

1 **Title**

2 The effect of 12-month participation in osteogenic and non-osteogenic sports on bone  
3 development in adolescent male athletes. The PRO-BONE study.

4

5

6 **Abstract**

7

8 *Objectives:* Research investigating the longitudinal effects of the most popular sports on bone  
9 development in adolescent males is scarce. The aim is to investigate the effect of 12-month  
10 participation in osteogenic and non-osteogenic sports on bone development.

11 *Design:* A 12-month study was conducted in adolescent males involved in football, swimming and  
12 cycling and compared with an active control group.

13 *Methods:* 116 adolescent males ( $13.1 \pm 0.1$  years at baseline): 37 footballers, 37 swimmers, 28 cyclists  
14 and 14 active controls were followed for 12 months. Bone mineral content (BMC) was measured by  
15 dual-energy x-ray absorptiometry, and bone stiffness was measured by quantitative ultrasound. Bone  
16 outcomes at 12 months were adjusted for baseline bone status, age, height, lean mass and moderate to  
17 vigorous physical activity.

18 *Results:* Footballers had higher improvement in adjusted BMC at the total body, total hip, shaft,  
19 Ward's triangle, legs and bone stiffness compared to cyclists (6.3 to 8.0 %). Footballers had  
20 significantly higher adjusted BMC at total body, shaft and legs compared to swimmers (5.4 to 5.6 %).  
21 There was no significant difference between swimmers and cyclists for any bone outcomes.  
22 Swimming and cycling participation resulted in non-significant lower bone development at most sites  
23 of the skeleton compared to controls (-4.3 to -0.6 %).

24 *Conclusions:* Football participation induces significantly greater improvements in BMC and bone  
25 stiffness over 12 months compared to cycling and swimming.

26

27 *Keywords:* Adolescence; Bone mass; Bone stiffness; Cycling; Football; Swimming; Weight-bearing  
28 exercise.

## 29 **1. Introduction**

30 Bone development occurs most rapidly during childhood and adolescence, with 80-90 % of peak  
31 bone mass (PBM) acquired by late adolescence depending on the site of the skeleton <sup>1</sup>. PBM is largely  
32 determined by genetics <sup>2</sup> and by modifiable factors, such as nutrition and physical activity (PA) <sup>3,4</sup>.  
33 Exercise during this period of life can enhance bone mineral content (BMC) and bone mineral density  
34 (BMD) <sup>5</sup> and be maintained into adulthood <sup>6</sup>. Football, cycling and swimming are among the most  
35 popular sports performed by adolescents around the world <sup>7</sup>. However, participation in these sports  
36 may have different effects on bone development <sup>8</sup>. Participation in “osteogenic” sports, such as  
37 football, can augment BMC at the loaded sites of the skeleton <sup>9,10</sup>. However, participation in “non-  
38 osteogenic sports”, such as swimming and cycling, may have a negative or no impact on bone  
39 outcomes<sup>11</sup>, which may compromise the achievement of a higher PBM and increase the risk of  
40 osteoporotic fractures in adulthood. From a public health perspective, understanding how the most  
41 popular sports worldwide among youth affect bone development is of great importance.

42 Cross-sectional studies have evaluated differences in BMC between adolescents engaged in  
43 different sports in comparison to a control group<sup>11</sup>. Specifically, footballers were found to have higher  
44 adjusted-BMC and BMD at most sites of the skeleton compared with age-matched controls <sup>9</sup>. In  
45 contrast, previous evidence found that adolescent male swimmers had lower adjusted-BMC and BMD  
46 at several sites compared to controls <sup>12</sup>, but a recent systematic review concluded that swimmers have  
47 similar bone mass compared to sedentary controls <sup>13</sup>. Similarly, in a cross-sectional analysis we found  
48 that adolescent male swimmers and cyclists had lower bone outcomes compared to footballers <sup>8</sup>.  
49 However, other studies showed that cycling during adolescence may negatively influence bone health  
50 <sup>11,14</sup>. To date, there are only a few longitudinal studies on this topic and it was found that 3 years of  
51 football participation increased femoral neck BMD by 10 % and improved femoral neck and  
52 intertrochanteric BMC twice as much compared to age-matched controls in prepubertal males <sup>15</sup>.  
53 Previously, 8 months of football training significantly improved bone outcomes at total body,  
54 intertrochanteric site, lumbar spine and femoral neck in female adolescent footballers, whereas 8  
55 months of swimming training had no effect on bone outcomes in female adolescent swimmers <sup>16</sup>.

