

1 **Title Page**

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

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21 JUMPING, SPORTS.

22 **ABSTRACT**

23 **Purpose:** Weight-bearing and non weight-bearing sports have different **effects** on bone  
24 geometry during growth **and there is need to identify effective interventions to improve bone**  
25 **geometry of adolescent athletes.** The purpose of this study was to investigate the effect of a 9-  
26 month jumping intervention on bone geometry and metabolism in adolescent male athletes  
27 **Methods:** **Nighty three adolescent (14.1 years old) male swimmers (SWI), footballers (FOO)**  
28 **and cyclists (CYC) were randomized to an intervention and sport (INT-SWI=19, INT-**  
29 **FOO=15, INT-CYC=14) or sport only control (CON-SWI =18, CON-FOO =15, CON-CYC**  
30 **=12) groups.** Cross-sectional area (CSA), cross-sectional moment of inertia (CSMI) and  
31 section modulus (Z) at the femoral neck were assessed using hip structural analysis, and  
32 trabecular texture of the lumbar spine using trabecular bone score (TBS). Bone mineral content  
33 (BMC) at femoral neck and lumbar spine was assessed using dual-energy x-ray absorptiometry.  
34 Serum N-terminal propeptide of procollagen type I (PINP), isomer of the Carboxi-terminal  
35 telopeptide of type 1 collagen (CTX-I), total serum calcium and 25 hydroxyvitamin D  
36 [25(OH)D] were analysed. **Results:** INT-CYC gained significantly higher lumbar spine BMC  
37 (4.6 %) and femoral neck BMC (9.8 %) than CON-CYC. INT-CYC gained significantly higher  
38 CSA (11.0 %), CSMI (10.1 %) and TBS (4.4 %) than CON-CYC. INT-SWI gained  
39 significantly higher femoral neck BMC (6.0 %) and CSMI (10.9 %) than CON-SWI. There  
40 were no significant differences between INT-FOO and CON-FOO in any bone outcomes. PINP  
41 significantly decreased in CON-SWI, INT-FOO, CON-FOO and CON-CYC. CTX-I  
42 significantly decreased in CON-SWI and CON-CYC. 25(OH)D significantly increased in INT-  
43 CYC, CON-CYC, INT-FOO and CON-FOO. **Conclusions:** A 9-month jumping intervention  
44 improved bone outcomes in adolescent swimmers and cyclists, but not in footballers. This  
45 intervention might be used by sports clubs to improve bone health of adolescent athletes.

46

## 47 INTRODUCTION

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

56 Previous evidence indicates that weight-bearing sports, such as football, have higher aBMD  
57 and BMC at the loaded sites of the skeleton compared to non-weight bearing sports, such as  
58 cycling and swimming (8-10). Prolonged participation in non-weight-bearing sports, such as  
59 swimming and cycling, may have a negative or no impact on bone status compared to controls  
60 (11, 12), which may compromise the achievement of a higher peak bone mass (13). As part of  
61 the cross-sectional analysis of the PRO-BONE study we have recently shown that footballers  
62 had significantly higher bone outcomes compared to swimming and cycling participation in  
63 adolescent males at baseline (10). Additionally, we followed the adolescent athletes for 12  
64 months and found that cyclists and swimmers had lower bone BMC (14) and bone geometry  
65 (15) compared to footballers after controlling for baseline bone outcomes, lean mass, age,  
66 height and moderate to vigorous physical activity. Collectively, these studies highlight the need  
67 to improve bone development of athletes involved in non-weight-bearing sports, such as  
68 swimming and cycling.

