

1 **Modelling Changes in Bone and Body Composition over a Season in Elite Male**  
2 **Footballers.**

3

4 **Abstract**

5 This study investigated the change in bone and body composition characteristics of elite  
6 football players and recreationally active control participants across the course of a season.  
7 Forty-six participants (20 footballers and 26 recreationally active controls) were assessed by  
8 dual-energy x-ray absorptiometry and peripheral Quantitative Computed Tomography for a  
9 range of bone and body composition characteristics at four points over the course of a  
10 competitive season. Multilevel modelling was used to examine changes.

11 Footballers had higher characteristics than controls for 24 out of 29 dual-energy x-ray  
12 absorptiometry and peripheral Quantitative Computed Tomography variables (all  $p < 0.05$ ),  
13 however, there was also significant random inter-individual variation in baseline values for  
14 all variables, for both footballers and controls ( $p < 0.05$ ). Whole-body bone mineral density,  
15 leg and whole-body bone mineral content, tibial bone mass and area (38%) increased across  
16 the season in footballers ( $p < 0.05$ ), and there was significant random inter-individual variation  
17 in the rate of increase of leg and whole-body bone mineral content ( $p < 0.05$ ).

18 Whole-body bone mineral density, leg and whole-body bone mineral content, tibial bone  
19 mass and area (38%) increased over the course of the season in elite football players. The  
20 modelling information on expected changes in bone characteristics provides practitioners  
21 with a method of identifying those with abnormal bone response to football training and  
22 match-play.

23

24 **Introduction**

25 Long-term weight bearing exercise has a positive effect on bone accrual [1, 2]. The  
26 physiological benefits of football participation on bone health are wide-ranging [3]. Habitual  
27 football participation has been associated with a greater whole-body bone mineral density  
28 (BMD) [4] and BMD at specific anatomical locations, such as the proximal femur and the  
29 femoral shaft [5], when compared to participation in other sports and to untrained control

30 participants. Others have reported similar associations for bone size and bone strength [6].. The  
31 osteogenic effect of football participation is likely to be due to the high magnitude of loading  
32 that takes place during football training and match-play [7], which stimulates the bone  
33 remodelling cycle through mechotransductive-related mechanisms [8].

34 Despite the osteogenic effect of habitual football participation, elite football players can suffer  
35 stress-related bone conditions that result in long-term absence from training and match-play,  
36 with the most common sites of stress fracture injury being the metatarsals and tibial shaft [9].  
37 Whilst whole body dual-energy x-ray absorptiometry (DXA) measurements have been used as  
38 a method to assess an individual's risk of stress fracture injury [10], there is some debate over  
39 the efficacy of this with some studies showing positive associations between bone structural  
40 properties and stress fracture incidence [10,11] and others showing no association [12, 13, 14].  
41 These contrasting findings may be due to the premise that inadequate bone adaptation in  
42 response to mechanical loading could lead to stress-related bone injury [15], rather than 'low'  
43 bone structural properties, such as BMD [14]. Moreover, whole-body DXA also lacks  
44 specificity when attempting to highlight bone weakness at a specific anatomical site.  
45 Furthermore, associations between bone characteristics and body composition have been  
46 demonstrated due to the interaction between adipose tissue [16] and skeletal muscle [17] with  
47 bone. Muscle size has been implicated in bone adaptation [18], with a larger muscle size and a  
48 greater amount of external loading force being diffused by a larger muscle mass prior to acting  
49 upon the bone [15]. Indeed, muscle-generated forces have been shown to play a role in bone  
50 adaptation (for review see Avin et al.,[19]), furthermore muscle-driven biochemical and  
51 endocrine stimuli are also known to mediate bone adaptation (for review see Brotto and  
52 Bonewald [20]).

53 Data on the change in bone characteristics expected in response to a period of physical activity  
54 would provide a useful insight for practitioners seeking information on bone adaption and

55 potential injury risk. Accurate information quantifying the expected change in bone  
56 characteristics, such as BMD, bone size and bone strength, over the course of a competitive  
57 football season is yet to be established. Previous studies have used a cross-sectional study  
58 designs [14,21] or a limited number of scans (2 scans, [22]; 3 scans, [23]). Cortical area and  
59 cortical thickness have been shown to increase following a collegiate football season, although  
60 it is difficult to truly determine seasonal changes from these data, as the study only scanned  
61 players at two time points during the season [22]. Site specific (pelvis, upper and lower limbs)  
62 changes in BMC have also been shown between pre-season and end-of-season and between  
63 mid-season and end-of-season in elite football players, but individual responses to specific  
64 training modalities were not recorded [23]. Another issue is that many of these studies have  
65 only utilised DXA for the measurement of bone characteristics [11,14,21], meaning that no  
66 measures of volumetric bone characteristics, which are vital in accurately determining bone  
67 strength [24], have been made. A prospective longitudinal study design, with multiple  
68 measurement points over the course of a season, and the attainment of both volumetric and  
69 areal bone density measurements is required to provide accurate data on bone structural  
70 characteristics. Within such a design, it is also important to have a comparison group to  
71 examine whether changes across a season are particular to elite footballers or are common to  
72 the more recreationally active population. Most studies examining bone characteristics in elite  
73 athletes have not, however, employed an active comparison group [11,14,21], and so season-  
74 long changes in bone remain unclear.

