the bone outcomes.

(Figures 2 and 3 here)

Bone turnover and nutrition markers

Table 3 shows the biochemical markers of the participants PRE- and POST-intervention. Bone formation, as measured by PINP, was reduced in all CON sport groups (4.4 % in SWI, 3.3% in FOO and 4.2% in CYC). Interestingly, bone formation did not decline in INT-SWI and INT-CYC but it slightly did in INT-FOO (1.8 %). Bone resorption, as measured by CTX-I, was reduced by 3.8% in CON-SWI and CON-CYC. However, bone resorption did not vary in any of the INT groups or in CON-FOO. 25(OH)D significantly increased in INT-CYC (3.1 %), CON-CYC (3.7 %), INT-FOO (3.1 %) and CON-FOO (3.5 %), but not in INT-SWI and CON-SWI. In all groups serum calcium significantly increased from PRE- and POST-intervention.

(Table 3 here)

DISCUSSION

This is the first study to examine the effect of jumping intervention on BMC at clinically relevant sites, hip geometry estimates, TBS and bone turnover markers in adolescent male athletes involved in weight-bearing (football) and non weight-bearing sports (swimming and cycling). The findings demonstrate that a 9-month progressive jumping intervention programme can significantly improve BMC, HSA and TBS bone outcomes at the clinically relevant skeletal sites of lumbar spine and femoral neck in non weight-bearing sport athletes, such as swimmers and cyclists, but not in the weight-bearing sport athletes, such as footballers. In addition, bone formation (PINP) was maintained in the non weight-bearing INT sport groups while decreased in the CON non weight-bearing sport groups. Moreover, bone resorption (CTX-I) significantly decreased in the CON non weight-bearing sport groups but did not vary in any of the INT groups, suggesting an increased bone turnover in these groups.

Jumping intervention effects on BMC at femoral neck and lumbar spine

Currently, there are no jumping intervention studies conducted in an athletic population to improve bone outcomes, therefore the findings of the present study were compared with jumping interventions applied in non-athletic children and adolescents (16-18). The present jumping intervention significantly improved femoral neck BMC (6.0 – 9.8 %) in INT-SWI and INT-CYC compared to CON-SWI and CON-CYC, and lumbar spine BMC (4.6) % in INT-CYC compared to CON-CYC. Previously, an 8-month school-based jumping intervention reported that non-athletic adolescent males and females gained 6.0 % higher femoral neck BMC and 2.3 % higher lumbar spine BMC compared to controls (17). Also, a 7-month school-based jumping intervention reported that non-athletic prepubescent children had 4.5 % and 3.1 % significantly higher gains at femoral neck and lumbar spine BMC respectively compared to age-matched controls (37). The greater magnitude of improvements observed in HSA at the femoral neck and TBS at lumbar spine of swimmers and cyclists in the current study may be explained by the ability of the unloaded skeletons of the non weight-bearing groups to respond

better to the external stimulus of the jumping intervention (8). Another explanation might be the longer duration of the present intervention (9 months vs 7-8 months) and the greater number of jumps performed in the present study (160 vs 90 jumps per week) (18, 20) by increasing the ground reaction forces applied to the skeleton progressively using the weight vests. These improvements may indicate a window of opportunity to counteract the lack of weight-bearing stimulus observed in adolescent swimmers and cyclists (10, 12, 38). In contrast, but consistent with our hypothesis, the stimulus provided by the jumping intervention was not enough to induce significant bone gains in INT-FOO compared to CON-FOO. This is in accordance with the mechanostat theory indicating that the bones adapt their strength and content to respond to the strain caused by external physiological loads up to a certain point (39). Footballers may have reached a threshold for bone improvements as we have previously shown to have greater bone outcomes compared to swimmers and cyclists (10). However, a longer duration jumping intervention programme may be needed to improve further bone outcomes in weight-bearing sports, such as football.