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

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

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

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

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

## 104 **METHODS**

### 105 **Cohort and study design**

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

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

119 affect participation in their respective sports and/or any variable considered in the present study  
120 and non-Caucasian participants. Informed consent was obtained from all parents and  
121 participants included in the study. All procedures performed in studies involving human  
122 participants were in accordance with the ethical standards of the institutional and/or national  
123 research committee and with the 1964 Helsinki declaration and its later amendments or  
124 comparable ethical standards. Ethics approval received from the following committees: 1) the  
125 Ethics Review Sector of Directorate-General of Research (European Commission, ref. number  
126 618496); 2) the Sport and Health Sciences Ethics Committee (University of Exeter, ref. number  
127 2014/766) and 3) the National Research Ethics Service Committee (NRES Committee South  
128 West – Cornwall & Plymouth, ref. number 14/SW/0060). For the present study, data obtained  
129 at pre (autumn/winter 2015/16) and post (summer/autumn 2016) the intervention programme  
130 (mean difference of visits = 289 days) are used. The Consolidated Standards of Reporting Trials  
131 (CONSORT) flow diagram is presented in Figure 1. A total of 93 adolescent males ( $14.1 \pm 1.0$   
132 years at PRE-intervention) completed all pre and post measurements. The sports groups were  
133 simply randomized by an independent researcher into two different groups: INT and sport  
134 (INT-SWI=19, INT-FOO=15, INT-CYC=14) and sport only (without any additional  
135 intervention) (CON-SWI=18, CON-FOO=15, CON-CYC=12).

136 (Figure 1 here)

### 137 **PRO-BONE study jumping intervention programme**

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

143 jumps, 0 kg, 3 sets/day, 3 times/week; Level 2= 20 jumps, 2 kg, 4 sets/day, 3 times/week; Level  
144 3= 20 jumps, 5 kg, 4 sets/day, 4 times/week). A jump diary was used to record the number of  
145 jumps performed at each level and was returned to the research group every 3 months. Before  
146 the intervention, trained research assistants explained and demonstrated the CMJ only to INT  
147 groups, and participants executed the CMJ to ensure proper technique. The CMJ was chosen  
148 for the intervention as it has a high rate of change in force (493 times body weight/s) and ground  
149 reaction forces (5 times body weight) in 8.3 - 11.7 years old boys and girls (24). The reliability  
150 and validity of the CMJ has been previously reported (25).

151 (Table 1 here)

#### 152 **Bone outcomes: DXA, HSA, TBS and biochemical markers**

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

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

165 The hip geometry estimates of the femoral neck were obtained and the following variables  
166 used: 1) the cross sectional area (CSA, mm<sup>2</sup>), 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,  $\text{mm}^4$ ), 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 ( $Z$ ,  $\text{mm}^3$ ), 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  $4^\circ\text{C}$ . Serum samples were stored at  $-80^\circ\text{C}$  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:  $6\text{-}400\text{ pg}\cdot\text{mL}^{-1}$ , sensitivity:  $1.2\text{ pg}\cdot\text{mL}^{-1}$ , inter and  
185 intra-assay CVs: 8.6 % and 9.1 % respectively), CTX-I (test range:  $0.1\text{-}7.0\text{ ng}\cdot\text{mL}^{-1}$ , sensitivity:  
186  $0.03\text{ ng}\cdot\text{mL}^{-1}$ , inter and intra-assay CVs: 8.3 % and 9.2 % respectively), and 25(OH)D (test  
187 range:  $3\text{-}80\text{ ng}\cdot\text{mL}^{-1}$ , sensitivity:  $1.2\text{ ng}\cdot\text{mL}^{-1}$ , 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  $0.25\text{ mg}\cdot\text{dL}^{-1}$  and the  
190 absorbance was read at 570-590 nm (inter and intra-assay CVs: 7.9 % and 9.0 % respectively).