75         Within a prospective longitudinal study design, appropriate statistical analyses are  
76 required to adequately investigate changes in bone across time in elite footballers and an active  
77 comparison group. Previous research has tended to use traditional regression- and ANOVA-  
78 based statistical analyses to examine change in bone across time in athletes (e.g.,[1, 25,26]).  
79 However, such analyses tend to ignore the hierarchical nature of repeated measures data (i.e.,

80 repeated scans over time nested within individuals), ignore variation in individual response,  
81 and can be overly restrictive in their assumptions. Conversely, longitudinal multilevel  
82 modelling is a flexible and robust technique that can describe the underlying pattern of response  
83 in a population (fixed part) but can also model the unexplained variation around the pattern  
84 (random part) [27]. Thus, as well as mean group effects, normal variation between individuals  
85 in terms of levels and changes in body composition and bone across time can be estimated,  
86 which has not been done previously. Comprehensive and accurate information on the expected  
87 levels and changes in bone characteristics and body composition in elite footballers could be  
88 used as a benchmark by clinicians and sports practitioners. The identification of players outside  
89 of the expected change parameters could be used as part of a multifaceted approach to reduce  
90 susceptibility to stress-related bone injuries. Therefore, the aim of the present study was to  
91 describe changes in bone and body composition characteristics over the course of a competitive  
92 season in elite football players and active controls, using multilevel modelling.

93

## 94 **Methods**

### 95 *Participants*

96 A total of 46 male participants volunteered to take part in the study, as part of the Bone Health  
97 in Elite Athlete Cohort (BEA-C), with twenty being senior professional football players (mean  
98  $\pm$  SD: stature of  $80.89 \pm 7.68$  kg and  $1.82 \pm 0.07$  m) and twenty-six being recreationally-active  
99 individuals (mean  $\pm$  SD: stature of  $77.91 \pm 13.37$  kg and  $1.78 \pm 0.06$  m), who acted as controls.  
100 The footballers were recruited via convenience sampling from the same professional football  
101 club. Control participants were then age-matched to the footballer group. An independent t-  
102 test revealed no significant differences in age between groups (mean  $\pm$  SD age for footballers  
103 vs. controls:  $25.2 \pm 4.7$  y vs.  $23.7 \pm 4.6$  y;  $p > 0.05$ ). Football players were all contracted to the

104 same professional football club in England and were in full time training. Across the season,  
105 this typically consisted of four 120-minute training sessions per week incorporating football  
106 training, strength and conditioning, tactical and technical drills and one or two competitive  
107 matches per week. Controls were recreationally active (defined as performing 2-3 unstructured  
108 weight-bearing activities per week), engaging in their normal physical activity across the study  
109 period. The study was approved by the National Health Service Research Ethics Committee  
110 **\*\*number will be inserted following review\*\***and conformed to Ionising Radiation  
111 Regulations. Informed consent was received from all participants prior to any study procedures  
112 being undertaken. The research has been conducted ethically according to the principles of the  
113 World Medical Association Declaration of Helsinki.

114

#### 115 *Design*

116 This was a prospective longitudinal study. Participants underwent DXA (iDXA, GE Healthcare,  
117 United Kingdom) and peripheral Quantitative Computed Tomography (pQCT) (XCT2000L,  
118 Stratec Medizintechnik) scans on four occasions across the study period. Prior to the  
119 commencement of the study (visit 1), the footballers had 7 weeks between the end of the  
120 previous season and the start of the new season. During this time, footballers were advised by  
121 club support and medical staff to participate in exercise training 4 times per week, which  
122 consisted of running, cycling and strength maintenance activities. Visit 1 / baseline (0 weeks)  
123 coincided with the start of the footballers' pre-season training period. At baseline, 46  
124 participants were assessed. Visit 2 ( $8 \pm 6$  weeks) coincided with the end of footballers' pre-  
125 season training period / the start of competitive matches. At visit 2, 46 participants were  
126 assessed. Visit 3 occurred in the middle of players' competitive season ( $25 \pm 7$  weeks). At visit  
127 3, 46 participants were assessed. Visit 4 took place at the end of the players' competitive season

128 (42 ± 4 weeks). At visit 4, 30 participants were assessed (participant drop-out at visit 4 was  
129 related to illness and unavailability). This resulted in a mixed-longitudinal sample of 166  
130 individual (participant-occasion) data points.

