

**Influence of different playing surfaces on bone mass accretion in male adolescent football players: a one-season study.**

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	<p>classified into two groups according to the surface they trained on: 14 on third-generation artificial turf with elastic layer (3G-EL) and 13 on third-generation artificial turf without elastic layer (3G-NEL). Bone mineral content (BMC) and areal bone mineral density (aBMD) were measured with dual-energy X-ray absorptiometry. Bone mineral apparent density (BMAD) variables were calculated. Bone geometry and strength of the non-dominant tibia were assessed with peripheral quantitative computed tomography. For both football players and controls, bone variables measured at subtotal body, lumbar spine, legs and tibia (<math>p &lt; 0.05</math>) significantly increased. Based on the time spent practicing football, the increase in aBMD for the legs (<math>p &lt; 0.05</math>) was higher in football players than controls. Moreover, lumbar spine BMAD increased more in 3G-NEL players in comparison with 3G-EL players (<math>p &lt; 0.05</math>). Playing football on 3G-EL and 3G-NEL seems to positively affect bone mass during growth. After playing for one season on these playing surfaces, football practice on 3G-NEL with the lower shock absorption seems to have produced the highest increment in aBMD at lumbar spine. Thus, football practice on surfaces with lower shock absorption could provide an extra benefit on bone health.</p>



# Influence of different playing surfaces on bone mass accretion in male adolescent football players: a one-season study

Gabriel Lozano-Berges, Ángel Matute-Llorente, Alejandro Gómez-Bruton, Alex González-Agüero, Germán Vicente-Rodríguez, José A. Casajús.

## Abstract

There are different surfaces on which football is played, but their influence on bone mass accretion still remains unknown. The aims of this study were to compare bone mass accretion between football players and controls and evaluate the influence of two different playing surfaces on bone accretion. Twenty-seven male football players (13.2±0.5 y) and 15 controls (12.6±1.1 y) participated in this study. Football players were classified into two groups according to the surface they trained on: 14 on third-generation artificial turf with elastic layer (3G-EL) and 13 on third-generation artificial turf without elastic layer (3G-NEL). Bone mineral content (BMC) and areal bone mineral density (aBMD) were measured with dual-energy X-ray absorptiometry. Bone mineral apparent density (BMAD) variables were calculated. Bone geometry and strength of the non-dominant tibia were assessed with peripheral quantitative computed tomography. For both football players and controls, bone variables measured at subtotal body, lumbar spine, legs and tibia ( $p<0.05$ ) significantly increased. Based on the time spent practicing football, the increase in aBMD for the legs ( $p<0.05$ ) was higher in football players than controls. Moreover, lumbar spine BMAD increased more in 3G-NEL players in comparison with 3G-EL players ( $p<0.05$ ). Playing football on 3G-EL and 3G-NEL seems to positively affect bone mass during growth. After playing for one season on these playing surfaces, football practice on 3G-NEL with the lower shock absorption seems to have produced the highest increment in aBMD at lumbar spine. Thus, football practice on surfaces with lower shock absorption could provide an extra benefit on bone health.

## Keywords

Soccer, body composition, bone density, bone mass, third-generation artificial turf

## 34 Introduction

35 Childhood and adolescence are crucial periods for bone building and children should  
36 reduce the risk of having low bone mass<sup>1</sup> by means of physical exercise and sports  
37 participation.<sup>2</sup> In fact, a recent review by Weaver et al.<sup>3</sup> graded the positive effects of  
38 physical activity on bone mass with a grade A (maximum level of evidence). However,  
39 the review did not include the effects of individual sports. A study by Mautalen<sup>4</sup>  
40 highlighted the positive effects of football practice on bone mass during adolescent  
41 growth. These positive effects on bone mass are mainly explained by the fact that  
42 football is a weight-bearing sport which is characterized by high-impact actions, such as  
43 accelerations, decelerations, changes of direction, jumps and kicks.<sup>5</sup> Furthermore, this  
44 sport has great importance for young people because football is one of the most, if not  
45 the most, practiced sport worldwide.<sup>6</sup>

46 The positive benefits of football practice on bone tissue have been amply  
47 demonstrated;<sup>7</sup> higher levels of bone mineral content (BMC) or areal bone mineral  
48 density (aBMD) levels when compared to a control group (CG) have been reported in  
49 youth football players.<sup>8-10</sup> More importantly, the positive effects generated by football  
50 have been shown to remain after 1- and 3-year follow-ups.<sup>8, 11-15</sup> To assess bone mass,  
51 most of studies performed on youth football teams have used dual-energy X-ray  
52 absorptiometry (DXA). Although DXA is capable of explaining up to 60% of the  
53 variance in bone strength, it cannot directly measure bone geometry variables.<sup>16</sup> For this  
54 reason, some studies have used other techniques, such as hip structural analysis (HSA)<sup>14</sup>  
55 and peripheral quantitative computed tomography (pQCT)<sup>17-19</sup> for measuring bone  
56 geometry in football players. HSA is derived from hip scan images acquired by DXA.  
57 According to the International Society for Clinical Densitometry (ISCD), the hip is not  
58 the recommended site for evaluating BMC and aBMD in children and adolescents due  
59 to its high variability during bone development.<sup>20</sup> Thus, bone geometry measured with  
60 HSA in young populations could be biased as described above. In contrast, pQCT,  
61 which is not influenced by bone size like DXA, measures trabecular and cortical bone,  
62 allowing for evaluation of the tibia, which is directly affected by football. Up to now,  
63 only one study has compared young male football players and the CG, showing higher  
64 bone geometry in football players than the CG.<sup>19</sup> On the other hand, to the authors'  
65 knowledge, there are no studies which have evaluated the effects of playing football  
66 compared to a population not engaged in any sport on bone geometry and strength  
67 measured by pQCT during growth.

68 Ground reaction forces could be described as one of the main contributing factors  
69 influencing bone accretion. However, properties of playing surfaces change over time,  
70 which may affect this relationship. The number of natural grass football fields is  
71 decreasing, whereas the number of artificial turf pitches is increasing.<sup>21</sup> To replicate the  
72 playing properties of natural grass football fields, new developments in construction  
73 methods of artificial turf fields include the use of materials such as rubber and sand  
74 infill.<sup>21</sup> At the same time, different infill materials create different mechanical  
75 characteristics.<sup>22</sup> Thus, the inclusion or lack of an elastic layer in the installation process  
76 generates differences in shock absorption and vertical deformation forces.<sup>22</sup> Due to  
77 these mechanical properties, different types of surfaces may evoke different loads to the  
78 bone. To the authors' knowledge, only Plaza-Carmona et al.<sup>23</sup> compared the influence  
79 of third-generation artificial turf fields and soil football fields on bone mass accrual in  
80 male children football players, finding no differences for BMC and aBMD. However,  
81 the influence of recent third-generation artificial turfs with an elastic layer (3G-EL) and  
82 without an elastic layer (3G-NEL) on bone tissue in young football players is yet

unknown. During adolescent growth, the short-term effects on bone mass while playing football versus not playing a sport should be studied more deeply, especially bone geometry and strength. Therefore, the aims of this study were: 1) to compare BMC, aBMD, bone mineral apparent density (BMAD), bone geometry and bone strength between young male football players and the CG; and 2) to evaluate the influence of training and playing football on two playing surfaces (3G-EL or 3G-NEL) on previous bone values.