Jumping intervention effects on HSA and TBS outcomes

In addition to BMC adaptations, the present 9-month jumping intervention significantly improved HSA and TBS bone parameters. More specifically, INT-CYC gained significantly higher CSA (11.0 %), CSMI (10.1 %) and TBS (4.4 %) compared to CON-CYC, and INT-SWI gained significantly higher CSMI (10.9 %) compared to CON-SWI. Previously, only two studies previously used HSA to describe bone geometry and structural strength adaptations from a jumping intervention in non-athletic populations (18). Petit et al (20) reported that a 7-month jumping intervention induced significantly greater increase in CSA (2.3 %) and section modulus (4.0 %) in the intervention group compared to an age-matched non-athletic control group. McKay et al (18) did not find significant improvements in HSA parameters after an 8-month jumping intervention in non-athletic pubertal children, but section modulus (3.3 %) and

CSA (2.0 %) had similar magnitude of increase with the study of Petit et al. In the present study, the greater improvements in bone outcomes of swimmers and cyclists compared to footballers may be explained by mechanoadaptation that converts the external stimulus of the jumping intervention to greater structural adaptations of previously unloaded bones (40). The present study is the first to present findings on TBS adaptations after a jumping intervention in adolescent athletes. Currently, there are no jumping intervention studies using TBS and only a recent cross-sectional study in adults reported that moderate impact loading sports was associated with a lower TBS score and increased fracture risk compared to high impact loading sports (41). The present study indicates that trabecular structure at the lumbar spine may be adapted to the forces produced from the jumping intervention after controlling for potential confounders (42). The compliance in the present study was slightly lower compared to a different study (70 % vs 80 %) (16) and this might be due to the longer duration of the present intervention (9 months vs 7 months) (16). However, the present jumping intervention had 1-2 months greater duration and progressive loading compared to previous studies, which might be responsible for the higher gains observed. The latter is similar with a 20-month exercise randomized control trial in prepubertal non-athletic males that found greater magnitude of improvements in CSMI (12.3 %) and section modulus (7.4 %) in the intervention group compared to age-matched controls (43).

Jumping intervention effects on biochemical markers

The analysis of biochemical markers in the present study showed that the jumping intervention prevented the significant decline of bone formation (PINP) and resorption (CTX-I) markers in INT-SWI and INT-CYC. In contrast, bone formation significantly decreased in INT-FOO and all CON-SPORT groups, and bone resorption significantly decreased in CON-SWI and CON-CYC. Previous studies have shown that bone turnover markers are associated with bone outcomes during growth and can provide additional information about bone remodelling (22).

In addition, the intensity of physical activity and the type of sports practised may be potent regulators of bone remodelling (15). Recently, a study has shown that one session of plyometric jumping exercises can stimulate bone formation in in boys and young men, with boy's response to be more pronounced (44). However, there are no studies investigating the response of bone turnover markers after a longer jumping intervention in combination with clinically relevant bone outcomes. The findings of the present study suggest that the cellular activity of bone turnover markers (both formation and resorption) in INT-SWI and INT-CYC was protected from declining due to the jumping intervention. In addition, serum calcium significantly increased from PRE- and POST-intervention, and 25(OH)D significantly increased in INT-CYC, CON-CYC, INT-FOO and CON-FOO, but not in INT-SWI and CON-SWI. There was an expected increase in serum calcium levels by age in adolescents, and the significant increase of 25(OH)D in cyclists and footballers might be explained by the higher exposure to sunlight during training in these sports, although other parameters such as dietary intake and the sampling period have been reported to affect 25(OH)D levels (45).

Strengths and limitations

The strengths of the present study include the evaluation for first time of a novel 9-month progressive jumping intervention programme in adolescent athletes participating in weight-bearing and non weight-bearing sports. In addition, the combination of DXA, HSA, TBS and biochemical markers can provide novel and clinically relevant findings regarding the bone changes induced from a jumping intervention programme in adolescent male athletes. The low cost and relative ease jumping programme for young athletes represents an additional strength, and almost any sport club could implement the programme with minimal training for the coach and the athlete. The limitations of this intervention include the lack of genetic data, and the unavailability of three dimensional imaging techniques to assess bone strength and structure, such as peripheral quantitative computed tomography. We studied Caucasian athletes due to

the evidence that each ethnicity can have different bone acquisition and responses to an intervention (16, 46), which requires a larger sample size and resources. Future interventions could be conducted in more diverse population including females, other ethnic and sports groups. .