131

### 132 *Procedures*

133 Participants were tested for body composition and bone characteristics using DXA and pQCT.  
134 Each participant completed a health status questionnaire prior to each testing session. Height  
135 (Stadiometer, Seca, Hamburg, Germany) and body mass (Seca, Birmingham, U.K.) were  
136 recorded with participants wearing minimal clothing. DXA scans assessed participant BMD  
137 ( $\text{g}/\text{cm}^2$ ), Bone Mineral Content (BMC, g), lean mass (g) and fat mass (g). pQCT assessed the  
138 following tibial measures: mass (4%, 14%, 38%, g), polar, Y and X stress strain index (14%,  
139 38%,  $\text{mm}^3$ ), trabecular Area (4%), trabecular density (4%,  $\text{mg}\cdot\text{cm}^3$ ), cortical area (14%, 38%,  
140  $\text{mg}\cdot\text{cm}^3$ ), cortical density (14%, 38%, 66%,  $\text{mg}\cdot\text{cm}^3$ ) cortical thickness (14%, 38%, mm),  
141 periosteal circumference (14%, 38%, mm) and total area (14%, 38%, 66%,  $\text{mm}^2$ ). A  
142 manufacturer-trained operator performed all scans consistent with the manufacturer's  
143 guidelines. Calibration of the DXA and pQCT was completed prior to scanning using a  
144 phantom of a known density. Participants were asked to wear minimal clothing or a cotton  
145 examination gown and remove any jewellery or metal prior to the scan to avoid measurement  
146 distortion. Participants fasted for at least 2 hours, emptied their bladder immediately before and  
147 were asked to be euhydrated prior to the scan.

148

### 149 *Dual-energy X-ray absorptiometry (DXA)*

150 Participants were positioned supine on the DXA bed within the scanner range, with ankles and  
151 knees held in place by Velcro straps or medical tape to minimise unintended movements. The  
152 participants lay with arms by their sides and were asked to remain motionless for the duration  
153 of the scan. Whole-body scans lasted <10 min depending upon the size of the participant.  
154 Subsequent segmental analyses for all scans were completed by the same trained operators.  
155 Coefficients of variation for the model of scanner used in the present study are 0.08–1.30% for  
156 BMD and 0.6% for fat mass [28,29].

157 The following measures were analysed: whole body lean mass and percentage body fat,  
158 whole body and legs BMD, whole body and legs BMC, T-score and Z-score. If any  
159 movement artefacts (inaccuracies in the measurement caused by motion) were present  
160 following the scan, the image was classified as invalid and a repeat scan was performed.

161

#### 162 *Peripheral Quantitative Computed Tomography pQCT*

163 pQCT scans were taken of the dominant lower leg (defined as the leg that the participant most  
164 comfortably kicked a ball with). For quality assurance, all scans were performed by the same  
165 operator. Before scanning commenced, the scanner was cross-calibrated using phantoms of  
166 known density in accordance with manufacturer guidelines. pQCT has previously been shown  
167 to provide a reliable measurement of bone characteristics in humans (Intraclass correlation  
168 coefficient, CC: 0.76-0.99; [30]). Each participant's tibial length was measured to the nearest  
169 1 mm, determined as the midpoint of the medial malleolus to the medial aspect of the tibial  
170 plateau. The participant's leg was then placed in the scanner with their foot secured in a  
171 purpose-built attachment. The leg was aligned with use of an integral laser and a clamp was  
172 placed to the knee to reduce movement, with the participant instructed to remain as still as  
173 possible for the duration of the scan. Initially, a preliminary reference point locating scout-view

174 scan was performed in the frontal plane to confirm the location of the middle of the distal end  
175 plate, which would act as a positioning line. Sectional images were then obtained at distal sites  
176 (4%, 14%) and the diaphysis of the tibia (38% and 66%) from the positioning line, with a voxel  
177 size set at 0.5mm and a slice thickness of 2.5mm for all measurements. A contour mode, with  
178 a threshold of  $180\text{mg}\cdot\text{cm}^3$ , was used to separate soft tissue and bone. To analyse trabecular  
179 bone, a constant default threshold of  $711\text{mg}\cdot\text{cm}^3$  was used to identify and remove cortical bone.  
180 The integral XCT2000L software (version 6.20A) was used to analyse the pQCT images. If  
181 any movement artefacts were present following the scan, the image was classed as invalid, and  
182 a repeat scan was performed. If an artefact was present in the second image, the participant was  
183 removed from the study in line with the radiation exposure guidelines. In the present study, no  
184 participants were removed from the analysis due to artefacts.

185

#### 186 *Data analysis*

187 The mixed-longitudinal sample represented a hierarchically structured data set, with  
188 measurement occasion nested within participant. Thus, multilevel models were developed  
189 using MLwiN (v 3.05, Bristol, U.K.) to investigate changes in DXA and pQCT variables across  
190 time, in controls and football players. Longitudinal multilevel modelling does not require the  
191 same number of measurement occasions per individual, meaning all data can be included  
192 within the analysis. Following Rasbash et al. [27], a two-level multilevel structure was defined,  
193 with measurement occasion (level 1) nested within participant (level 2), with a given DXA or  
194 pQCT variable as the continuous response variable for each model. For each model, relevant  
195 parameters were added to an empty model to observe their effect on explaining and partitioning  
196 variation in the continuous response variable. Parameters were accepted or rejected based upon  
197 changes in model fit, as indicated by changes in -2 loglikelihood. Independent intercepts for