The authors hypothesized that all adolescents will improve bone mass and strength values throughout the season, but football players will have increased bone mass, geometry and strength compared to the CG. Also, those football players who play on 3G-NEL during this period will exhibit additional bone mass gain in comparison with players on the 3G-EL due to the fact that the 3G-NEL surface will have lower shock absorption than the 3G-EL. Therefore, the football players will receive increased loads.

## Methods

### *Participants*

Two football clubs and two high schools in Aragon (Spain) were invited to participate. Although 35 football players and 23 controls agreed to participate, 16 participants were excluded because of the following reasons: three football players and three controls did not perform the second measurement citation, four football players and two controls did not wear the accelerometer, and one football player and three controls had blurred DXA or pQCT images. Consequently, the final sample of 27 male football players (13.17±0.52 years) and 15 male controls (CG; 12.58±1.11 years) participated in this study (Fig. 1). Football players were split into two groups according to the surface where they trained and played: 3G-EL (n=14; 13.01±0.61 years) and 3G-NEL (n=13; 13.35±0.34 years). Although the CG were physically active, they were not regularly engaged in any sport. Measurements were performed in Zaragoza (Spain) at the beginning of the season (October-December 2013) and end of the season (May-July 2014) with a mean measurement time of 31.5±6.2 weeks, which followed the protocol recommended by the ISCD<sup>20</sup> to evaluate bone changes between DXA scans at a minimum interval of six months.

The years of exposure to football practice prior to the beginning of this study were 5±2 years in 3G-EL players and 5±1 years in 3G-NEL players. Hours of training per week were individually quantified based on the number of training sessions in which the player participated (3G-EL players=2.6±0.2 hours/week; 3G-NEL players=2.3±0.3 hours/week). A sport scientist monitored the type of exercises performed by each team during their training throughout the season. Each training session lasted approximately 90 min, including 5-min warm-up consisting of low-intensity running; 5-10 min of low-intensity games; 60 min of technical football exercises (passing, kicking, running, dribbling); and 5-10 minutes of cool down stretching exercises. Taking into account training sessions and matches played at home and away grounds, the percentage of time spent on surfaces included in the present study were 80.2% for 3G-EL players and 78.1% for 3G-NEL players (i.e. away games reduced the percentages of per time on the study field).

Participants, parents and coaches of each club were informed about the protocol, and the possible benefits and risks associated with this study. Written informed consent from parents and verbal assent from the participants were obtained. This study was performed in accordance with the Declaration of Helsinki 1961 (revision of Fortaleza

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3 131 2013). The protocol was approved by the Ethics Committee of Clinical Research from  
4 132 the Government of Aragon (CEICA, Spain) [C.I. PI13/0091]. The research was  
5 133 registered in a public database Clinicaltrials.gov [NCT02399553]. This longitudinal  
6 134 study is part of a larger randomized controlled trial that evaluated the effect of football  
7 135 surfaces and boot model on bone mass and strength in male and female adolescent  
8 136 football players. However, female football players were not included in the present  
9 137 manuscript because of the low number of participants that could be evaluated in the  
10 138 second assessment (some players stopped playing and others did not perform to the  
11 139 evaluation). The Transparent Reporting of Evaluations with Nonrandomized Designs  
12 140 (TREND) Statement was used as a guideline for reporting non-randomized trials.<sup>24</sup>  
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### 142 *Inclusion criteria*

143 The inclusion criteria established for the project included: Caucasian, a minimum of one  
144 year of football practice on the playing surface prior to the beginning of the  
145 measurements (football players), or not regularly engaged in any sport (controls),  
146 between the ages of 11 and 14 and free of any medications affecting bone properties.  
147

### 148 *Anthropometric measurements*

149 Height (stadiometer SECA 225, SECA, Hamburg, Germany; to the nearest 0.1 cm) and  
150 weight (scale SECA 861, SECA, Hamburg, Germany; to the nearest 0.1 kg) were  
151 measured without shoes and with minimum clothing. Body mass index (BMI) was  
152 calculated by dividing the participant's weight (kg) by the squared height (m<sup>2</sup>).  
153

### 154 *Maturity status*

155 Pubertal maturity was determined according to the stages proposed by Tanner and  
156 Whitehouse<sup>25</sup> and using a self-assessment method which has been shown to be a valid  
157 and reliable technique.<sup>26</sup>  
158

### 159 *Calcium intake*

160 Milligrams of daily calcium intake were calculated by a validated calcium food  
161 frequency questionnaire.<sup>27</sup> Participants were asked how many times per day, week or  
162 months they consumed calcium-rich foods.  
163

### 164 *Physical activity measurements*

165 Physical activity was assessed with triaxial accelerometers (GENEActiv developed by  
166 Unilever Discover, Colworth, UK; and distributed by ActivInsights Ltd., Kimbolton,  
167 Cambridge, UK). These accelerometers have been calibrated and validated for  
168 measuring physical activity in children and adolescents on different body locations,  
169 including the right and the left wrists.<sup>28</sup> All participants wore GENE devices on their  
170 non-dominant wrist for seven days, except for football players who had to remove the  
171 accelerometer during official matches. Data were recorded at 30 Hz and analysed at 1-s  
172 epochs. These data were taken within a duration of seven days towards the end of the  
173 season (May-July 2014). Accelerometer data were analysed using the software RStudio  
174 (version RStudio Desktop 1.0.153, Boston, MA, United States). Minutes of valid time



175 in light, moderate and vigorous physical activity, as well as sedentary time, were  
 176 calculated using cut-points proposed by Phillips et al.<sup>28</sup> for right wrist as follows: light,  
 177 2.4 – 7.9 g·s; moderate, 8.0 – 21.0 g·s; vigorous >21.1 g·s; sedentary, <2.3 g·s; and for  
 178 left wrist as follows: light, 2.7 – 7.1 g·s; moderate, 7.2 – 22.5 g·s; vigorous >22.6 g·s;  
 179 sedentary, <2.6 g·s.  
 180

## 181 *Bone measurements*

### 182 DXA

183 Bone and lean masses were measured with DXA QDR-Explorer (pediatric version of  
 184 the software QDR-Explorer, Hologic Corp. Software version 12.4, Bedford, MA, USA).  
 185 DXA equipment was calibrated daily following the manufacturer guidelines. Whole  
 186 body, non-dominant hip and lumbar spine scans were conducted with participants in  
 187 supine position by the same technician who had been fully trained to perform the  
 188 scans. The positioning of the subjects and analysis of the results were performed  
 189 according to the manufacturer's guidelines. The non-dominant limb was determined by  
 190 asking which leg would be used to kick a ball.<sup>29</sup>

191 Subtotal (total body less head) body BMC (g), legs (calculated as a mean of both  
 192 legs) aBMD (g/cm<sup>2</sup>), subtotal lean mass (g) and subtotal percentage of body fat (%)  
 193 were obtained from whole body scans and lumbar spine BMC was obtained from  
 194 lumbar spine scans (L1-L4). Femoral neck values used to calculate BMAD were  
 195 obtained from hip scans. Coefficients of variation for BMC and aBMD at whole body in  
 196 the laboratory in this study were 2.3% and 1.3%, respectively. However, subtotal body  
 197 BMC and lumbar spine BMAD were the preferred bone sites for evaluating bone  
 198 changes during growth.<sup>20</sup> Legs aBMD and femoral neck BMAD have also been  
 199 included in the present study because they could be skeletal sites directly influenced by  
 200 football actions.