CONCLUSIONS

This is the first randomized control trial to investigate the effects of a 9-month progressive jumping intervention programme on bone mass, geometry, texture and biochemical markers in adolescent male athletes. The findings indicate that the jumping intervention programme can significantly improve bone quantity, geometry and TBS bone outcomes at the femoral neck and lumbar spine, and maintain the bone turnover in adolescent male athletes involved in swimming and cycling, but not in football. The present jumping intervention programme can be implemented by non weight-bearing sports clubs and athletes to improve bone health.

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.

Author contributions

DV collected the data and drafted the manuscript. LGM designed the study and approved the final manuscript as submitted. ARB and CAW coordinated and supervised data collection critically reviewed the manuscript and approved the final manuscript as submitted. EUG contributed to data collection and critically reviewed the manuscript, and approved the final manuscript as submitted. 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. Dr. Gracia-Marco acknowledges "Plan Propio" of the University of Granada, Spain. The authors also gratefully acknowledge the researchers of Children's Health and Exercise Research Centre for their continuous support and help with the study.

Ethical approvals

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.

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

25(OH)D: 25-hydroxyvitamin D, BMC: Areal Bone mineral content; aBMD: Bone mineral density; CTX-I: Carboxi-terminal telopeptide of type 1 collagen, CON: Controls; CSA: Cross-sectional area; CSMI: cross-sectional moment of inertia; CYC: Cyclists; DXA: Dual Energy X-Ray Absorptiometry; FOO: Footballers; HSA: Hip structural analysis, INT: Intervention; MVPA: Moderate to vigorous physical activity; PHV: Peak height velocity; PINP: N-terminal propeptide of procollagen type I, TBS: SWI: Swimmers; Trabecular bone score; Z: Section modulus.

Conflict 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.

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Figure captions

Figure 1. PRO-BONE study flow chart. CONSORT, Consolidated Standards of Reporting Trials.

Figure 2. Nine-month adjusted changes (%) in Bone Mineral Content (BMC) at femoral neck and lumber spine between the sport specific intervention and control groups. Results were adjusted for baseline bone outcomes, changes in lean mass and post peak height velocity. * denotes significant differences compared to the sport specific control group, $p<0.05$.

Figure 3. Nine-month adjusted changes (%) in HSA and TBS bone outcomes at PRE and POST of the jumping intervention in footballers, swimmers and cyclists. The results were adjusted for baseline bone outcomes, change in lean mass and post peak height velocity. CSMI: Cross sectional moment of inertia, CSA: cross-sectional area, TBS: Trabecular Bone score, Z: Section modulus. The figures represent unadjusted results of participants of similar peak height velocity and training hours. *denotes significant differences compared to the sport specific control group, $p<0.05$.

TABLES

Table 1. PRO BONE study plyometric jump intervention training progression and compliance.

Level	Exercise	Vest weights (kg)	Repetitions	[2]Sets / day ([3]Rest)	[4]Trainings / week	Jumps / week	[5]Compliance in % and number (SD) of jumps completed		
							INT-SWI	INT-FOO	INT-CYC
1	[1]CMJ	-	20	3	3	180	90.3 %	95.3 %	91.3 %
Total level 1 (12 weeks)					180 x 12 =	2160	1949 (204)	2059 (155)	1971 (240)
2	[1]CMJ	2	20	4	3	240	75.0 %	83.9 %	83.1 %
Total level 2 (12 weeks)					240 x 12 =	2880	2159 (434)	2416 (444)	2393 (454)
3	[1]CMJ	5	20	4	4	320	46.0 %	56.8 %	47.1 %
Total level 3 (12 weeks)					320 x 12 =	3840	1765 (298)	2181 (434)	1807 (598)
Total intervention (36 weeks)						8880	66.0 % 5858 (1051)	75.0 % 6656 (1281)	69.5 % 6171 (1097)

[1]Countermovement jump. [2]Sets = 20 Counter Movement Jump. [3]Rest between sets = 30 seconds. [4]When 3 sets/day, jumps suggested to be performed in the morning before going to school (1 set), after school (1 set) and before going to bed (1 set). When 4 sets/day, jumps performed in the morning before going to school (1 set), after school (2 sets) and before going to bed (1st set).[5]No significant differences between the intervention groups at any level of the intervention.