191 **Anthropometry and maturity status**

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

199 **Physical activity and training characteristics**

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

206 **Statistical analyses**

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

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

## 223 RESULTS

### 224 Cohort characteristics

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

236 (Table 2 here)

237 **Bone quantity, geometry and texture**

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

247 (Figures 2 and 3 here)

248 **Bone turnover and nutrition markers**

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

257 (Table 3 here)

258 **DISCUSSION**

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

#### 270 **Jumping intervention effects on BMC at femoral neck and lumbar spine**

271 Currently, there are no jumping intervention studies conducted in an athletic population to  
272 improve bone outcomes, therefore the findings of the present study were compared with  
273 jumping interventions applied in non-athletic children and adolescents (16-18). The present  
274 jumping intervention significantly improved femoral neck BMC (6.0 – 9.8 %) in INT-SWI and  
275 INT-CYC compared to CON-SWI and CON-CYC, and lumbar spine BMC (4.6) % in INT-  
276 CYC compared to CON-CYC. Previously, an 8-month school-based jumping intervention  
277 reported that non-athletic adolescent males and females gained 6.0 % higher femoral neck  
278 BMC and 2.3 % higher lumbar spine BMC compared to controls (17). Also, a 7-month school-  
279 based jumping intervention reported that non-athletic prepubescent children had 4.5 % and 3.1  
280 % significantly higher gains at femoral neck and lumbar spine BMC respectively compared to  
281 age-matched controls (37). The greater magnitude of improvements observed in **HSA at the**  
282 **femoral neck and TBS at lumbar spine** of swimmers and cyclists in the current study may be  
283 explained by the ability of the unloaded skeletons of the non weight-bearing groups to respond

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

### 298 **Jumping intervention effects on HSA and TBS outcomes**

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

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

### 327 **Jumping intervention effects on biochemical markers**

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

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

#### 348 **Strengths and limitations**

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

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

363

## 364 **CONCLUSIONS**

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



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376 **Author contributions**

377 DV collected the data and drafted the manuscript. LGM designed the study and approved the  
378 final manuscript as submitted. ARB and CAW coordinated and supervised data collection  
379 critically reviewed the manuscript and approved the final manuscript as submitted. EUG  
380 contributed to data collection and critically reviewed the manuscript, and approved the final  
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382

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389 **Ethical approvals**

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

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

398 **List of abbreviations**

399 25(OH)D: 25-hydroxyvitamin D, BMC: Areal Bone mineral content; aBMD: Bone mineral  
400 density; CTX-I: Carboxy-terminal telopeptide of type 1 collagen, CON: Controls; CSA: Cross-  
401 sectional area; CSMI: cross-sectional moment of inertia; CYC: Cyclists; DXA: Dual Energy  
402 X-Ray Absorptiometry; FOO: Footballers; HSA: Hip structural analysis, INT: Intervention;  
403 MVPA: Moderate to vigorous physical activity; PHV: Peak height velocity; PINP: N-terminal  
404 propeptide of procollagen type I, TBS: SWI: Swimmers; Trabecular bone score; Z: Section  
405 modulus.

406

407 **Conflict of interest**

408 The authors declare that they have no competing interests.

409 The results of the study are presented clearly, honestly, and without fabrication, falsification,  
410 or inappropriate data manipulation, and the present study do not constitute endorsement by  
411 ACSM.

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535

536 **Figure captions**

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

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

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

550

## TABLES

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

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

<sup>1</sup>Countermovement jump. <sup>2</sup>Sets = 20 Counter Movement Jump. <sup>3</sup>Rest between sets = 30 seconds. <sup>4</sup>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). <sup>5</sup>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.