56 Research investigating the longitudinal effects of the most popular sports on bone development in  
57 adolescent males is scarce<sup>17</sup>. It should be noted that a comprehensive analysis of potential  
58 confounders, such as lean mass and objectively measured moderate-to-vigorous PA (MVPA) should  
59 be used to control for important predictors of bone status in these sports<sup>18</sup>.

60 In addition to Dual energy X-ray Absorptiometry (DXA), Quantitative Ultrasound (QUS) can  
61 indicate the risk of osteoporotic fractures at the calcaneus site that is particularly important for  
62 adolescent athletes due to their high prevalence of injuries<sup>19,20</sup>. In a cross-sectional study, it was  
63 shown that swimming had no effect on bone stiffness compared to age-matched controls in adolescent  
64 males and females<sup>12</sup>. Also, in a cross-sectional analysis it was found that footballers had higher bone  
65 stiffness than controls but there were no differences in swimmers and cyclists compared to controls<sup>8</sup>.  
66 However, there is lack of longitudinal studies comparing the effects of osteogenic and non-osteogenic  
67 sports on QUS bone outcomes in adolescent males athletes<sup>21</sup>. Therefore, the purpose of this study is to  
68 investigate the effect of 12-month participation on BMC and bone stiffness in osteogenic (football)  
69 and that non-osteogenic sports (swimming and cycling) compared to an active control group after  
70 controlling for baseline bone outcomes, age, height, lean mass and MVPA.

71

## 72 **2. Methods**

73 The present study represents a 12-month analysis of sport participation as part of the PRO-BONE  
74 study, whose purpose and methodology have been described elsewhere<sup>22</sup>. For the present study, data  
75 obtained at baseline (T0) during autumn/winter 2014/15 and at follow-up (T1) during autumn/winter  
76 2015/2016 were used (mean difference of visits = 372 days). Five participants were excluded because  
77 they did not complete the second visit (n=3) or they had missing data (n=2). For the present study, 116  
78 adolescent males (37 swimmers, 37 footballers, 28 cyclists and 14 active controls not engaged in these  
79 sports more than 3 hour per week) aged 13.1 years  $\pm$  1.0 at T0 and 14.1 years  $\pm$  1.0 at T1 were  
80 included. The inclusion criteria at T0 were: 1) males 12–14 years old, engaged ( $\geq$ 3 h/week) in  
81 osteogenic (football) and/or non-osteogenic (swimming and cycling) sports for the last 3 years or

82 more; 2) males 12–14 years old not engaged in any of these sports ( $\geq 3$  h/week) in the last 3 or more  
83 years (control group). The exclusion criteria were at T0 were: 1) participants not taking part in another  
84 clinical trial; 2) participants not having any acute infection lasting until  $< 1$  week before inclusion; 3)  
85 participants free of any medical history of diseases or medications affecting bone metabolism or  
86 injured; 4) white Caucasian ethnicity. Ethics approval received from the following committees: 1) the  
87 Ethics Review Sector of Directorate-General of Research (European Commission, ref. number  
88 618496); 2) the Sport and Health Sciences Ethics Committee (University of Exeter, ref. number  
89 2014/766) and 3) the National Research Ethics Service Committee (NRES Committee South West –  
90 Cornwall & Plymouth, ref. number 14/SW/0060).

91 A DXA scanner (GE Lunar Prodigy Healthcare Corp., Madison, WI, USA, 2006) was used to  
92 measure BMC (g), fat mass (g) and lean mass (g, excluding bone and fat mass). The total body scan  
93 was used to obtain BMC at the arms, legs, and total body (excluding head). Dual hip scans were  
94 performed to obtain BMC for total hip, femoral neck, Ward's triangle, trochanter and shaft sub-  
95 regions and the mean of right and left hip scans was used. The coefficient of variation (CV) for  
96 measurement reliability was not determined in the present study. Previous paediatric studies have  
97 shown that the DXA between-day CV was between 1.0 % and 2.9 % depending on the region<sup>23</sup>. In  
98 addition, QUS measurements were performed with a Lunar Achilles Insight (TM Insight GE  
99 Healthcare, Milwaukee, WI, USA). This portable device measures bone stiffness using ultrasound  
100 waves. QUS is a non-ionising radiation technique and evaluates bone stiffness based on broadband  
101 ultrasound attenuation (dB/MHz) and speed of sound (m/s)<sup>24</sup>. The real-time image of the calcaneus  
102 and the region of interest ensures that the measurement is reliable and valid to assess bone health as  
103 demonstrated in paediatric population<sup>25</sup>. Daily calibration was completed at all visits and  
104 measurements were taken according to the standard procedure provided by the manufacturer. The  
105 positioning was standardised between visits by using an adapter for the children's feet in order to get  
106 the same position of the calcaneus. Both feet were measured twice and the mean of the two measures  
107 was used for statistical analyses.