Table 2. Characteristics of the sports groups and the control group before (PRE) and after (POST) the 9-month intervention programme.

	SWIMMERS				FOOTBALLERS				CYCLISTS			
TOTAL (N=105)	INTERVENTION (N=19)		CONTROL (N=18)		INTERVENTION (N=15)		CONTROL (N=15)		INTERVENTION (N=14)		CONTROL (N=12)	
	PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST
Age (years)	14.5 ± 0.9	**15.3 ± 0.9**	14.7 ± 1.1	**15.4 ± 1.1**	13.8 ± 1.0	**14.6 ± 1.0**	13.7 ± 0.8	**14.5 ± 0.8**	14.1 ± 1.1	**14.9 ± 1.1**	14.1 ± 0.9	**14.9 ± 0.9**
Height (cm)	170.3 ± 10.0	**174.1 ± 9.6**	172.8 ± 7.6	**176.0 ± 6.9**	160.5 ± 11.0	**165.4 ± 11.1**	163.3 ± 9.3	**168.9 ± 9.1**	168.2 ± 10.7	**174.5 ± 8.2**	162.7 ± 9.7	**166.4 ± 9.6**
Body mass (kg)	57.2 ± 9.0	**62.6 ± 7.8**	60.6 ± 7.2	**63.6 ± 7.7**	49.3 ± 10.8	**54.8 ± 11.6**	49.6 ± 7.4	**55.4 ± 7.1**	57.7 ± 13.0	**62.6 ± 12.2**	51.0 ± 12.4	**55.2 ± 13.9**
Lean mass (kg)	46.7 ± 9.4	**51.2 ± 8.5**	48.7 ± 8.2	**51.9 ± 6.7**	41.5 ± 10.0	**46.1 ± 10.5**	39.6 ± 7.4	**44.9 ± 7.2**	45.0 ± 7.9	**49.5 ± 7.6**	40.3 ± 8.6	**44.0 ± 9.2**
Fat mass (kg)	7.6 ± 3.1	**8.5 ± 2.9**	8.2 ± 3.4	8.7 ± 3.8	5.4 ± 2.2	**6.1 ± 2.3**	7.1 ± 2.3	7.6 ± 3.5	9.9 ± 9.7	10.0 ± 9.7	8.1 ± 5.5	8.3 ± 6.5
Years from PHV	1.0 ± 1.0	**1.7 ± 1.0**	1.2 ± 1.0	**1.8 ± 0.9**	0.0 ± 1.0	**0.7 ± 1.0**	0.1 ± 0.8	**0.9 ± 0.8**	0.7 ± 1.1	**1.4 ± 1.0**	0.3 ± 0.9	**1.0 ± 1.0**
Maturation (I/II/III/ IV/V) (%)	(10/10/10 /50/20)	**(0/0/11 /52/37)**	(0/11/16 /47/26)	**(0/0/6 /61/33)**	(10/10/4 3/37/0)	**(0/13/13 /40/34)**	(0/22/28 /50/0)	**(0/7/27 /40/27)**	(7/7/20 /53/13)	**(0/0/14 /43/43)**	(8/15/8 /61/8)	**(0/8/17 /42/33)**
MVPA (min/day)	61.3 ± 19.6	67.5 ± 19.6	59.6 ± 25.2	60.0 ± 18.1	97.7 ± 18.8	83.3 ± 18.5	89.6 ± 33.5	76.3 ± 22.4	85.6 ± 22.1	93.9 ± 15.7	88.9 ± 21.8	78.7 ± 13.4
Training volume (hrs/week)	7.9 ± 3.6	**11.8 ± 5.4**	10.2 ± 3.1	**12.9 ± 5.4**	10.2 ± 1.2	**12.0 ± 2.8**	8.8 ± 2.0	**10.7 ± 1.8**	5.3 ± 2.0	**7.9 ± 3.8**	5.3 ± 1.6	**8.2 ± 2.8**
Energy intake (kcal/day)	2534 ± 382	2465 ± 221	2603 ± 425	2386 ± 133	2237 ± 517	2379 ± 262	2419 ± 620	2309 ± 234	2320 ± 280	2244 ± 166	2221 ± 325	2226 ± 152
Protein intake (g/day)	85.8 ± 30.9	89.8 ± 31.2	77.9 ± 28.5	82.8 ± 24.8	95.3 ± 21.6	97.1 ± 29.3	92.0 ± 35.3	90.1 ± 20.4	80.3 ± 13.3	88.0 ± 23.0	84.9 ± 28.9	86.4 ± 21.1
Calcium intake (mg/day)	1237 ± 280	1118 ± 289	1155 ± 257	1109 ± 189	1342 ± 350	1231 ± 167	1177 ± 378	1179 ± 178	1183 ± 370	1253 ± 196	1385 ± 280	1279 ± 149