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
Age (years)	14.5 ± 0.9	<b>15.3 ±</b> <b>0.9</b>	14.7 ± 1.1	<b>15.4 ±</b> <b>1.1</b>	13.8 ± 1.0	<b>14.6 ±</b> <b>1.0</b>	13.7 ± 0.8	<b>14.5 ±</b> <b>0.8</b>	14.1 ± 1.1	<b>14.9 ±</b> <b>1.1</b>	14.1 ± 0.9	<b>14.9 ±</b> <b>0.9</b>
Height (cm)	170.3 ± 10.0	<b>174.1 ±</b> <b>9.6</b>	172.8 ± 7.6	<b>176.0 ±</b> <b>6.9</b>	160.5 ± 11.0	<b>165.4 ±</b> <b>11.1</b>	163.3 ± 9.3	<b>168.9 ±</b> <b>9.1</b>	168.2 ± 10.7	<b>174.5 ±</b> <b>8.2</b>	162.7 ± 9.7	<b>166.4 ±</b> <b>9.6</b>
Body mass (kg)	57.2 ± 9.0	<b>62.6 ±</b> <b>7.8</b>	60.6 ± 7.2	<b>63.6 ±</b> <b>7.7</b>	49.3 ± 10.8	<b>54.8 ±</b> <b>11.6</b>	49.6 ± 7.4	<b>55.4 ±</b> <b>7.1</b>	57.7 ± 13.0	<b>62.6 ±</b> <b>12.2</b>	51.0 ± 12.4	<b>55.2 ±</b> <b>13.9</b>
Lean mass (kg)	46.7 ± 9.4	<b>51.2 ±</b> <b>8.5</b>	48.7 ± 8.2	<b>51.9 ±</b> <b>6.7</b>	41.5 ± 10.0	<b>46.1 ±</b> <b>10.5</b>	39.6 ± 7.4	<b>44.9 ±</b> <b>7.2</b>	45.0 ± 7.9	<b>49.5 ±</b> <b>7.6</b>	40.3 ± 8.6	<b>44.0 ±</b> <b>9.2</b>
Fat mass (kg)	7.6 ± 3.1	<b>8.5 ±</b> <b>2.9</b>	8.2 ± 3.4	8.7 ± 3.8	5.4 ± 2.2	<b>6.1 ±</b> <b>2.3</b>	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	<b>1.7 ±</b> <b>1.0</b>	1.2 ± 1.0	<b>1.8 ±</b> <b>0.9</b>	0.0 ± 1.0	<b>0.7 ±</b> <b>1.0</b>	0.1 ± 0.8	<b>0.9 ±</b> <b>0.8</b>	0.7 ± 1.1	<b>1.4 ±</b> <b>1.0</b>	0.3 ± 0.9	<b>1.0 ±</b> <b>1.0</b>