201 Due to the fact that DXA results are highly influenced by skeletal dimensions and  
 202 BMAD is less sensitive to size changes than aBMD, Carter et al.<sup>31</sup> and Katzman et al.<sup>32</sup>  
 203 developed new mathematical models to calculate BMAD. In this study, Eqs. (1) – (3)  
 204 have been used:

$$205 \text{ Whole body BMAD} = \text{BMC (whole body)} / [\text{Ap}^2 / \text{h}] \quad (1)$$

206 where Ap is the projected area (whole body) from DXA and h is the height of the  
 207 participant.<sup>32</sup>

$$208 \text{ Lumbar spine BMAD} = \text{BMC (L1-L4)} / \text{Ap}^{3/2} \quad (2)$$

209 where Ap is the projected area (L1-L4) from DXA<sup>31</sup>

$$210 \text{ Femoral neck BMAD} = \text{BMC (femoral neck)} / \text{Ap}^2 \quad (3)$$

211 where Ap is the projected area (femoral neck) from DXA<sup>31</sup>  
 212

### 213 pQCT

214 Bone mass, geometry and strength were measured at the non-dominant tibia using a  
 215 Stratec XCT-2000 L pQCT scanner (Stratec Medizintechnik, Pforzheim, Germany).  
 216 This device is a rotate-translate scanner that obtains a trans-axial image. Following the  
 217 guidelines provided by the manufacturer, the pQCT calibration was performed daily  
 218 using a quality control phantom. Coefficients of variation for each pQCT variable used  
 219 in the present study were as follows: 0.71% for total volumetric bone mineral density  
 220 (Tt.vBMD), 0.93% for trabecular vBMD (Tb.vBMD), 0.25% for total BMC (Tt.BMC),  
 221 0.25% for cortical vBMD (Ct.vBMD), 0.73% for cortical cross sectional area (Ct.Ar),

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3 222 1.12% for cortical thickness (Ct.Th), 2.51% for fracture load in X-axis (Frc.LdX); and  
4 223 2.08% for polar strength strain index (SSIp).

5 224 Tibia length was determined as the inner border of the medial condyle to the farthest  
6 225 point of the medial malleolus of the tibia and it was always measured by the same  
7 226 technician using a wooden ruler (to the nearest 1 mm). Then, the non-dominant leg was  
8 227 centred in the imaging field and the foot and knee were secured to reduce movement.  
9 228 The scanner was positioned on the distal tibia, and a scout view was performed to  
10 229 manually set the reference line on the midpoint of the distal tibia end plate. Bone  
11 230 parameters were assessed at 4% (distal tibia) and 38% (diaphyseal tibia) of the length of  
12 231 the tibia with a voxel dimension of 0.5 mm and a slice thickness of 1 mm. Following  
13 232 ISCD<sup>34</sup> recommendations for evaluating bone geometry and strength with pQCT,  
14 233 Tt.vBMD (mg/cm<sup>3</sup>) and Tb.vBMD (mg/cm<sup>3</sup>) at the 4% site of the tibia were analysed.  
15 234 Moreover, the parameters measured at 38% of the length of the tibia were total Tt.BMC  
16 235 (g), Ct.vBMD (mg/cm<sup>3</sup>), Ct.Ar (mm<sup>2</sup>), Ct.Th (mm), Frc.LdX (N) and SSIp (mm<sup>3</sup>).

17 236 All pQCT images were analysed with version 6.20 of the manufacturer's software.  
18 237 Contour mode 1 with a threshold of 180 mg/cm<sup>3</sup> for the 4% site of the tibia and 280  
19 238 mg/cm<sup>3</sup> for the 38% site of the tibia was used to determine the periosteal surface of the  
20 239 bone. At 4% site of the tibia, trabecular bone was determined from a central area  
21 240 covering 45% of the total bone cross-sectional area. At 38% site of the tibia, cortical  
22 241 bone was obtained using cortical mode 1 with a threshold of 710 mg/cm<sup>3</sup>. Additionally,  
23 242 cortical mode 1 with a threshold of 280 mg/cm<sup>3</sup> was used to obtain bone strength  
24 243 variables (Frc.LdX and SSIp). After that, bone mineralization of 1200 mg/cm<sup>3</sup> was  
25 244 assumed.  
26 245

### 31 246 *Mechanical properties of the pitches*

32 247 Two different surfaces were included in the present study: 3G-EL and 3G-NEL. By the  
33 248 time the study was performed, no more than six years had passed since they were  
34 249 installed. Both pitches presented similar infill characteristics and were constructed by  
35 250 the same manufacturer.

36 251 Assessments of mechanical characteristics of the football fields used in the present  
37 252 study were performed according to the quality standards proposed by the European  
38 253 Committee for Standardisation (EN 15530-1:2007) and the Handbook of Test Methods  
39 254 for Football Turf.<sup>35</sup> This standard is applied for amateur, educational and recreational  
40 255 sport and evaluates the performance and durability of outdoor sport surfaces. Thus, test  
41 256 requirements used for evaluating mechanical properties of the football pitches are as  
42 257 follows: ball rebound has to be between 0.608 and 1.012 m; ball roll between 4 and 10  
43 258 m; shock absorption between 55 and 70%; vertical deformation between 4 and 10 mm;  
44 259 and rotational resistance between 25 and 50 N·m. Although maintenance of these  
45 260 football fields could vary, both 3G-EL (ball rebound: 0.825 m; ball roll: 10 m; shock  
46 261 absorption: 62%; vertical deformation: 7 mm; and rotational resistance: 50 N·m) and  
47 262 3G-NEL (ball rebound: 0.944 m; ball roll: 10 m; shock absorption: 56%; vertical  
48 263 deformation: 6 mm; and rotational resistance: 41 N·m) were within these parameters.

49 264 These mechanical characteristics were measured in five field positions following the  
50 265 quality standards guidelines. Each test was performed three or five times (according to  
51 266 the required attempts) in all field positions. An Advanced Artificial Athlete was used  
52 267 for measuring shock absorption and vertical deformation variables. The other  
53 268 mechanical characteristics were measured with the equipment proposed by the  
54 269 Handbook of Test Methods for Football Turf.<sup>35</sup>



270 All tests were performed at stable meteorological conditions at temperatures between  
271 10 and 22°C, wind speed between 0 and 1.2 m/s and humidity between 45 and 60%.  
272 Pocket Weather Tracker 4000 (Kestrelmeters, Birmingham, UK) was used to evaluate  
273 meteorological conditions. These measurements were performed in April (3G-NEL) and  
274 May (3G-EL).  
275

## 276 *Statistical analyses*

### 277 Sample size calculation

278 To the authors knowledge, there have been no studies that have calculated whole body  
279 or lumbar spine BMAD. Therefore, data from a Zouch et al.<sup>13</sup> study evaluating aBMD  
280 at whole body in football players and the CG ( $1.098 \pm 0.093$  and  $1.010 \pm 0.087$  g/cm<sup>2</sup>,  
281 respectively) was used to calculate sample size. Due to the fact that the main analysis of  
282 the present study was the repeated measures, the sample size calculation was performed  
283 for these analyses. The sample size for repeated measures was calculated in whole body  
284 aBMD to get a power of 80% at the 5% alpha level and to reject the null hypothesis  
285  $H_0: \mu_1 = \mu_2$ . Thus, assuming a small to medium effect size ( $f = 0.20$ ) and a correlation  
286 among repeated measures of 0.7 at pre- and post-season, a total sample size of 32 (16  
287 per group) would be needed.  
288

### 289 Outcome measures treatment

290 Statistical Package for the Social Sciences (SPSS) version 22.0 for Mac OS X (SPSS  
291 Inc., Chicago, IL, USA) was used for the statistical analyses. All variables showed  
292 normal distribution tested with the Kolmogorov-Smirnov test.