Values are mean ± standard deviation. No differences observed at PRE and POST between INT and CON groups of each specific sport, $p>0.05$. Bold values denote significant different values between PRE and POST, $p<0.05$. MVPA: Moderate to vigorous physical activity; PHV: peak height velocity.

Table 3. PRE and 9-month adjusted gain in bone mineral content (BMC, g) and bone stiffness of the intervention and control groups.

TOTAL (N=105)	SWIMMERS				FOOTBALLERS				CYCLISTS			
	INTERVENTION (N=19)		CONTROL (N=18)		INTERVENTION (N=15)		CONTROL (N=15)		INTERVENTION (N=14)		CONTROL (N=12)	
	PRE	9-month adjusted gains Δ (95% CI)	PRE	9-month adjusted gains Δ (95% CI)	PRE	9-month adjusted gains Δ (95% CI)	PRE	9-month adjusted gains Δ (95% CI)	PRE	9-month adjusted gains Δ (95% CI)	PRE	9-month adjusted gains Δ (95% CI)
TBLH BMC	1892 ± 339	322 (276-368)	1952 ± 325	251 (203-300)	1730 ± 479	386 (335-438)	1769 ± 398	325 (274-377)	1848 ± 379	**341 (290-394)**	1582 ± 401	203 (145-260)
Legs BMC	888 ± 147	**141 (124-159)**	922 ± 135	95 (77-114)	877 ± 246	150 (130-170)	886 ± 201	131 (111-150)	899 ± 170	**148 (129-168)**	759 ± 168	87 (65-109)
Arms BMC	290 ± 68	44 (37-51)	303 ± 67	40 (33-47)	226 ± 76	46 (38-53)	228 ± 61	48 (41-56)	271 ± 68	49 (40-56)	233 ± 74	40 (32-48)
Bone stiffness	95.9 ± 12.0	**10.3 (7.2-13.5)**	94.5 ± 16.3	-1.8 (-5.1-1.6)	104.4 ± 11.9	15.3 (11.6-8.9)	105.7 ± 12.4	10.9 (7.2-14.6)	95.6 ± 15.6	**9.5 (5.9-13.1)**	93.6 ± 14	-2.2 (-6.0-1.7)

Raw values at PRE are mean ± standard deviation. Values at 9-month were adjusted for pre bone values, change in lean mass and post peak height velocity, and presented as mean and 95% CI. BMC: Bone mineral content, TBLH: Total body less head. No differences observed in bone outcomes at PRE between INT and CON groups of each specific sport, $p>0.05$. Bold values denote significant higher adjusted bone gains between the intervention and control group of each specific sport, $p<0.05$.

Table 4. Physical fitness measurements of the sports groups and the control group before (PRE) and after (POST) the 9-month intervention programme.