Maturation (I/II/III/ IV/V) (%)	(10/10/10 /50/20)	<b>(0/0/11 /52/37)</b>	(0/11/16 /47/26)	<b>(0/0/6 /61/33)</b>	(10/10/4 /3/37/0)	<b>(0/13/13 /40/34)</b>	(0/22/28 /50/0)	<b>(0/7/27 /40/27)</b>	(7/7/20 /53/13)	<b>(0/0/14 /43/43)</b>	(8/15/8 /61/8)	<b>(0/8/17 /42/33)</b>
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	<b>11.8 ±</b> <b>5.4</b>	10.2 ± 3.1	<b>12.9 ±</b> <b>5.4</b>	10.2 ± 1.2	<b>12.0 ±</b> <b>2.8</b>	8.8 ± 2.0	<b>10.7 ±</b> <b>1.8</b>	5.3 ± 2.0	<b>7.9 ±</b> <b>3.8</b>	5.3 ± 1.6	<b>8.2 ±</b> <b>2.8</b>
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 $\Delta$ (95% CI)	PRE	9-month adjusted gains $\Delta$ (95% CI)	PRE	9-month adjusted gains $\Delta$ (95% CI)	PRE	9-month adjusted gains $\Delta$ (95% CI)	PRE	9-month adjusted gains $\Delta$ (95% CI)	PRE	9-month adjusted gains $\Delta$ (95% CI)
TBLH	1892 $\pm$	322	1952 $\pm$	251	1730 $\pm$	386	1769 $\pm$	325	1848 $\pm$	<b>341</b>	1582 $\pm$	203
BMC	339	(276-368)	325	(203-300)	479	(335-438)	398	(274-377)	379	<b>(290-394)</b>	401	(145-260)
Legs	888 $\pm$	<b>141</b>	922 $\pm$	95	877 $\pm$	150	886 $\pm$	131	899 $\pm$	<b>148</b>	759 $\pm$	87
BMC	147	<b>(124-159)</b>	135	(77-114)	246	(130-170)	201	(111-150)	170	<b>(129-168)</b>	168	(65-109)
Arms	290 $\pm$	44	303 $\pm$	40	226 $\pm$	46	228 $\pm$	48	271 $\pm$	49	233 $\pm$	40
BMC	68	(37-51)	67	(33-47)	76	(38-53)	61	(41-56)	68	(40-56)	74	(32-48)
Bone	95.9 $\pm$	<b>10.3</b>	94.5 $\pm$	-1.8	104.4 $\pm$	15.3	105.7 $\pm$	10.9	95.6 $\pm$	<b>9.5</b>	93.6 $\pm$	-2.2
stiffness	12.0	<b>(7.2-13.5)</b>	16.3	(-5.1-1.6)	11.9	(11.6-8.9)	12.4	(7.2-14.6)	15.6	<b>(5.9-13.1)</b>	14	(-6.0-1.7)