293 Chi-square test was performed to evaluate differences between pubertal stages.  
294 Independent t-tests were applied to examine differences among groups for descriptive  
295 characteristics and bone parameters at pre- and post-season. ANOVA for repeated  
296 measures were applied to check differences within all football players and the CG, as  
297 well as within 3G-EL and 3G-NEL between pre- and post-season without adjusting by  
298 covariates (Model 1). After that, these analyses were repeated, including two covariates  
299 as follow: Model 1 + minutes per day of moderate-vigorous physical activity (MVPA;  
300 Model 2); and Model 2 + total lean mass less head for DXA parameters or tibia muscle  
301 area for pQCT parameters (Model 3). MVPA was selected as a covariate to analyse the  
302 possible effect of these high intensity activities on bone mass. In addition, lean mass  
303 was used as a covariate due to its influence on bone mass.<sup>36</sup> Moreover, weight was not  
304 used as covariate to avoid multicollinearity in the analysis due to its high correlation  
305 with lean mass (Pearson correlation coefficient = 0.922;  $p < 0.001$ ). Group by time  
306 interactions for changes in bone values were also performed by repeated measures  
307 analyses.

308 Effect size statistics using Cohen's  $d$  was calculated for independent t-test and partial  
309 eta squared ( $\eta^2_p$ ) for repeated measures analyses. The effect size for Cohen's  $d$  can be  
310 small (0.2 – 0.5), medium (0.5 – 0.8) or large (>0.8) and partial eta squared ( $\eta^2_p$ ) can be  
311 small (0.01 – 0.06), medium (0.06 – 0.14) or large (>0.14). Statistical significance was  
312 set at  $p < 0.05$ .  
313

## 314 **Results**

### 315 *Descriptive data*

316 The physical characteristics of the participants are shown in Table 1. No differences  
 317 were found in any descriptive data between football players and controls (Cohen's  $d$   
 318 ranged from 0.05 to 0.69;  $p > 0.05$ ). Between different surfaces (3G-EL and 3G-NEL),  
 319 no differences were found either (Cohen's  $d$  ranged from 0.06 to 0.70;  $p > 0.05$ ).

320 As expected, there were significant differences for the age, weight, height, BMI,  
 321 subtotal lean mass, tibia length and tibia muscle area between pre- and post-season in all  
 322 groups ( $\eta^2_p$  ranged from  $<0.001$  to 0.943;  $p < 0.05$ ). Moreover, football players who  
 323 trained on 3G-NEL demonstrated lower percentage of body fat at post- than pre-season  
 324 ( $\eta^2_p$  was 0.357;  $p < 0.05$ ).

325 No significant differences were found in MVPA between football players and the CG  
 326 ( $93.29 \pm 19.93$  vs  $95.50 \pm 33.46$  minutes per day; 95% CI, -17.54 to -21.95; Cohen's  $d$   
 327 was 0.08;  $p > 0.05$ ) and between football players who trained in 3G-EL and 3G-NEL  
 328 ( $100.25 \pm 21.69$  vs  $85.80 \pm 15.29$  minutes per day; 95% CI, -29.29 to 0.40; Cohen's  $d$  was  
 329 0.77;  $p > 0.05$ ).

### 331 *BMC, aBMD and BMAD*

#### 332 Comparisons between football players and the CG

333 Table 2 summarizes BMC and aBMD measured at pre- and post-season in football  
 334 players and CG. Higher legs aBMD was found in football players than the CG at post-  
 335 season (95% CI = -0.02 -0.19; Cohen's  $d$  was 0.72;  $p < 0.05$ ). Football players and the  
 336 CG significantly increased subtotal body BMC, lumbar spine BMC, legs aBMD and  
 337 lumbar spine BMAD ( $\eta^2_p$  ranged from 0.192 to 0.713;  $p < 0.05$ ). Furthermore, a  
 338 significant group by time interaction was found for legs aBMD (percentage changes of  
 339 football players and CG were 7.0% and 4.0%, respectively;  $\eta^2_p$  was 0.097;  $p < 0.05$ ).  
 340 This interaction showed that the increase in the legs aBMD was significantly greater in  
 341 football players in comparison with the CG. The same result was obtained when MVPA  
 342 were included as covariate (percentage changes of football players and the CG were  
 343 7.0% and 4.1%, respectively;  $\eta^2_p$  was 0.099;  $p < 0.05$ ), but it became non-significant  
 344 when subtotal lean mass was introduced as covariate (percentage changes of football  
 345 players and the CG were 6.9% and 4.5%, respectively;  $\eta^2_p$  was 0.063;  $p > 0.05$ ; Fig. 2).  
 346 Therefore, a lean mass correction could underestimate the effects of this high-impact  
 347 sport on bone mass.

#### 349 Comparison between 3G-EL and 3G-NEL football players

350 Table 3 summarizes BMC and aBMD measured at pre- and post-season in 3G-EL and  
 351 3G-NEL football players. 3G-EL showed higher lumbar spine and femoral neck BMAD  
 352 at pre- and post-season than 3G-NEL (Cohen's  $d$  ranged from 0.80 to 1.45;  $p < 0.05$ ).  
 353 Both football groups increased subtotal body BMC, lumbar spine BMC, legs aBMD and  
 354 lumbar spine BMAD from pre- to post-season ( $\eta^2_p$  ranged from 0.203 to 0.674;  $p <$   
 355  $0.05$ ). Moreover, 3G-NEL also increased femoral neck BMAD ( $\eta^2_p$  was 0.096;  $p <$   
 356  $0.05$ ). There was a group by time interaction for lumbar spine BMAD (percentage  
 357 changes of 3G-EL and 3G-NEL players were 1.8% and 5.9%, respectively;  $\eta^2_p$  was  
 358 0.169;  $p < 0.05$ ). This interaction demonstrated that during one year of football practice,  
 359 BMAD at lumbar spine increased more in football players who trained on 3G-NEL than  
 360 in those who trained on 3G-EL. On the other hand, no group by time interaction was  
 361 found for lumbar spine BMAD when MVPA was included as covariate (percentage

362 changes of 3G-EL and 3G-NEL players were 2.6% and 5.9%, respectively;  $\eta^2_p$  was  
363 0.109;  $p > 0.05$ ; Fig. 3).