TOTAL (N=105)	SWIMMERS				FOOTBALLERS				CYCLISTS			
	INTERVENTION (N=19)		CONTROL (N=18)		INTERVENTION (N=15)		CONTROL (N=15)		INTERVENTION (N=14)		CONTROL (N=12)	
	PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST
Counter movement jump (cm)	46.8 ± 7.2	**49.9 ± 7.7***	46.5 ± 9.4	45.9 ± 8.8	45.3 ± 6.1	**47.5 ± 5.7**	41.7 ± 5.8	**43.4 ± 6.4**	42.7 ± 5.9	**45.9 ± 5.3***	45.4 ± 7.8	46.1 ± 8.0
Standing Long Jump (cm)	195.8 ± 27.8	**203.1 ± 27.9**	191.1 ± 27.7	194.7 ± 28.1	188.1 ± 24.9	**194.6 ± 23.4**	184.1 ± 21.2	191.7 ± 22.3	173.8 ± 34.1	**182.9 ± 31.8**	180.7 ± 24.5	185.3 ± 28.4
20mSRT (shuttles)	79.2 ± 17.6	**85.7 ± 17.3**	74.2 ± 24.4	76.1 ± 18.0	94.5 ± 14.4	98.3 ± 16.1	92.0 ± 21.9	94.9 ± 25.0	82.4 ± 24.2	**88.5 ± 22.6**	82.8 ± 19.2	85.6 ± 25.1

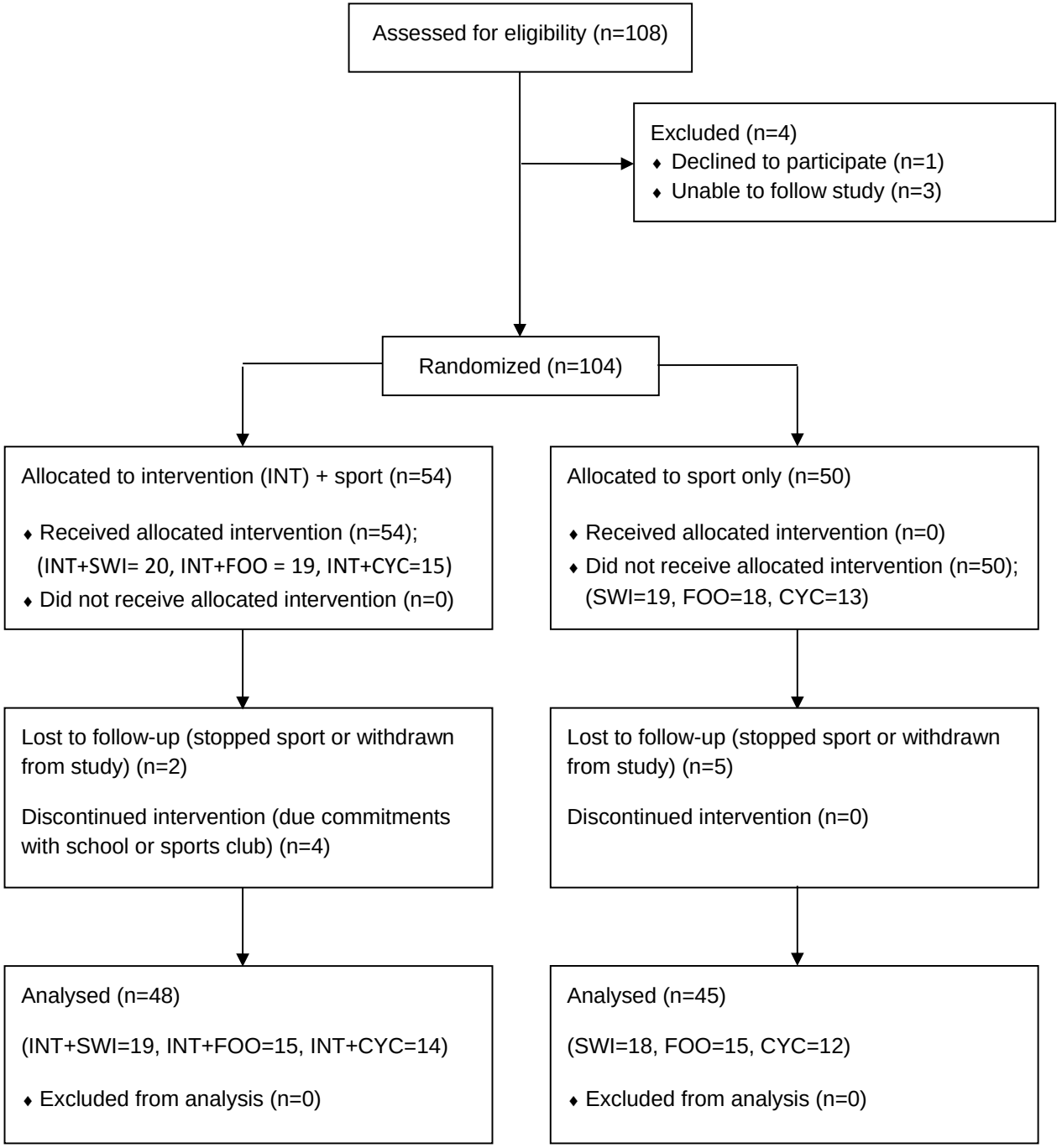
20mSRT: 20meter shuttle run test. Values are mean ± standard deviation. No differences observed at PRE and POST between INT and CON groups of each specific sport, $p>0.05$. Bold values denote significant different values between PRE and POST, $p<0.05$. * denote significant higher 9-month increase in CMJ of the INT group compared to the CON group of the specific sport, $p<0.05$.