198 the control group and the football player group were considered. The effect of allowing the  
199 control group intercept and football player group intercept to randomly vary was then  
200 examined. This allows the inter-individual variation in the response variable to be modelled  
201 separately for the two groups. Subsequently, the fixed effect of 'visit number' (centred at  
202 baseline / time point 1) was considered for each group, to examine whether the response  
203 variable changed across time for each group. The effect of allowing the control group slope for  
204 time and football player group slope for time to randomly vary was then examined. This allows  
205 the inter-individual variation in the rate of change in the response variable to be modelled  
206 separately for the two groups. The fixed effect of 'group' was also considered, to examine  
207 differences between controls and football players in relation to the response variable. The size  
208 of the effects when comparing controls and football players were examined using Cohen's *d*  
209 adapted for multilevel modelling by Feingold [31]. Effect sizes were evaluated based upon  
210 Cohen's guidance using the following boundaries: <0.20 (trivial), 0.20-0.49 (small), 0.50-0.79  
211 (medium), and >0.79 (large) [32]. The assumption that variance in random effects followed a  
212 normal distribution with a mean of zero, was checked following each analysis [27]. Statistical  
213 significance was accepted at the 95% confidence level ( $p < 0.05$ ). Mean  $\pm$  SD were used to  
214 describe the average and variability of data, unless stated otherwise.

215

## 216 **Results**

217 The average values (mean  $\pm$  SD) for controls and footballers at each visit for DXA  
218 variables and pQCT variables are displayed in Tables 1 and 2. The multilevel models  
219 predicting changes across the study period in controls and footballers are displayed in Table 3  
220 for DXA outcome variables and Table 4 for pQCT outcome variables. For stature and body  
221 mass, modelling revealed that controls were significantly shorter than footballers at the start of

222 the study (1.78 m vs. 1.82 m,  $p<0.05$ ,  $d=0.52$ ) and that stature did not significantly change  
223 across the study period ( $p>0.05$ ). Furthermore, there were no significant differences between  
224 controls and footballers in body mass at the start of the study (77.85 kg vs. 81.25 kg,  $p>0.05$ ,  
225  $d=0.32$ ) and body mass did not change significantly across the study period ( $p>0.05$ ,  $d=0.23$ ).

226 For DXA variables, modelling revealed that at baseline footballers had lower total fat  
227 mass compared to controls, and had higher total lean mass, bone mineral density (total and  
228 legs), bone mineral content (total and legs), and area (total and legs) (all  $p<0.05$ ,  $d>0.49$ ) (see  
229 Table 3). For pQCT, modelling revealed that at baseline, footballers had higher estimates for  
230 17 out of 21 variables (all  $p<0.05$ ,  $d>0.19$ ) compared to controls. Exceptions were that there  
231 were no differences between groups in bone density at the 4% and 14% sites, and in endosteal  
232 circumference at the 14% and 38% sites (see Table 4). Allowing the intercepts to randomly  
233 vary for controls and footballers, improved the fit of every model. This allows variation in  
234 baseline values for each group to be estimated using a 95% coverage range for each variable  
235 using the standard deviations from the random part of models displayed in Tables 3 and 4.  
236 There were changes across the study period in total fat mass and total lean mass in both controls  
237 and footballers, and changes in total BMD, Legs BMC, Total BMC, Tibial Mass (38%), and  
238 Tibial Area (38%) in footballers. We also allowed slopes to randomly vary. This improved  
239 model fit for footballers for legs BMC and total BMC. This allows variation in the rate of  
240 change to be estimated using a 95% coverage range using the standard deviations from the  
241 random part of models displayed in Table 3.

242 On average, total fat mass at baseline was predicted to be 11.13 kg for footballers, 5.34  
243 kg lower than controls ( $p<0.05$ ,  $d=0.62$ ) (Table 3). However, modelling also revealed that there  
244 was significant random inter-individual variation in total fat mass for both controls (SD=8.17  
245 kg) and footballers (SD=2.38kg) at baseline (Table 3). This information can be used to  
246 construct a 95% coverage range (CR) for predicted total fat mass for the two groups. Controls

247 were predicted to have a total fat mass of 16.47 kg, but with a random intercept SD of 8.17 kg,  
248 the coverage range within which 95% of controls are expected to lie can be estimated as 0.46  
249 kg to 32.48 kg ( $16.47 \text{ kg} \pm (1.96 * 8.17 \text{ kg})$ ). Conversely, footballers were predicted to have a  
250 total fat mass of 11.13 kg, but with a random intercept SD of 2.38 kg, the coverage range within  
251 which 95% of footballers are expected to lie can be estimated as 6.47 kg to 15.80 kg ( $11.13 \text{ kg}$   
252  $\pm (1.96 * 2.38 \text{ kg})$ ).