108 Stature (cm) and body mass (kg) were measured by using standard procedures and sexual maturity  
109 was self-reported using adapted drawings of the five stages of pubic hair development<sup>26</sup>. Physical

110 activity was measured for seven consecutive days at T0 and T1 using wrist accelerometers  
111 (GENEAActiv, GENEActiv, UK). The validity and reliability of the accelerometer has been established  
112 previously in children and adolescents<sup>27</sup>. Data were collected at 100 Hz and analysed at 1 s epoch  
113 intervals to establish time spent in MVPA using a validated cut-point<sup>27</sup>. Weekly training hours were  
114 obtained by face to face interviews at T0 at T1. In addition, the coaches indicated participation in  
115 weight-training exercises for a subsample of participants.

116 Statistical analyses were performed using the SPSS IBM statistics (version 21.0 for Windows,  
117 Chicago, IL, USA). Data were normally distributed and presented as mean and standard deviation.  
118 Data were analysed in two stages: 1) raw (unadjusted) data using one-way analysis of variance  
119 (ANOVA) with Bonferroni post hoc or Chi-Square tests at T0 and T1 to detect the differences in  
120 BMC, and 2) adjusted data using one-way analysis of covariance (ANCOVA) with Bonferroni post  
121 hoc to detect the differences between the groups at T1 after controlling for: bone status at T0, age,  
122 height, lean mass, MVPA and maturity status<sup>18,28,29</sup>. Paired t-tests were used to compare differences  
123 in values between T0 and T1. Preliminary analyses showed bone outcome results did not change when  
124 maturity was used as confounder instead of age. Thus, maturity was not included in the model.  
125 Percentages of difference between groups were used to quantify the magnitude of the differences.  
126 Significance was set at  $p < 0.05$  and  $p < 0.01$ .

127

### 128 **3. Results**

129 Table 1 presents the descriptive characteristics of the participants at T0 and T1. From T0 to T1 all  
130 the descriptive characteristics significantly increased in all groups except MVPA in all groups and  
131 body fat percentage in sports groups that significantly decreased. Between-group differences at T1  
132 showed that swimmers were older, taller, heavier and had more lean mass than the footballers and  
133 controls. Swimmers were more mature than footballers and controls. Swimmers trained more hours  
134 per week and had more years of training than cyclists. Footballers spent more time doing MVPA than  
135 swimmers and controls. In addition, footballers trained more hours per week and had more training

136 years than cyclists and swimmers. Cyclists were older than controls and spent more time doing MVPA  
137 compared to swimmers and controls. Controls had a higher body fat percentage than all sports groups.

138 (Table 1 here)

139 Table 2 shows the adjusted BMC and bone stiffness at T0 and T1 between the groups and Figure 1  
140 shows the adjusted BMC and bone stiffness differences (%) between the sports groups and controls at  
141 T1. At T1 footballers had significantly higher BMC at total body, shaft and legs compared to  
142 swimmers (5.4 to 5.6 %). Also, at T1 footballers had significantly higher BMC at total body, total hip,  
143 Ward's triangle, shaft and legs compared to cyclists (6.3 to 8.0 %). At T1 footballers had non-  
144 significantly higher bone outcomes than controls (3.3 to 8.4 %). The adjusted bone stiffness was  
145 significantly higher in footballers compared to cyclists (7.8 %) at T1. Swimmers and cyclists had  
146 similar bone outcomes at T1 (-0.6 to 4.3 %) and both groups had no significant differences at any of  
147 the bone outcomes compared to controls (-4.5 to 4.7 %).

148 (Table 2 and Figure 1 here)

149 Supplementary table 1 shows the unadjusted change in bone outcomes at T0 and T1. At T1 BMC  
150 significantly increased at all skeletal sites in swimmers (10.3 to 21.0 %), footballers (13.6 to 23 %),  
151 cyclists (9.9 to 19.0 %) and controls (14.8 to 21.0 %) compared to T0. In addition, bone stiffness  
152 significantly increased in swimmers (4.5 %), footballers (6.9 %) and controls (5.1 %) from T0 to T1,  
153 but the increase was not significantly different in cyclists (0.9 %).

154

#### 155 **4. Discussion**

156 The main findings of the present study are: 1) after 12 months of sports participation, footballers  
157 had significantly higher BMC and bone stiffness gains compared to swimmers and cyclists, and higher  
158 but non-significant BMC and bone stiffness compared to active controls; 2) after 12 months swimmers  
159 and cyclists had similar BMC and bone stiffness, and both groups had no significant differences in  
160 BMC and bone stiffness compared to controls.