## 365 *Bone geometry and strength*

### 366 Comparisons between football players and the CG

367 Bone geometry and strength measured at the 4% and 38% sites of the tibia in football  
368 players and the CG are shown in Table 2. Higher Ct.Ar at pre-season and SSIp at pre-  
369 and post-season were found in football players than the CG (Cohen's  $d$  ranged from  
370 0.63 to 0.80;  $p < 0.05$ ). Both groups improved Tt.BMC, Ct.Ar, Ct.Th, Frc.LdX and  
371 SSIp ( $\eta^2_p$  ranged from 0.106 to 0.635;  $p < 0.05$ ). Furthermore, the CG decreased  
372 Tt.vBMD and Tb.vBMD at distal tibia ( $\eta^2_p$  were 0.102 and 0.128;  $p < 0.05$ ). No group  
373 by time interactions were found in bone geometry and strength values ( $\eta^2_p$  ranged from  
374  $< 0.001$  to 0.053;  $p < 0.05$ ); however, when tibia muscle area was added as covariate,  
375 there was a group by time interaction for Tt.BMC (percentage changes of football  
376 players and the CG were 5.7% and 8.1%, respectively;  $\eta^2_p$  was 0.105;  $p < 0.05$ ; Figure  
377 2). This interaction demonstrated that Tt.BMC increased more in the CG than football  
378 players. As determined in DXA parameters, a tibia muscle area correction could modify  
379 the differences between groups and, consequently, under-estimate the effects of this  
380 high-impact sport on bone mass.

### 382 Comparison between 3G-EL and 3G-NEL football players

383 Bone geometry and strength measured at the 4% and 38% sites of the tibia in 3G-EL  
384 and 3G-NEL football players are shown in Table 3. At pre- and post-season, 3G-EL  
385 players showed higher Tt.vBMD and Tb.vBMD than 3G-NEL (Cohen's  $d$  ranged from  
386 1.01 to 1.31;  $p < 0.05$ ). 3G-EL and 3G-NEL players improved Tt.BMC, Ct.Ar, Ct.Th,  
387 Frc.LdX and SSIp ( $\eta^2_p$  ranged from 0.199 to 0.683;  $p < 0.05$ ). There were no group by  
388 time interactions between both 3G-EL and 3G-NEL players even when MVPA and tibia  
389 muscle area were used as covariates ( $\eta^2_p$  ranged from  $< 0.001$  to 0.086;  $p < 0.05$ ).

## 391 **Discussion**

392 The main finding of the present study is that one season of football practice,  
393 independent of the playing surface, positively affect bone accretion in the lower limbs  
394 of young players. Subtotal body BMC and lumbar spine BMAD are the variables that  
395 ISCD recommends to compare densitometry results in children and adolescents.<sup>20</sup>  
396 However, the analysis of legs would help to explain how football practice affects bone  
397 mass because legs are the closest site of the body to the floor and support more impact  
398 than the other bone sites. On the other hand, football players playing on 3G-EL and 3G-  
399 NEL demonstrated similar bone mass, geometry and strength increases in most  
400 variables studied, except for lumbar spine BMAD that increased more in football  
401 players who played on 3G-NEL than those who played on 3G-EL.

402 The present study has demonstrated that legs aBMD improved more in football  
403 players than the CG. To the best of the authors' knowledge, six studies have analysed  
404 the effects of football practice on DXA parameters in young football players and the  
405 CG.<sup>8, 11-15</sup> Most of them demonstrated that football practice seems to be a good strategy  
406 for increasing BMC and aBMD during growth. These results were higher for football  
407 players than those obtained by the CG. Moreover, they also reported higher BMC and

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3 408 aBMD at lower limbs in football players than the CG. Most of them also demonstrated  
4 409 differences in lumbar spine, a preferred site to assess densitometry variables during  
5 410 growth.<sup>20</sup> Nonetheless, none of them included subtotal body site and BMAD parameters  
6 411 in their study. Therefore, their results could be slightly influenced by bone mass of the  
7 412 skull (site not responsive to physical activity and their loads<sup>37</sup>) and bone size of their  
8 413 participants.<sup>31</sup> In the present study, although subtotal body BMC and lumbar spine  
9 414 BMAD were included, no significant differences in these variables were found between  
10 415 football players and the CG. These results could be explained by the fact that the  
11 416 number of hours per week of football training could not be sufficient to have significant  
12 417 bone differences between groups. In summary, football practice during childhood and  
13 418 adolescence might help to attain a higher peak of bone mass and, consequently, to  
14 419 reduce the risk of suffering osteoporosis during adulthood.

15 420 In terms of bone geometry and strength parameters, the present study showed that  
16 421 bone strength was higher in football players than the CG, with larger SSIP values. Up to  
17 422 now, there are only a cross-sectional study<sup>19</sup> and a 1-year follow-up study<sup>14</sup> that have  
18 423 compared bone geometry and strength values between male football players and the CG  
19 424 using pQCT and HSA, respectively. Despite the use of different techniques, all of them  
20 425 found greater, but not significant, bone geometry and strength in football players than  
21 426 the CG. The lack of differences between these groups could be explained by the fact  
22 427 that cortical bone parameters increase sharply after the age of 14<sup>38</sup> and the age of  
23 428 participants of the present study was lower. Thus, future studies evaluating bone  
24 429 geometry and strength acquisition before and after 14 years of age will help to clarify  
25 430 the effects of football practice on these bone variables during growth.

26 431 To date, only a cross-sectional study performed by Plaza-Carmona et al.<sup>23</sup> analysed  
27 432 bone mass in football players who trained on different playing surfaces (artificial and  
28 433 soil fields). These authors showed that neither BMC nor aBMD were different between  
29 434 football players according to playing surface. The present study demonstrated that  
30 435 lumbar spine BMAD, femoral neck BMAD, Tt.vBMD and Tb.vBMD were higher in  
31 436 3G-EL than 3G-NEL players at both pre- and post-season. Although no significant  
32 437 differences in hours of trainings per week were found between football groups (3G-EL  
33 438 players=2.6±0.2 hours/week; 3G-NEL players=2.3±0.3 hours/week), the extra 15-20  
34 439 minutes per week of training performed by players who trained in 3G-EL might explain  
35 440 the observed bone differences between both football groups. In addition to this, as  
36 441 demonstrated Varley et al.<sup>18</sup>, an increase of training volume improved bone geometry  
37 442 and strength parameters.

38 443 Artificial fields aim to emulate physical and mechanical characteristics of natural  
39 444 surfaces. In fact, since rubber and sand were added in artificial turf, differences in  
40 445 mechanical variables and the number of injuries between both surfaces were reduced.<sup>21</sup>  
41 446 <sup>39</sup> Afterwards, the inclusion of the elastic layer behind the artificial turf systems  
42 447 increased shock absorption,<sup>22</sup> and consequently, reduced the amount of load received by  
43 448 football players. Although shock absorption characteristics measured in the present  
44 449 study in 3G-EL (62%) and 3G-NEL (56%) were different, the effects of each surface on  
45 450 bone mass, geometry and strength seem to be similar between fields with the exception  
46 451 of lumbar spine BMAD. The closest bone sites to the ground receive the highest  
47 452 impacts produced by football and progressively, as they move away from the ground to  
48 453 other bone sites, these impacts dissipate. Impacts produced in both 3G-EL and 3G-NEL  
49 454 at tibia and femoral neck sites are high and cause similar bone adaptations. However,  
50 455 only loads produced by football actions in 3G-NEL are capable of causing an extra  
51 456 lumbar spine BMAD compared to those produced in 3G-EL. On the other hand, the fact  
52 457 that both football groups included in this study trained less than three hours per week



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3 458 could be limiting the differences between football players. To reinforced this idea,  
4 459 Zouch et al.<sup>11</sup> and Varley et al.<sup>18</sup> demonstrated that higher training volume improved  
5 460 both DXA and pQCT parameters.