253 On average, total fat mass was predicted to decrease in footballers by 0.51 kg between  
254 baseline and visit 4 (0.17 kg per visit,  $p < 0.05$ ), decreasing from 11.13 kg in pre-season to 10.62  
255 kg at the end of the season. When modelling changes in controls, a similar pattern emerged.  
256 On average, total fat mass was predicted to decrease in controls by 0.51 kg between baseline  
257 and visit 4 (0.17 kg per visit,  $p < 0.05$ ), decreasing from 16.47 to 15.96 kg. There was no random  
258 inter-individual variation in the rate of change (slope) for either group.

259 On average, total lean mass at baseline was predicted to be 8.58 kg higher in footballers  
260 versus controls ( $p < 0.05$ ,  $d = 0.90$ ) (Table 3). Total lean mass was predicted to be 58.01 kg, 95%  
261 CR [43.68, 72.34 kg] for controls, and 66.59 kg, 95% CR [54.89, 78.29 kg] for footballers. On  
262 average, total lean mass was predicted to increase in both groups (controls = 0.30 kg per visit,  
263  $p < 0.05$ ; footballers = 0.35 kg per visit,  $p < 0.05$ ). Thus, footballers' total lean mass was predicted  
264 to increase from 66.59 kg in pre-season to 67.64 kg at the end of the season and controls' total  
265 lean mass was predicted to increase from 58.01 kg to 58.91 kg. There was no random inter-  
266 individual variation in the rate of change (slope) for either group.

267 On average, total BMD at baseline was predicted to be 0.106 g/cm<sup>2</sup> higher in footballers  
268 versus controls ( $p < 0.05$ ,  $d = 0.71$ ). Total BMD was predicted to be 1.309 g/cm<sup>2</sup>, 95% CR [1.078,  
269 1.540 g/cm<sup>2</sup>] for controls and 1.415 g/cm<sup>2</sup>, 95% CR [1.241, 1.589 g/cm<sup>2</sup>] for footballers. Total  
270 BMD was predicted to increase in footballers but not in controls. On average, footballers

271 increased total BMD by 0.012 g/cm<sup>2</sup> (0.004 g/cm<sup>2</sup> per visit, p<0.05) from 1.415 g/cm<sup>2</sup> in pre-  
272 season to 1.427 g/cm<sup>2</sup> at the end of the season. There was no random inter-individual variation  
273 in the rate of change (slope).

274 Figure 1 displays the observed data and associated model predictions of total BMC for  
275 each participant across the study period, and also the average predicted changes across the  
276 season for controls (A) and footballers (B). On average, total BMC at baseline was predicted  
277 to be 486 g higher in footballers versus controls (p<0.05, *d*=0.77). At baseline total BMC was  
278 predicted to be 3315 g, 95% CR [2411, 4219g] for controls and 3801g, 95% CR [2954, 4648g]  
279 for footballers. Total BMC was predicted to increase in footballers but not in controls. On  
280 average, footballers increased total BMC by 54 g (18 g per visit, p<0.05) from 3801 g in pre-  
281 season to 3855g at the end of the season. However, modelling also revealed that there was  
282 significant random inter-individual variation in the rate of increase in total BMC for footballers.  
283 Footballers were predicted to increase total BMC by 18 g per visit, 95% CR[-25, 61g].  
284 Furthermore, there was a positive relationship between players' baseline levels of total BMC  
285 and their changes across time, whereby those with higher baseline levels of total BMC tended  
286 to increase total BMC more between the start and end of the season (see figure 1).

287 On average, leg BMC at baseline was predicted to be 274 g higher in footballers versus  
288 controls (p<0.05, *d*=0.99). At baseline leg BMC was predicted to be 1286 g, 95% CR [894,  
289 1678g] for controls and 1560 g, 95% CR [1188, 1932g] for footballers. Leg BMC was  
290 predicted to increase in footballers but not in controls. On average, footballers increased leg  
291 BMC by 12 g (4 g per visit, p<0.05) from 1560g in pre-season to 1572g at the end of the season.  
292 However, modelling also revealed that there was significant random inter-individual variation  
293 in the rate of increase in leg BMC for footballers. Footballers were predicted to increase leg  
294 BMC by 4 g per visit, but with a random intercept SD of 7 g, the coverage range within which  
295 95% of footballers' slopes are expected to lie can be estimated as -10 to 18 (4 ± (1.96\*7)) per

296 visit. Furthermore, there was positive covariance between players' intercepts and slopes,  
297 indicating that those with high intercepts at baseline tend to have higher slopes and those with  
298 lower intercepts tend to have lower slopes.