161 The present study shows that after 12 months footballers had higher adjusted BMC compared to  
162 cyclists and swimmers at most skeletal sites. The only study comparing these sports was conducted in  
163 female adolescent swimmers and footballers and showed that 8 months period of sport-specific  
164 training increased total body BMD by 2.9 % in footballers, whereas BMD remained constant in  
165 swimmers<sup>16</sup>. The present study found that footballers had 2.4 % higher adjusted BMC compared to  
166 swimmers after 12 months. Cross-sectional evidence in adolescent males found that footballers had  
167 greater BMD at the femoral neck compared to swimmers<sup>30</sup>. The differences observed in BMC gains  
168 among the sports groups in the present study might be explained by the plyometric exercises included  
169 in the football training that can induce higher bone mass in adolescent athletes despite the reduced lean  
170 mass in footballers compared to swimmers<sup>31</sup>. In this regard, Larsen et al. found that a 10-month  
171 programme that included small-sided ball games improved BMD at the legs and total body compared  
172 to controls, and BMD at the legs compared to a circuit strength training<sup>32</sup>.

173 In the present study, BMC development over 12 months was similar between adolescent male  
174 swimmers and cyclists at any skeletal sites. This is in line with studies showing that swimming and  
175 cycling seem to have no additional effect on bone growth<sup>11,12</sup>, which could be due to the low ground  
176 reaction forces produced during participation in the non-osteogenic environment. In regards to bone  
177 stiffness, the present study showed that footballers significantly increased bone stiffness compared to  
178 cyclists. The latter is in accordance with cross-sectional analysis from this cohort showing that  
179 footballers had significantly higher bone stiffness compared to swimmers and cyclists<sup>8</sup>.

180 Football participation during adolescence may induce higher bone outcomes compared to leisure  
181 active controls according to cross-sectional evidence<sup>9,10</sup>. However, evidence from a study in  
182 prepubescent boys found that footballers had non-significant but higher bone outcomes compared to  
183 active controls after 10 months of training<sup>33</sup>. These results are in line with our findings showing that  
184 footballers had higher (3.3 % to 8.4 %) but not significant bone outcomes compared to active controls  
185 after 12 months. It should be noted that the control group was physically active (MVPA= 64 min/day)  
186 and some controls engaged in other weight-bearing sports (< 3 hours per week) which might explain  
187 the non-significant difference compared to footballers. A previous cross-sectional study showed that

188 footballers had significantly higher bone stiffness at lower extremities compared to active controls <sup>34</sup>.  
189 The differences in bone outcomes between adolescent footballers and controls might increase in the  
190 future due to the previous findings showing that 3 years of football training exhibited significantly  
191 greater adjusted BMC in total body, legs and intertrochanteric sites compared to age-matched controls  
192 <sup>15</sup>.

193 Swimming is considered a non-osteogenic sport and does not promote positive changes on bone  
194 development above that observed due to growth. According to a recent meta-analysis, swimmers and  
195 sedentary controls have similar bone outcomes <sup>13</sup>. In addition, adolescent males that participated only  
196 in swimming had lower BMD and BMC at several sites of the skeleton compared to age-matched  
197 controls <sup>12</sup>. In the present study swimmers had similar BMC gains with active controls after  
198 controlling for relevant covariates (including T0 BMC). Similarly, we found swimmers to have similar  
199 bone outcomes with controls at baseline after controlling for the same covariates <sup>8</sup>. A possible  
200 explanation is that swimming has non-gravitational training characteristics and despite swimmers  
201 having augmented higher lean mass it was not enough to produce bone adaptations after 12-months of  
202 training <sup>35</sup>. Regarding bone stiffness, previous cross-sectional findings showed similar values between  
203 swimmers and controls <sup>12</sup>.

204 Cycling is a widely practised sport that applies low mechanical forces to the skeleton during  
205 training <sup>36</sup> and the present analysis showed that cyclists had lower but non-significant adjusted BMC  
206 and bone stiffness than controls. Previous evidence exist only from cross-sectional studies indicating  
207 that adolescent female cyclists had similar or lower bone outcomes compared to non-athletic  
208 controls<sup>16</sup>. Another cross-sectional study found that males cyclists (< 17 years) had significantly lower  
209 BMC at the legs compared to age-matched controls <sup>11</sup>. According to the baseline cross-sectional  
210 analysis of this cohort, cyclists had non-significantly higher adjusted BMC at the most skeletal sites <sup>8</sup>.  
211 However, after one year cyclists had non-significant lower bone development in BMC and bone  
212 stiffness than controls. The differences observed in the current study might be explained by the non-  
213 osteogenic environment of both swimming and cycling and by the mechanical loading produced by the  
214 sports-specific patterns. In addition, participation in plyometric training or other weight-bearing



215 activities might explain the difference on bone outcomes between adolescent athletes and needs  
216 further investigation to quantify the impact of weight training on bone outcomes.