6 461 Prior to the beginning of this study, sample sizes of each group were higher than that  
7 462 obtained in the sample size calculation. Nevertheless, some participants were excluded  
8 463 because of the above-mentioned reasons (see Methods section). Consequently, the main  
9 464 limitation of the present study was that sample sizes of 3G-EL (n = 14), 3G-NEL (n =  
10 465 13) and the CG (n = 15) were lower than the predicted number obtained in the sample  
11 466 size calculation (16 participants per group). Therefore, bone comparisons between  
12 467 groups may have been affected by type II error. Moreover, the type of football exercises  
13 468 performed by each team was similar but not equal. Therefore, the variation in football  
14 469 training exercises could cause slight differences in bone mass. Participants' calcium  
15 470 intake data may be somewhat unreliable due to the fact that questionnaires were  
16 471 undertaken by the youth participants with supervision by the researchers, as opposed to  
17 472 their parents or guardians. Another limitation was that the number of practice hours per  
18 473 week for both teams was lower than those in other studies performed with football  
19 474 players (approximately 2.4 vs 10.0 and 11.9 hours per week).<sup>14, 18</sup> Moreover, football  
20 475 players could not use accelerometers during matches and accelerations registered could  
21 476 not accurately represent the accelerations of lower limbs as they had to be placed on the  
22 477 wrist. On the other hand, the main strength was that this is the first study that evaluated  
23 478 the influence of two third-generation artificial turf surfaces (3G-EL and 3G-NEL) on  
24 479 bone mass, geometry and strength in male adolescent football players. Moreover, this is  
25 480 also the first study that compares bone geometry and strength between football players  
26 481 and the CG.  
27 482

## 28 483 **Conclusions**

29 484 The present study demonstrates that football practice on artificial surfaces with or  
30 485 without an elastic layer seems to increase bone mass in lower limbs during growth  
31 486 compared to not playing football. Moreover, after one-season follow-up, football  
32 487 practice on a 3G-NEL surface with lower shock absorption seems to be an adequate  
33 488 alternative to improve BMAD in the lumbar spine. Thus, soccer practice on surfaces  
34 489 with lower shock absorption could provide an extra benefit to bone health.  
35 490

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## 46 501 **Declaration of conflicting interests**

47 502 The authors declare that there is no conflict of interest.  
48 503



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For Peer Review

**Table 1.** Subject characteristics of football players who played in different surfaces and controls.

	Pre-season moment		Post-season moment		Pre-season moment		Post-season moment	
	All players (n = 27)	CG (n = 15)	All players (n = 27)	CG (n = 15)	3G-EL (n = 14)	3G-NEL (n = 13)	3G-EL (n = 14)	3G-NEL (n = 13)
Age (year)	13.17±0.52	12.58±1.11	13.75±0.51*	13.37±1.15*	13.01±0.61	13.35±0.34	13.61±0.61*	13.90±0.34*
Weight (kg)	50.57±11.19	46.26±8.94	54.07±12.09*	50.32±9.77*	50.89±12.01	50.23±10.72	54.41±12.57*	53.69±12.05*
Height (cm)	158.32±8.77	153.37±8.82	162.97±9.11*	158.62±9.15*	157.26±10.09	159.47±7.31	161.96±10.62*	164.05±7.42*
BMI (kg·m <sup>-2</sup> )	19.99±3.06	19.54±2.51	20.18±3.27*	19.86±2.61*	20.35±3.24	19.60±2.93	20.54±3.32*	19.80±3.30*
Daily calcium intake (mg)	819.88±210.85	793.18±309.33	855.09±329.62	1001.54±505.92	812.27±213.77	828.08±216.06	879.42±432.92	828.88±175.88
Subtotal lean mass (g)	34269.71±6804.01	30965.97±5982.53	37438.26±7871.54*	34295.15±7183.19*	34677.22±7597.57	33830.86±6113.04	37979.51±8586.61*	36855.38±7325.65*
Percentage of body fat (%)	23.98±6.86	24.35±6.76	22.50±6.59*	23.92±6.61	23.57±7.53	24.42±6.34	22.00±7.69	23.04±5.43*
Tibia Length (mm)	359±23	349±26	366±24*	363±32*	357±25	362±21	364±26*	369±21*
Tibia Muscle Area (mm <sup>2</sup> )	5637.13±972.37	5185.67±985.63	5958.94±1085.76*	5538.15±1335.06*	5823.79±1066.85	5436.12±855.09	6108.96±1168.91*	5797.37±1009.69*
Tanner (I/II/III/IV/V)	0/6/11/9/1	0/4/4/7/0	0/3/8/14/2	0/2/6/6/1	0/3/7/3/1	0/3/4/6/0	0/2/5/6/1	0/1/3/8/1

Data presented as mean ± standard deviation. 3G-EL: football players who trained in third-generation artificial turf with elastic layer; 3G-NEL: football players who trained in third-generation artificial turf without elastic layer; CG: control group; BMI: body mass index. \* significant differences between values obtained at the beginning and end of the season.