299 For pQCT variables, the only significant changes across time were in tibial mass and  
300 area at the 38% site in footballers, which will be discussed in detail henceforth. On average,  
301 tibial mass at the 38% site at baseline was predicted to be 0.65 g higher in footballers than in  
302 controls ( $p < 0.05$ ,  $d = 0.83$ ). Mass at the 38% site was predicted to be 4.45 g, 95% CR [3.23, 5.67  
303 g] for controls and 5.10 g, 95% CR [4.22, 5.98 g] for footballers. Mass at the 38% site was  
304 predicted to increase in footballers but not controls. On average, footballers increased mass at  
305 the 38% site by 0.09 g (0.03 g per visit,  $p < 0.05$ ) from 5.10 g in pre-season to 5.19 g at the end  
306 of the season. There was no random inter-individual variation in the rate of change (slope). On  
307 average, area at the 38% site at baseline was predicted to be 43 mm<sup>2</sup> higher in footballers than  
308 in controls ( $p < 0.05$ ,  $d = 0.55$ ). Area at the 38% site was predicted to be 489 mm<sup>2</sup>, 95% CR [364,  
309 614 mm<sup>2</sup>] for controls and 532 mm<sup>2</sup>, 95% CR [454, 610 mm<sup>2</sup>] for footballers. Area at the 38%  
310 site was predicted to increase in footballers but not controls. On average, footballers increased  
311 area at the 38% site by 0.09 mm<sup>2</sup> (4 mm<sup>2</sup> per visit,  $p < 0.05$ ) from 532 mm<sup>2</sup> in pre-season to 536  
312 mm<sup>2</sup> at the end of the season. There was no random inter-individual variation in the rate of  
313 change (slope).

314

315 **Discussion**

316           The findings from the present study show the modelling of changes in bone and body  
317 composition characteristics derived from both DXA and pQCT over the course of a competitive  
318 football season. The baseline DXA and pQCT derived bone characteristics of the footballer  
319 cohort in the present study were similar to previously published findings in elite footballers [15,  
320 33]. Until now, the specific effects of a competitive season on body composition and bone  
321 characteristics in male professional footballers had yet to be fully determined. Lean mass  
322 increased, while fat mass decreased across time in footballers and the control group. Increases  
323 in whole-body BMD, leg BMC, whole-body BMC, tibial area and tibial mass (38% site) were  
324 shown across over the course of the season in elite footballers, but not in control participants.  
325 While footballers showed consistently higher bone characteristics, there was considerable  
326 variation within and between footballers and controls. By going beyond mean effects of change  
327 in body composition and bone characteristics across time and estimating individual variation  
328 in response to a competitive season, the current study provides novel insight into the osteogenic  
329 effect of football participation.

330           There were increases in whole-body BMD, leg and whole-body BMC over the course  
331 of the season in elite footballers, but not in control participants, which may be due to the greater  
332 volume and magnitude of loading that the footballers are likely to have experienced over the  
333 study period. The greater loading the footballers are expected to have experienced is likely to  
334 have resulted in mechonstrasductive mechanisms being stimulated, ultimately leading to a  
335 greater BMD and BMC [7, 34]. Although on average footballers' increase their BMC across  
336 the season, it is important to note that some players may respond differently. A major strength  
337 of the current study was that change in body composition and bone characteristics were  
338 measured across time and estimates of individual variation in response were assessed. Indeed,  
339 results suggest that some players may decrease BMC across the season. For both controls and

340 elite football players, there was significant between-person variation in levels of all body  
341 composition and bone characteristics. For example, despite footballers' average BMC being  
342 estimated as 3801 g at pre-season, results showed that 95% of footballers' values are expected  
343 to lie between 2954 and 4648 g. This may have implications for practitioners interpreting body  
344 composition and bone characteristics in professional footballers at the start of pre-season, given  
345 that values within range could be considered normal, yet values outside this range might be a  
346 cause for concern from a bone health perspective in elite football environments and warrants  
347 further investigation.

348         Having higher BMC at pre-season is related to having larger increases in BMC  
349 throughout the season. This may be indicative of the specific performance characteristics of a  
350 player in terms of running speed or style of play which are likely to be related to the osteogenic  
351 response shown [35]. Until now, data on how bone adapts to competitive sport has been  
352 produced as a result of cross-sectional studies which have not investigated individual variation.  
353 Therefore, previous studies [23, 36, 37] are not able to interpret how baseline differences in  
354 bone characteristics influence subsequent bone adaptation, something that could be important  
355 when trying to assess the expected change over the course of a season. Cross-sectional studies  
356 have shown, BMC has been shown to increase (Football [23]), decrease (Football [23]; Rugby  
357 League [36]) and fluctuate (Speed Skaters [37]) over the course of a season in athletic  
358 populations. The contrasting findings between previous studies could be attributed individual  
359 variation in athlete response to training being ignored the specific anatomical sites measured,  
360 the loading specific demands of individual sports and contextual factors, such as training  
361 characteristics and playing schedules that are likely to alter the loading experienced. The  
362 individual variation shown in the current study provides a detailed insight into how bone is  
363 likely to adapt at various seasonal timepoints in elite footballers.