Table 4. Physical fitness measurements of the sports groups and the control group before (PRE) and after (POST) the 9-month intervention programme.

	SWIMMERS				FOOTBALLERS				CYCLISTS			
TOTAL (N=105)	INTERVENTION (N=19)		CONTROL (N=18)		INTERVENTION (N=15)		CONTROL (N=15)		INTERVENTION (N=14)		CONTROL (N=12)	
	PRE	9-month change	PRE	9-month change	PRE	9-month change	PRE	9-month change	PRE	9-month change	PRE	9-month change
Counter movement jump (cm)	46.8 ± 7.2	**3.1 ± 0.4***	46.5 ± 9.4	-0.6 ± 0.3	45.3 ± 6.1	**2.2 ± 0.3**	41.7 ± 5.8	**1.7 ± 0.5**	42.7 ± 5.9	**3.2 ± 0.6***	45.4 ± 7.8	0.7 ± 0.4
Standing Long Jump (cm)	195.8 ± 27.8	**7.3 ± 1.8**	191.1 ± 27.7	3.6 ± 2.0	188.1 ± 24.9	**6.5 ± 2.6**	184.1 ± 21.2	7.6 ± 3.8	173.8 ± 34.1	**9.1 ± 2.2**	180.7 ± 24.5	4.6 ± 3.6
20mSRT (shuttles)	79.2 ± 17.6	**6.5 ± 1.7**	74.2 ± 24.4	1.9 ± 1.4	94.5 ± 14.4	3.8 ± 1.3	92.0 ± 21.9	2.9 ± 1.6	82.4 ± 24.2	**6.1 ± 1.0**	82.8 ± 19.2	2.8 ± 1.6

20mSRT: 20meter shuttle run test. Values are mean ± standard deviation. No differences observed at PRE between INT and CON groups of each specific sport, $p>0.05$. Bold ~~values~~ letters denote significant differents ~~values~~ between PRE and POST, $p<0.05$. * denotes a significant ~~higher 9-month~~ increase ~~in CMJ~~ of the INT group compared to the CON group of the specific sport, $p<0.05$.

Figure 1. PRO-BONE study flow chart. CONSORT, Consolidated Standards of Reporting Trials.