**Table 2.** Bone mineral content, density, strength and structure in football players and controls.

		All players N=27	CG N=15	<i>d</i>	Repeated Measures		
					Within Group		Group by time
					All players $\eta^2_p$	CG $\eta^2_p$	$\eta^2_p$
<b>DXA</b>							
<b>BMC (g)</b>							
Subtotal body	T0	1289.290±263.534	1130.329±267.328	0.60	0.713‡	0.521‡	0.011
	T1	1467.438±330.159	1288.363±322.811	0.55			
Lumbar Spine	T0	38.44±8.10	35.24±8.13	0.39	0.642‡	0.492‡	<0.001
	T1	43.96±10.14	40.68±10.56	0.32			
<b>aBMD (g/cm<sup>3</sup>)</b>							
Legs	T0	1.089±0.111	1.025±0.127	0.54	0.592‡	0.192‡	0.097*
	T1	1.165±0.131	1.066±0.144 <sup>#</sup>	0.72			
<b>BMAD (g/cm<sup>3</sup>)</b>							
Whole body	T0	0.093±0.005	0.094±0.006	0.20	0.074	0.006	0.051
	T1	0.094±0.005	0.094±0.005	0.07			
Lumbar Spine	T0	0.108±0.012	0.107±0.014	0.08	0.440‡	0.213‡	0.013
	T1	0.112±0.011	0.110±0.016	0.14			
Femoral Neck	T0	0.184±0.019	0.178±0.025	0.29	0.017	0.015	<0.001
	T1	0.186±0.019	0.180±0.031	0.23			
<b>pQCT</b>							
<b>4% site</b>							
Tt.vBMD (mg/cm <sup>3</sup> )	T0	323.239±36.539	322.503±48.332	0.02	0.078	0.102‡	0.009
	T1	318.962±30.043	315.847±48.179	0.08			
Tb.vBMD (mg/cm <sup>3</sup> )	T0	298.757±43.691	287.146±51.027	0.71	0.083	0.128‡	0.016
	T1	291.867±37.550	275.365±54.061	0.91			
<b>38% site</b>							
Tt.BMC (g)	T0	3.072±0.339	2.841±0.511	0.53	0.635‡	0.586‡	0.026
	T1	3.254±0.398	3.061±0.598	0.38			
Ct.vBMD (mg/cm <sup>3</sup> )	T0	1057.859±30.134	1055.431±30.778	0.22	0.014	0.041	0.053
	T1	1055.337±28.683	1061.363±32.942	0.28			
Ct.Ar (mm <sup>2</sup> )	T0	391.991±43.761	357.433±63.992 <sup>#</sup>	0.63	0.500‡	0.346‡	<0.001
	T1	412.398±51.080	382.033±79.129	0.46			
Ct.Th (mm)	T0	4.832±0.422	4.605±0.512	0.48	0.192‡	0.106‡	<0.001
	T1	5.017±0.418	4.779±0.603	0.46			
Frc.LdX (N)	T0	3142.342±497.580	2822.855±763.484	0.50	0.280‡	0.266‡	0.012
	T1	3317.777±510.716	3050.249±783.238	0.41			
SSIp (mm <sup>3</sup> )	T0	1410.744±228.345	1190.714±314.927 <sup>#</sup>	0.80	0.259‡	0.204‡	0.003
	T1	1502.870±221.134	1296.479±320.088 <sup>#</sup>	0.75			

Data presented as mean ± standard deviation. T0: pre-season moment; T1: post-season moment; CG: control group; DXA: dual-energy X-ray absorptiometry; BMC: bone mineral content; aBMD: areal bone mineral density; BMAD: bone mineral apparent density; pQCT: peripheral quantitative computed tomography; Tt.vBMD: total volumetric bone mineral density; Tb.vBMD: trabecular volumetric bone mineral density; Tt.BMC: total bone mineral content; Ct.vBMD: cortical volumetric bone mineral density; Ct.Ar: cortical cross sectional area; Frc.LdX: fracture load (axe X); SSIp: polar strain index; *d*: Cohen's *d*;  $\eta^2_p$ : partial eta square.

<sup>#</sup>Significant differences when compared to all players; ‡significant differences within groups between the beginning and the final of the season; \*significant group by time interaction.

Cohen's *d* can be small (0.2 – 0.5), medium (0.5 – 0.8) or large (>0.8) and  $\eta^2_p$  can be small (0.01 – 0.06), medium (0.06 – 0.14) or large (>0.14).



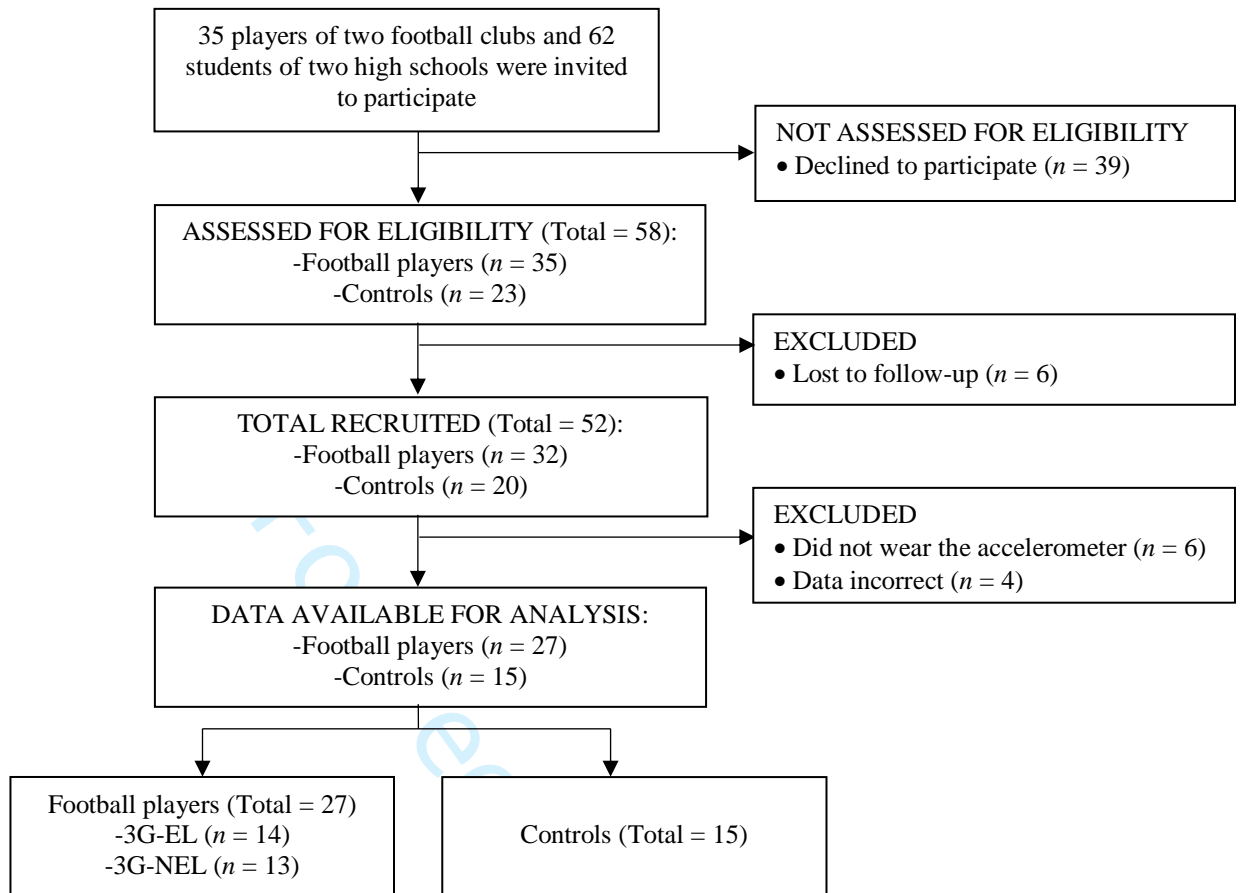
**Table 3.** Bone mineral content, density, strength and structure in football players who played in third-generation artificial turf with and without elastic layer.