364

365 Tibial area and tibial mass at the 38% site were also shown to increase over the course  
366 of the season in elite footballers, and not controls. The tibial shaft is likely to subjected to a  
367 greater amount of tension during football specific dynamic loading, relative to other tibial sites  
368 examined, which may explain which changes were not shown at all tibial lengths. The lack of  
369 seasonal change at other tibial sites may be reflective of a bone that is already adapted to  
370 football training and match-play. The increase in tibial area and mass suggests that a  
371 competitive football season is osteogenic for this site of the tibia, however as the epiphysis of  
372 the tibia is a common stress fracture site the change in tibial mass and area may have  
373 implications for injury prevention [38]. Knowledge of the expected changes in body  
374 composition and bone characteristics, particularly at bone sites where stress fracture commonly  
375 occurs, may assist with the identification of abnormal adaptation in response to exercise.  
376 Quantification of expected bone adaptations may have a greater utility in the identification of  
377 athletes susceptible to stress-related bone injuries than merely the quantification of bone  
378 strength, density and size characteristics alone. Recent data has shown that DXA derived bone  
379 measurements were not associated with stress fracture history [14], whilst the changes in bone  
380 characteristics across the lifespan in the general population are well characterised [39], until  
381 now, no such information for bone characteristics derived from DXA and pQCT is known in  
382 an elite footballer population. Due to the debilitating nature of stress fracture injury [9, 40], the  
383 findings from the present study could potentially be used as a benchmark for practitioners and  
384 clinicians as part of a multifaceted approach in the identification of individuals with a heighten  
385 risk of stress fracture injury.

386 At baseline, elite footballers had greater bone characteristics than recreationally active  
387 control participants in a range of characteristics, including whole-body lean mass, BMD, BMC,  
388 bone area, and tibial bone mass, strength strain index, bone area, cortical thickness, and  
389 periosteal circumference. The reason for the greater bone characteristics in footballers is likely



390 to be due to demands of football training and match-play, which necessitate frequent, high  
391 magnitude loading and physical strength, both of which are known to be osteogenic [7,17]  
392 Despite the footballers having greater bone characteristics at baseline, increases in a range of  
393 whole-body BMD, leg and whole-body BMC and tibial area and mass (38% site) were shown  
394 over the course of the season. This suggests that although the footballers were accustomed to  
395 the football specific training undertaken, however football participation still generated an  
396 osteogenic response in some bone characteristics. It can be speculated that a bone  
397 unaccustomed to football training may have an even greater osteogenic response if training  
398 load is monitored in order to avoid above-threshold loading. These data provide an insight into  
399 the osteogenic influence football training can have on bone that is accustomed to exercise and  
400 has implications for those using football specific training to improve bone health in a range of  
401 populations.

402         Studies do not typically utilise both pQCT and DXA measurements when assessing  
403 seasonal changes in elite footballers [22,25]. This may cause changes in some bone  
404 characteristics to have been missed. Furthermore, previous studies have also only implemented  
405 measurement points at two [22] or three [23] time points during the season. As a professional  
406 football season typically lasts  $\geq 9$  months, transient changes in bone characteristics over the  
407 entire season may be missed if only two or three measurement points are employed. In relation  
408 to body composition, lean mass increased, while body fat decreased in both groups across the  
409 study period. While the changes in body composition were expected in elite players due to the  
410 vigorous nature of professional football training and match-play, the changes in the control  
411 group were not expected. The reason for the changes in the control group could be due to their  
412 greater body fat and less lean mass at baseline and therefore the potential loses/gain are likely  
413 to have been greater. Previous research has shown that lean mass in footballers increases during  
414 pre-season and then be maintained for the rest of the season [23,41,42]. While fat mass has

415 been shown to increased towards the end of the season [23,41]. However, previous studies, like  
416 the present study, have mainly used players from only one club during the study period. This  
417 is likely to be due to the logistical challenges associated with recruiting numerous players from  
418 various clubs. Using only one team causes the data to be at a greater risk of influence from  
419 contextual factors, such as training volume and coaching tactics, which are likely to influence  
420 body composition.

421

422         The present study is not without limitation. A selection bias could have occurred in that  
423 elite football players could have had greater bone and body composition characteristics prior  
424 to involvement in elite football. The greater lean mass and lower body fat characteristics may  
425 have contributed to them becoming an elite football player. Playing position wasn't  
426 standardised in the present study, which, due to the differing demands of playing positions [43],  
427 may have influenced the findings. However, determining specific playing position is very  
428 difficult in modern football, due to differing managerial tactics and differing positional roles  
429 in and out of possession of the ball. As the study was conducted in elite athletes, control  
430 measures were not applied. Therefore, habitual diet and lifestyle preferences, such as sleep  
431 quantity and quality, alcohol consumption and use of anti-inflammation drugs could have  
432 influenced the findings. However, prescribing control measures to elite athletes is not possible  
433 as these measures could influence the athletes' performance and would have reduced the  
434 validity of the findings. The present study described changes in bone and body composition  
435 characteristics across a season. Future research is warranted to examine the factors that may be  
436 responsible for the observed changes. Specifically, training load information could be collected  
437 to examine whether the type and magnitude of training and match-play the players in engage  
438 in relate to changes in bone and body composition characteristics. Furthermore, it is  
439 recommended that future studies assess bone changes between the end of the season and the

440 start of a new season in order to investigate the impact of the off-season on bone characteristics  
441 and subsequent bone injury risk.