217 The strengths of the present study are 1) the investigation of bone outcomes across osteogenic and  
218 non-osteogenic male adolescent groups over 12 months; 2) the combination of DXA and QUS, which  
219 provides a comprehensive insight into BMC and bone stiffness outcomes and 3) the rigorous  
220 methodology that enabled the inclusion of a selection of specific confounders which increases the  
221 internal validity of the study. A limitation of the present study is the lack of nutrition-related  
222 covariates and the two time points of the longitudinal assessment. However, we have observed that  
223 dietary intakes (total energy, protein and calcium) were no different between the groups at T0 and T1  
224 (data not reported). In addition, despite the two measurements completed, this is the first stud to assess  
225 the differences in bone development of these sports over 12 months. Also, it should be noted that all  
226 sport groups were very active, but cyclists trained less compared to footballers and swimmers.

## 227 **5. Conclusions**

228 In summary, this is the first study to investigate the 12-month development on BMC and bone  
229 stiffness in adolescent males engaged in osteogenic (football) and non-osteogenic sports (swimming  
230 and cycling). The findings of this study suggest that 12 months of football participation induces  
231 greater BMC and bone stiffness compared to cycling or swimming participation. In addition,  
232 footballers had higher BMC although not significant compared to an active control group. Swimmers  
233 and cyclists had similar bone outcomes after 12 months, and both groups no significant differences in  
234 any of the bone outcomes compared to active controls. These findings suggest that participation in  
235 non-osteogenic sports during adolescence should be combined with weight-bearing activities in order  
236 to optimise bone development. Studies focusing on females and using specific interventions to  
237 improve bone mineralization in non-osteogenic sports during growth are needed.

238

239 **Practical implications**

- 240 • Football participation for 12-months induces significantly higher increase in bone mineral content  
241 and bone stiffness compared to cycling and swimming in adolescent males.
- 242 • Participation in cycling and swimming for 12-months has similar effects on bone development in  
243 adolescent males and both groups have non-significant lower bone outcomes compared to active  
244 controls.
- 245 • Cycling and swimming participation may compromise the optimal bone development during  
246 adolescence suggesting intervention studies are needed to improve bone development in  
247 adolescents participating in these sports.

248

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252 with the study.

253 **Abbreviations:** BMC: Bone mineral content; BMD: Bone mineral density; DXA: Dual Energy X-Ray  
254 Absorptiometry; MVPA: Moderate to vigorous physical activity; PA: Physical activity; QUS:  
255 Quantitative ultrasound; PBM: Peak bone mass; T0: baseline measurements; T1: 12-months  
256 measurements.

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352

353 **Tables**

354

**Table 1.**

Descriptive characteristics of the participants at baseline (T0) and after 12 months (T1) of sport participation