				<i>d</i>	Repeated Measures		
					Within Group		Group by time
					3G-EL	3G-NEL	
		N=14	N=13		$\eta^2_p$	$\eta^2_p$	$\eta^2_p$
<b>DXA</b>							
<b>BMC (g)</b>							
Subtotal body	T0	1334.216±305.267	1240.909±211.261	0.36	0.674‡	0.629‡	0.004
	T1	1517.670±375.491	1413.341±278.067	0.32			
Lumbar Spine	T0	38.72±9.76	38.14±6.22	0.07	0.602‡	0.591‡	<0.001
	T1	44.21±12.12	43.70±7.98	0.05			
<b>aBMD (g/cm<sup>2</sup>)</b>							
Legs	T0	1.125±0.127	1.050±0.079	0.70	0.549‡	0.572‡	0.005
	T1	1.198±0.150	1.130±0.100	0.53			
<b>BMAD (g/cm<sup>3</sup>)</b>							
Whole body	T0	0.093±0.004	0.094±0.006	0.20	0.057	0.090	0.003
	T1	0.094±0.004	0.095±0.007	0.25			
Lumbar Spine	T0	0.114±0.010	0.102±0.011 <sup>§</sup>	1.12	0.203‡	0.553‡	0.169*
	T1	0.116±0.011	0.108±0.010 <sup>§</sup>	0.80			
Femoral Neck	T0	0.195±0.018	0.173±0.012 <sup>§</sup>	1.45	0.002	0.096‡	0.064
	T1	0.195±0.021	0.177±0.013 <sup>§</sup>	1.01			
<b>pQCT</b>							
<b>4% site</b>							
Tt.vBMD (mg/cm <sup>3</sup> )	T0	340.621±37.567	304.520±25.136 <sup>§</sup>	1.13	0.109	0.025	0.016
	T1	334.884±28.814	301.816±21.023 <sup>§</sup>	1.31			
Tb.vBMD (mg/cm <sup>3</sup> )	T0	317.911±44.056	278.130±33.827 <sup>§</sup>	1.01	0.112	0.032	0.013
	T1	309.011±34.253	273.404±32.739 <sup>§</sup>	1.06			
<b>38% site</b>							
Tt.BMC (g)	T0	3.135±0.369	3.004±0.302	0.39	0.683‡	0.494‡	0.086
	T1	3.349±0.441	3.153±0.334	0.50			
Ct.vBMD (mg/cm <sup>3</sup> )	T0	1053.591±29.836	1062.454±30.967	0.29	0.017	0.023	<0.001
	T1	1051.289±32.829	1059.696±23.984	0.29			
Ct.Ar (mm <sup>2</sup> )	T0	398.911±46.288	384.539±41.377	0.33	0.606‡	0.462‡	0.036
	T1	423.714±56.310	400.212±43.680	0.47			
Ct.Th (mm)	T0	4.941±0.501	4.716±0.295	0.55	0.383‡	0.384‡	<0.001
	T1	5.122±0.476	4.903±0.326	0.54			
Frc.LdX (N)	T0	3210.438±553.960	3069.009±439.040	0.28	0.432‡	0.199‡	0.057
	T1	3428.474±535.839	3198.564±473.699	0.46			
SSIp (mm <sup>3</sup> )	T0	1455.894±245.232	1362.122±207.083	0.41	0.379‡	0.399‡	0.002
	T1	1544.487±224.998	1458.052±216.588	0.39			

Data presented as mean ± standard deviation. T0: pre-season moment; T1: post-season moment; 3G-EL: football players who trained in third-generation artificial turf with elastic layer; 3G-NEL: football players who trained in third-generation artificial turf without elastic layer; DXA: dual-energy X-ray absorptiometry; BMC: bone mineral content; aBMD: areal bone mineral density; BMAD: bone mineral apparent density; pQCT: peripheral quantitative computed tomography; Tt.vBMD: total volumetric bone mineral density; Tb.vBMD: trabecular volumetric bone mineral density; Tt.BMC: total bone mineral content; Ct.vBMD: cortical volumetric bone mineral density; Ct.Ar: cortical cross sectional area; Frc.LdX: fracture load (axe X); SSIp: polar strain index; *d*: Cohen's *d*;  $\eta^2_p$ : partial eta square.

<sup>§</sup>Significant differences when compared to 3G-EL; ‡significant differences within groups between the beginning and the final of the season; \*significant group by time interaction.

Cohen's *d* can be small (0.2 – 0.5), medium (0.5 – 0.8) or large (>0.8) and  $\eta^2_p$  can be small (0.01 – 0.06), medium (0.06 – 0.14) or large (>0.14).



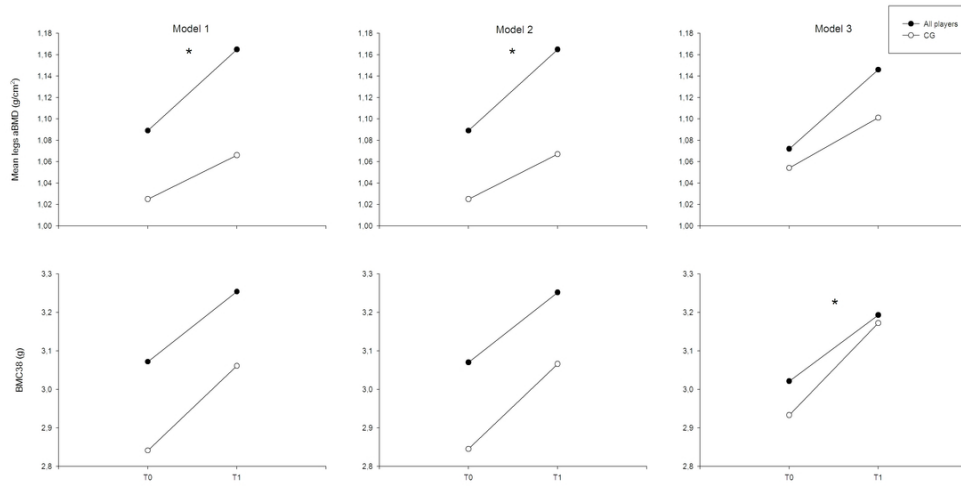


Figure 2 Legs aBMD and BMC38 interactions in football players and CG. aBMD: areal bone mineral density; BMC38: total bone mineral content at the 38% of the length of the tibia; CG: control group; T0: pre-season moment; T1: post-season moment; Model 1: unadjusted data; Model 2: adjusted data by MVPA; Model 3: adjusted data by MVPA and subtotal lean mass (legs aBMD)/tibia muscle area (BMC38). \*: Significant interactions were set at  $p < .05$ .

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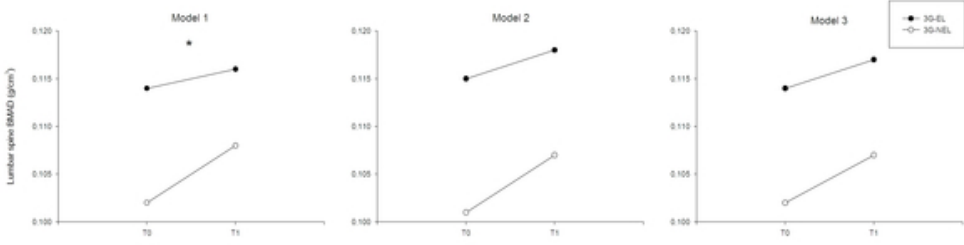


Figure 3 Lumbar spine BMAD interactions in football players. BMAD: bone mineral apparent density; 3G-EL: football players who trained in third-generation artificial turf with elastic layer; 3G-NEL: football players who trained in third-generation artificial turf without elastic layer; T0: pre-season moment; T1: post-season moment; Model 1: unadjusted data; Model 2: adjusted data by MVPA; Model 3: adjusted data by MVPA and subtotal lean mass. \*: Significant interactions were set at  $p < .05$ .

50x13mm (300 x 300 DPI)