Raw values at PRE are mean  $\pm$  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	<b>49.9 ± 7.7*</b>	46.5 ± 9.4	45.9 ± 8.8	45.3 ± 6.1	<b>47.5 ± 5.7</b>	41.7 ± 5.8	<b>43.4 ± 6.4</b>	42.7 ± 5.9	<b>45.9 ± 5.3*</b>	45.4 ± 7.8	46.1 ± 8.0
Standing Long Jump (cm)	195.8 ± 27.8	<b>203.1 ± 27.9</b>	191.1 ± 27.7	194.7 ± 28.1	188.1 ± 24.9	<b>194.6 ± 23.4</b>	184.1 ± 21.2	191.7 ± 22.3	173.8 ± 34.1	<b>182.9 ± 31.8</b>	180.7 ± 24.5	185.3 ± 28.4
20mSRT (shuttles)	79.2 ± 17.6	<b>85.7 ± 17.3</b>	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	<b>88.5 ± 22.6</b>	82.8 ± 19.2	85.6 ± 25.1

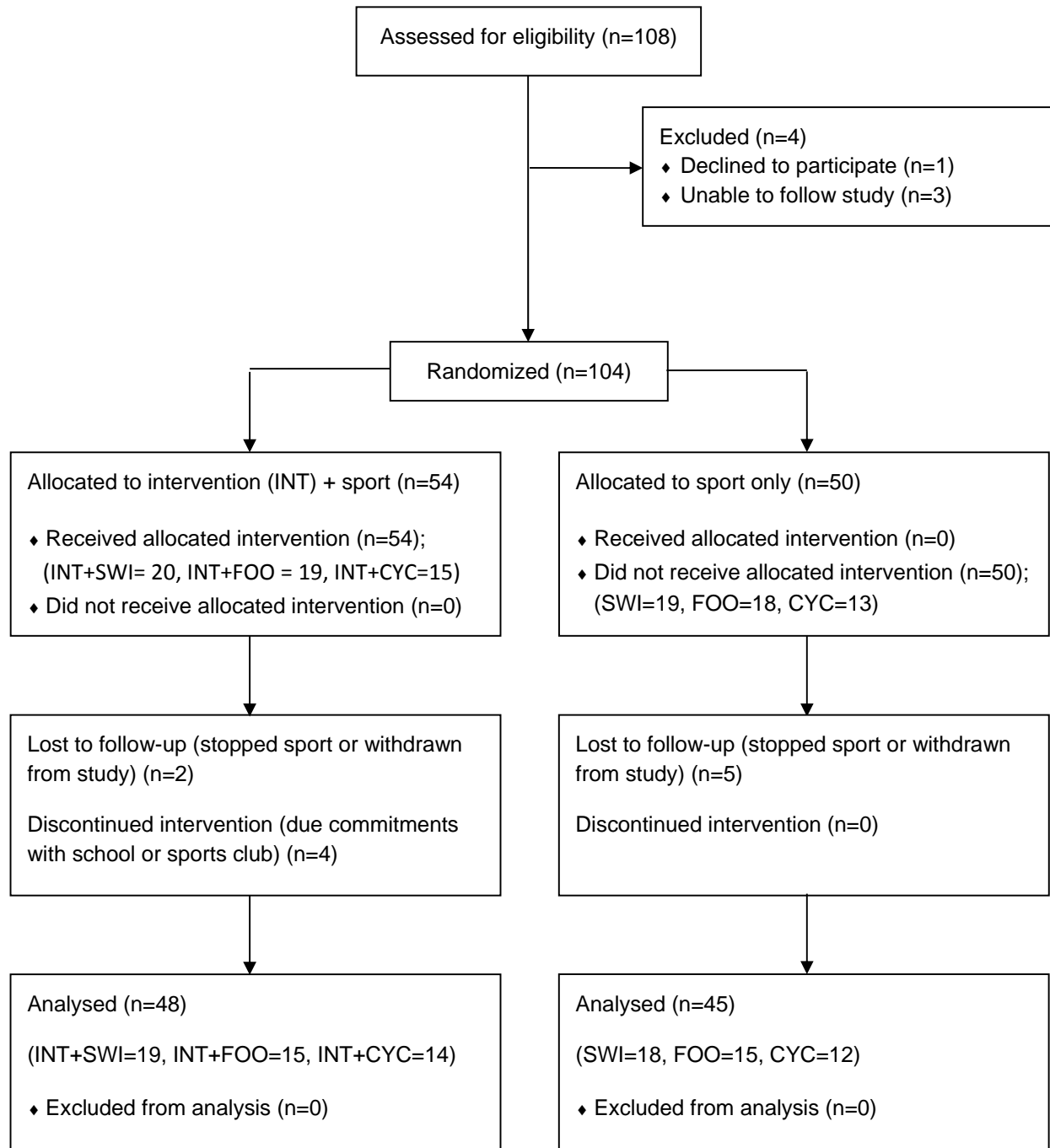
20mSRT: 20meter shuttle run test. Values are mean ± standard deviation. No differences observed at PRE and POST between INT and CON groups of each specific sport, p>0.05. Bold values denote significant different values between PRE and POST, p<0.05. \* denote significant higher 9-month increase in CMJ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