N = 116	Swimmers (N = 37)	Footballers (N = 37)	Cyclists (N = 28)	Controls (N = 14)
Age (yrs)				
T0	13.5 (1.0) <sup>b,dd</sup>	12.9 (0.9)	13.2 (1.0) <sup>d</sup>	12.3 (0.5)
T1	14.6 (1.0) <sup>b,dd,*</sup>	13.9 (0.9) <sup>*</sup>	14.2 (1.0) <sup>d,*</sup>	13.2 (0.5) <sup>*</sup>
Stature (cm)				
T0	165.1 (9.7) <sup>bb,d</sup>	155.2 (9.3)	160.7 (10)	154.5 (9.9)
T1	171.6 (8.9) <sup>bb,dd,*</sup>	162.7 (10.3) <sup>*</sup>	166.6 (10.7) <sup>*</sup>	160.7 (10.5) <sup>*</sup>
Body mass (kg)				
T0	51.9 (8.7) <sup>bb</sup>	44.3 (7.9)	49.3 (12.5)	48.3 (13.0)
T1	58.9 (8.2) <sup>b,*</sup>	50.8 (9.7) <sup>*</sup>	54.7 (12.5) <sup>*</sup>	55.2 (15.6) <sup>*</sup>
BMI (kg/m <sup>2</sup> )				
T0	18.9 (1.6)	18.3 (1.4)	18.9 (3.3)	20.0 (3.4)
T1	19.9 (2.0) <sup>*</sup>	19.0 (1.8) <sup>*</sup>	21.0 (3.1) <sup>*</sup>	21.0 (3.7) <sup>*</sup>
Lean mass (kg)				
T0	41.1 (9.0) <sup>b,dd</sup>	35.4 (7.2)	37.5 (7.5)	31.7 (5.5)
T1	47.8 (8.7) <sup>b,dd,*</sup>	41.2 (9.2) <sup>*</sup>	42.9 (8.2) <sup>*</sup>	36.8 (7.1) <sup>*</sup>
Body fat (%)				
T0	17.3 (7.3) <sup>*</sup>	15.7 (5.6) <sup>*</sup>	18.0 (9.0) <sup>*</sup>	29.0 (10.5) <sup>aa,bb,cc</sup>
T1	14.4 (6.4)	14.5 (6.0)	16.1 (9.2)	27.9 (10.9) <sup>aa,bb,cc</sup>
Tanner stages (1-5; %)				
T0	(16/25/16/43/0)	(24/35/24/16/0)	(14/28/25/28/4)	(29/21/21/29/0)
T1	(5/11/11/51/22) <sup>b,d,*</sup>	(6/16/35/43/0) <sup>*</sup>	(7/11/14/57/11) <sup>*</sup>	(0/21/43/36/0) <sup>*</sup>
Training (h/week)				
T0	9.4 (5.1) <sup>cc</sup>	10.0 (2.3) <sup>cc</sup>	5.2 (2.1)	-
T1	8.9 (3.6) <sup>cc</sup>	9.4 (1.7) <sup>cc</sup>	5.6 (2.0)	-
Years of training				
T0	5.9 (2.5) <sup>cc</sup>	7.5 (2.3) <sup>a,cc</sup>	3.9 (1.3)	-
T1	6.9 (2.5) <sup>cc,*</sup>	8.5 (2.3) <sup>a,cc,*</sup>	4.9 (1.3)	-
MVPA (min/day)				
T0	85.0 (30.9) <sup>*</sup>	119.8 (29.7) <sup>aa,dd,*</sup>	106.5 (33.7) <sup>a,*</sup>	83.2 (26.8) <sup>*</sup>
T1	62.9 (21.8)	92.4 (25.7) <sup>aa,dd</sup>	85.6 (21.8) <sup>aa,d</sup>	64.3 (18.1)

Values presented as mean (SD). BMI: Body mass index, MVPA: Moderate to vigorous physical activity. T0 = baseline values, T1 = 1 year values. Superscript letters denote a higher significant difference between sports: a (swimmers), b (footballers), c (cyclists), d (controls), <sup>a,b,c,d</sup> p<0.05, <sup>aa,bb,cc,dd</sup> p<0.001 and within each sports group at T0 and T1: \* p<0.05.

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**Table 2.**

Adjusted bone mineral content (BMC, g) and bone stiffness at baseline (T0) and after 12 months (T1) of sports participation in adolescent males

N = 116	Swimmers (N = 37)	Footballers (N = 37)	Cyclists (N = 28)	Controls (N = 14)
<b>TBLH (g)</b>				
T0	1453.9 (21.1)	1574.5 (21.5) <sup>a,c,d</sup>	1459.9 (22.7)	1451.8 (34.4)
T1	1752.9 (20.9)	1846.7 (20.9) <sup>a,cc</sup>	1737.0 (21.9)	1787.1 (33.6)
<b>Total hip (g)</b>				
T0	26.50 (0.50)	30.24 (0.51) <sup>aa,cc,d</sup>	26.62 (0.53)	24.61 (0.79)
T1	32.04 (0.42)	33.53 (0.44) <sup>c</sup>	31.26 (0.45)	31.70 (0.70)
<b>Ward's (g)</b>				
T0	2.15 (0.06)	2.48 (0.06) <sup>a,c,dd</sup>	2.14 (0.06)	1.92 (0.1)
T1	2.66 (0.05)	2.74 (0.05) <sup>c</sup>	2.55 (0.05)	2.63 (0.08)
<b>Trochanter (g)</b>				
T0	8.11 (0.23)	9.85 (0.23) <sup>aa,cc,d</sup>	8.22 (0.24)	7.60 (0.36)
T1	10.99 (0.27)	11.38 (0.28)	10.59 (0.28)	10.50 (0.43)
<b>Shaft(g)</b>				
T0	14.16 (0.26)	15.71 (0.26) <sup>a,cc,d</sup>	14.12 (0.27)	13.09 (0.41)
T1	16.20 (0.16)	17.09 (0.17) <sup>a,cc</sup>	16.08 (0.17)	16.30 (0.27)
<b>Arms (g)</b>				
T0	209.27 (3.23)	207.24 (3.19)	211.98 (3.48) <sup>d</sup>	193.43 (5.22)
T1	252.85 (2.95)	258.71 (2.85)	254.39 (3.15)	249.52 (4.84)
<b>Legs (g)</b>				
T0	215.69 (4.51)	253.79 (4.59) <sup>aa,cc,d</sup>	223.08 (4.89)	216.31 (7.43)
T1	854.67 (9.67)	902.89 (9.82) <sup>a,cc</sup>	836.26 (9.85)	873.90 (15.24)
<b>Stiffness index</b>				
T0	89 (2)	100 (2) <sup>a,c,d</sup>	91 (2)	86 (3)
T1	97 (1)	101 (1) <sup>c</sup>	93 (1)	98 (2)