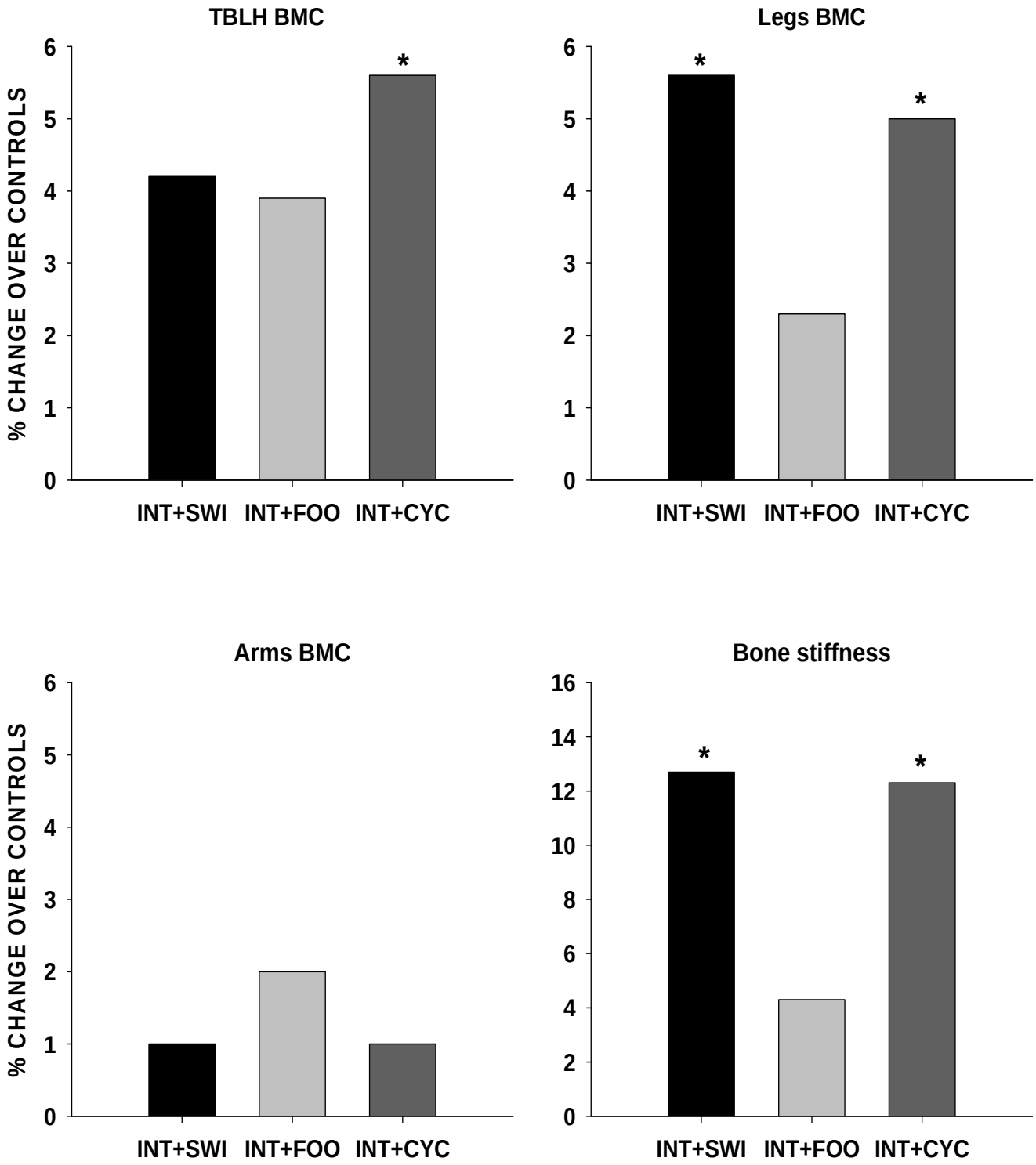


Figure 2. Adjusted percent change on Bone Mineral Content (BMC, g) and Quantitative Ultrasound (QUS) in the intervention groups over control groups. Results were adjusted for initial bone outcomes, change in lean mass and post peak height velocity. Superscript * denotes significant higher change compared to the sport specific control group, $p<0.05$.