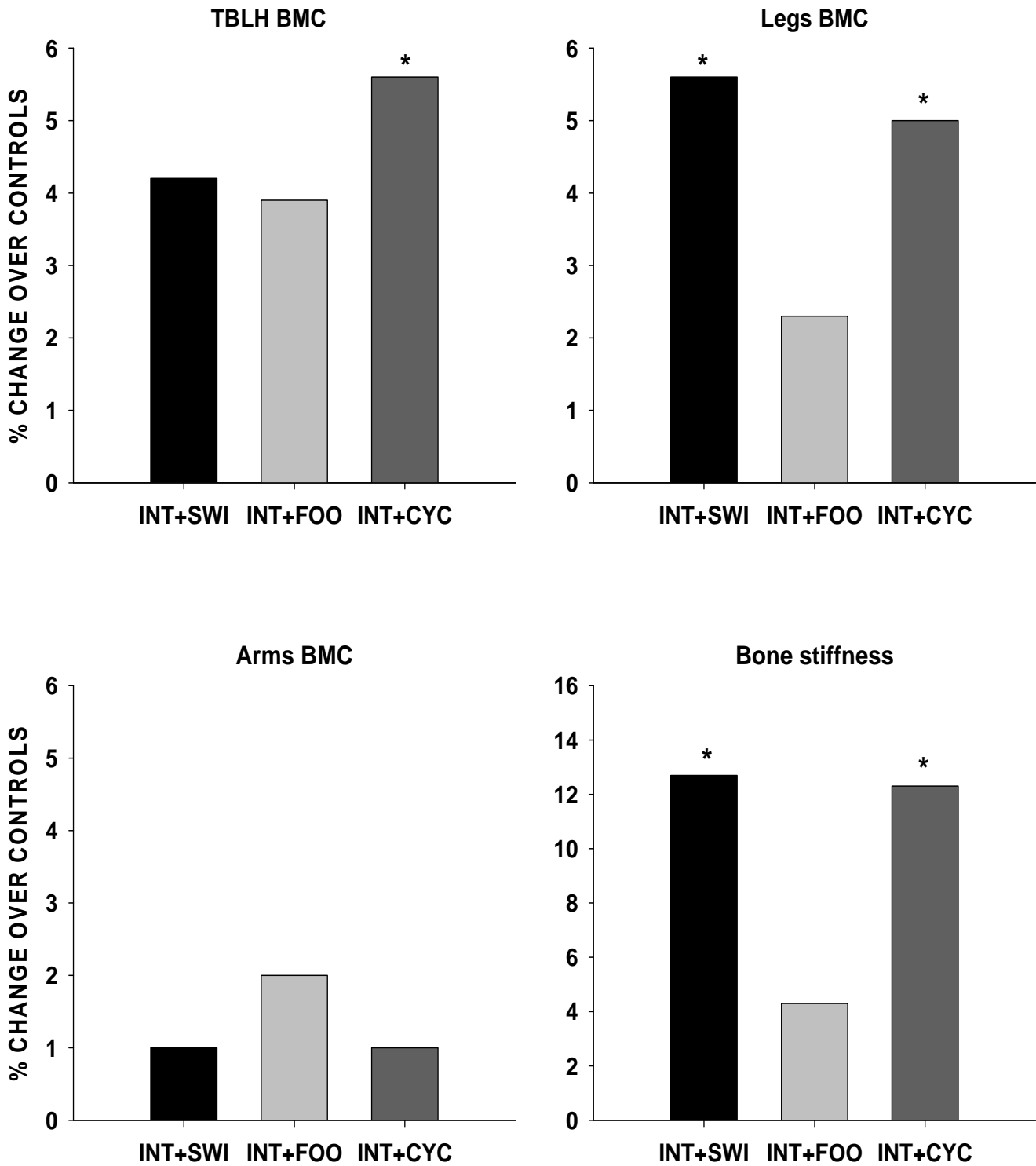
**Table 4.** Physical fitness measurements of the sports groups and the control group before (PRE) and after (POST) the 9-month intervention programme.

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 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	<b>3.1 ±</b> <b>0.4*</b>	46.5 ± 9.4	-0.6 ± 0.3	45.3 ± 6.1	<b>2.2 ±</b> <b>0.3</b>	41.7 ± 5.8	<b>1.7 ±</b> <b>0.5</b>	42.7 ± 5.9	<b>3.2 ±</b> <b>0.6*</b>	45.4 ± 7.8	0.7 ± 0.4
Standing Long Jump (cm)	195.8 ± 27.8	<b>7.3 ±</b> <b>1.8</b>	191.1 ± 27.7	3.6 ± 2.0	188.1 ± 24.9	<b>6.5 ±</b> <b>2.6</b>	184.1 ± 21.2	7.6 ± 3.8	173.8 ± 34.1	<b>9.1 ±</b> <b>2.2</b>	180.7 ± 24.5	4.6 ± 3.6
20mSRT (shuttles)	79.2 ± 17.6	<b>6.5 ±</b> <b>1.7</b>	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	<b>6.1 ±</b> <b>1.0</b>	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 different values between PRE and POST, p<0.05. \* denotes a significant higher-9-month increase in CMI 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,  $p < 0.05$ .