Values are presented as mean (SE). TBLH: Total body less head. Superscript letters denote a higher significant difference with: a (swimmers), b (footballers), c (cyclists) and d (controls). <sup>a,b,c,d</sup> p<0.05 and <sup>aa,bb,cc,dd</sup> p<0.001. At T0 BMC values were adjusted for age, stature, MVPA and lean mass. At T1 BMC values were adjusted for age, stature, MVPA, lean mass and for baseline BMC (T0).

**Supplementary table 1.**

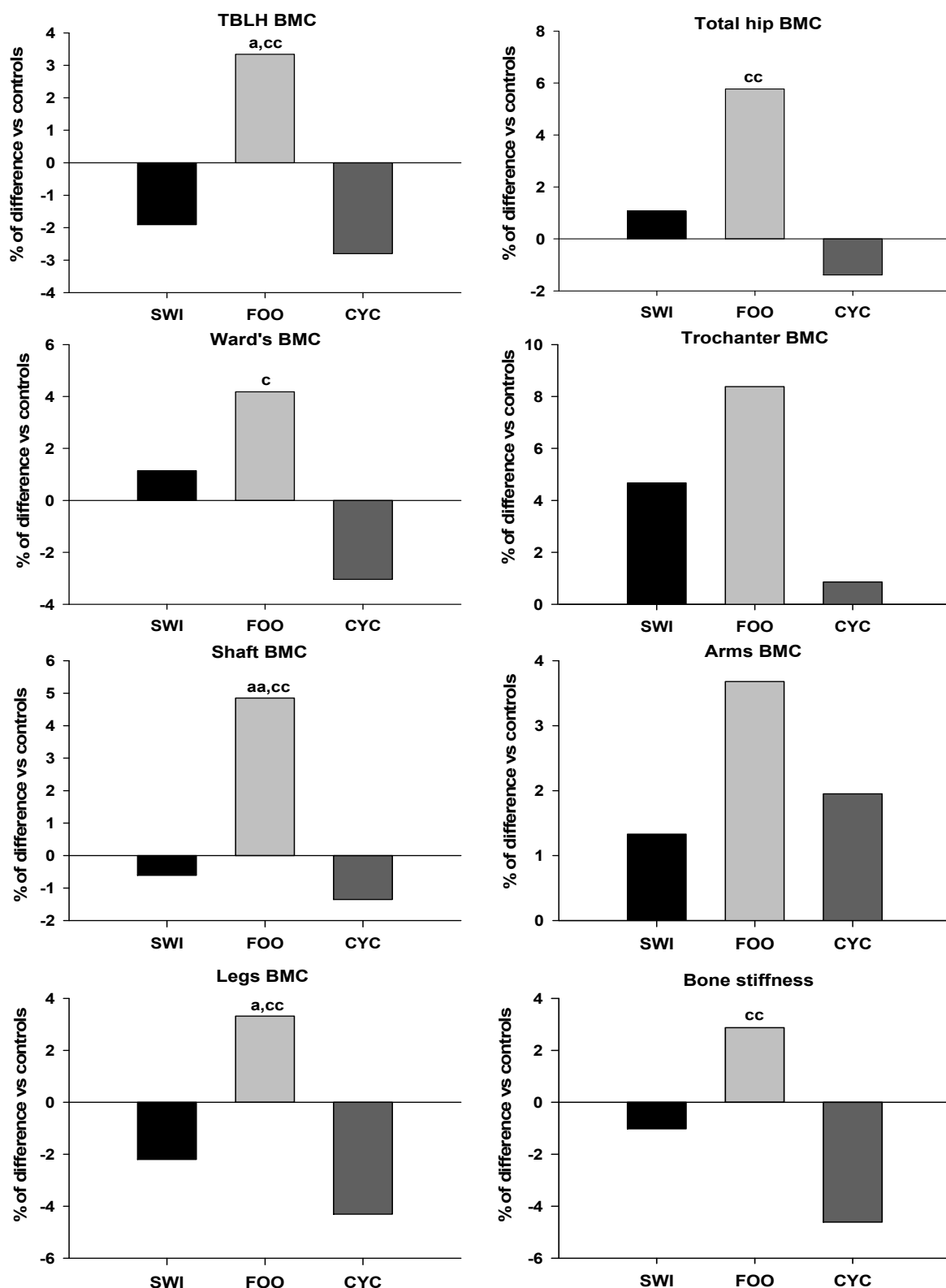
Unadjusted bone mineral content (BMC, g) and bone stiffness at baseline (T0) and after 12 months (T1) of sports participation in adolescent males

N = 116	Swimmers (N = 37)	Footballers (N = 37)	Cyclists (N = 28)	Controls (N = 14)
<b>TBLH (g)</b>				
T0	1622.8 (325.4) <sup>dd</sup>	1473.5 (338.6)	1478.9 (353.2)	1234.4 (347.9)
T1	1923.9 (327.8) <sup>dd</sup>	1791.3 (453.0)	1725.4 (396.2)	1504.8 (433.7)
<b>Total hip (g)</b>				
T0	28.79 (5.56) <sup>dd</sup>	28.78 (6.18) <sup>dd</sup>	27.31 (5.92) <sup>d</sup>	21.12 (5.55)
T1	33.52 (5.69) <sup>dd</sup>	34.56 (7.76) <sup>dd</sup>	31.17 (6.28) <sup>d</sup>	25.21 (6.49)
<b>Ward's (g)</b>				
T0	2.29 (0.50) <sup>dd</sup>	2.40 (0.59) <sup>dd</sup>	2.19 (0.51) <sup>d</sup>	1.64 (0.45)
T1	2.76 (0.63) <sup>d</sup>	2.92 (0.77) <sup>dd</sup>	2.51 (0.70)	1.98 (0.56)
<b>Trochanter (g)</b>				
T0	9.01 (2.35) <sup>d</sup>	9.31 (2.67) <sup>dd</sup>	8.49 (2.34) <sup>d</sup>	6.11 (2.17)
T1	11.41 (2.57) <sup>d</sup>	12.09 (3.84) <sup>dd</sup>	10.48 (2.76) <sup>d</sup>	7.73 (2.87)
<b>Shaft (g)</b>				
T0	15.31 (2.69) <sup>dd</sup>	14.94 (2.97) <sup>d</sup>	14.46 (2.96) <sup>d</sup>	11.49 (2.74)
T1	17.07 (2.59) <sup>d</sup>	17.30 (3.34) <sup>dd</sup>	16.05 (2.92)	13.49 (2.93)
<b>Arms (g)</b>				
T0	243.39 (64.01) <sup>bb,dd</sup>	188.34 (48.05)	210.62 (59.05) <sup>d</sup>	155.89 (40.58)
T1	297.38 (66.37) <sup>b,dd</sup>	235.65 (71.68)	254.04 (70.70) <sup>d</sup>	193.5 (57.54)
<b>Legs (g)</b>				
T0	775.78 (136.24) <sup>d</sup>	747.84 (175.02)	733.99 (171.45)	612.28 (179.47)
T1	906.04 (139.86) <sup>d</sup>	900.62 (224.16)	835.18 (177.26)	746.29 (217.99)
<b>Bone stiffness</b>				
T0	91 (12)	99 (11) <sup>a,dd</sup>	92 (23)	82 (11)
T1	95 (14)	106 (12) <sup>a,cc,dd</sup>	93 (14)	87 (14)

Values are presented as mean (SD). TBLH: Total body less head. Superscript letters denote a higher significant difference with: a (swimmers), b (footballers), c (cyclists) and d (controls). <sup>a,b,c,d</sup> p<0.05 and <sup>aa,bb,cc,dd</sup> p<0.001.

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 360 **Figure 1.** Differences (%) in adjusted bone mineral content (BMC) between the sports groups and  
 361 controls after 1 year. The results adjusted for age, height, lean mass, moderate to vigorous physical  
 362 activity and bone outcomes at baseline (T0). TBLH: Total body less head. Letters denote a significant  
 363 difference with: a (Swimmers, SWI), b (Footballers, FOO), c (Cyclists, CYC) and d (Controls). <sup>a,b,c,d</sup>  
 364  $p < 0.05$  and <sup>aa,bb,cc,dd</sup>  $p < 0.01$ .