442 In conclusion, whole-body BMD, leg and whole-body BMC, tibial bone mass and area  
443 (38%) increased over the course of the season in elite football players. The modelling  
444 information on expected changes in bone characteristics provides practitioners with normative  
445 data in order to benchmark their players, which may be used as a method of identifying those  
446 with abnormal bone response to football training and match-play.

#### 447 Perspective

448 Accurate information quantifying the expected change in bone characteristics, such as BMD,  
449 bone size and bone strength, as a result of exercise is yet to be established. Previous findings  
450 have shown football to be osteogenic [4,5], however the expected change in bone  
451 characteristics is not known. The findings from the present study demonstrate the bone and  
452 body composition adaptations that occur across the course of a season in professional footballers  
453 and a healthy active population. Furthermore, by going beyond mean effects of change across  
454 time and estimating individual variation in response to a competitive season, the findings show  
455 that although bone characteristics, such as BMC, increased across the season in professional  
456 footballers, there was between-person variation with some players showing a decrease. The  
457 reporting of the ‘normal’ range of bone adaptation in the present study allows for those  
458 responding outside of this range to be assessed from a bone health perspective. The results  
459 provide insight for practitioners and health professionals into changes in bone characteristics  
460 and can be used as a benchmark for similar populations.

#### 461 **References**

462 1) Weidauer L, Eilers M, Binkley T, Et al. Effect of different collegiate sports on cortical bone  
463 in the tibia. J Musculoskelet Neuronal Interact 2012;12(2),68-73.

- 464 2) Nilsson M, Ohlsson C, Odén A, Et al. Increased physical activity is associated with enhanced  
465 development of peak bone mass in men: A five-year longitudinal study. *JBMR*  
466 2012;27(5),1206-1214.
- 467 3) Creighton DL, Morgan AL, Boardley D, Et al. Weight-bearing exercise and markers of bone  
468 turnover in female athletes. *J Appl Physiol* 2001;90(2),565-570.
- 469 4) Krstrup P, Aagaard P, Nybo L, Et al. Recreational football as a health promoting activity:  
470 a topical review. *Scand J Med Sci Sports* 2010;20(s1),1-13.
- 471 5)Hagman M, Helge EW, Hornstrup T. Bone mineral density in lifelong trained male football  
472 players compared with young and elderly untrained men. *Journal of Sport and Health Science*  
473 2018; 7(2), 159-168
- 474 6)Varley I, Hughes DC, Greeves JP, Et al. Increased training volume improves bone density  
475 and cortical area in adolescent football players. *Int J Sports Med* 2017; 38(5),341-346. doi:  
476 10.1055/s-0042-124510
- 477 7)Vicente-Rodriguez G, Jimenez-Ramirez J, Ara I, Et al. Enhanced bone mass and physical  
478 fitness in prepubescent footballers. *Bone* 2003;33(5),853-859. doi:  
479 10.1016/j.bone.2003.08.003.
- 480 8) Scott A, Khan KM, Duronio V, Hart DA. Mechanotransduction in human bone: in vitro  
481 cellular physiology that underpins bone changes with exercise. *Sports Med* 2008;38(2),139-  
482 160.
- 483 9) Ekstrand J, Torstveit MK. Stress fractures in elite male football players. *Scand J Med Sci*  
484 *Sports* 2012;22(3), 341-346. doi: 10.1111/j.1600-0838.2010.01171.x
- 485 10) Johnston TE, Dempsey C, Gilman F, Et al. Physiological Factors of Female Runners  
486 With and Without Stress Fracture Histories: A Pilot Study. *Sports Health* 2020;12:334-40.
- 487 11) Alway P, Peirce N, King M, Et al. Lumbar bone mineral asymmetry in elite cricket fast  
488 bowlers. *Bone* 2019;127:537-543.
- 489 12) Duckham RL, Brooke-Wavell K, Summers GD, Et al. Stress Fracture Injury in Female  
490 Endurance Athletes in the United Kingdom: A 12-month Prospective Study. *Scand J Med Sci*  
491 *Sports* 2015;25:854-859.
- 492 13) Kraus E, Tenforde AS, Nattiv A, Et al. Bone Stress Injuries in Male Distance Runners:  
493 Higher Modified Female Athlete Triad Cumulative Risk Assessment Scores Predict Increased  
494 Rates of Injury. *Br J Sports Med* 2019;53:237-242.
- 495 14) Varley I, Stebbings G, Williams AG, Et al. An investigation into the association of bone  
496 characteristics and body composition with stress fracture in athletes. *J Sports Med Phys*  
497 *Fitness* 2021;22. doi: 10.23736/S0022-4707.21.11871-7.

- 498 15) Warden SJ, Edwards WB, Willy RW. Optimal Load for Managing Low-Risk Tibial and  
499 Metatarsal Bone Stress Injuries in Runners: The Science Behind the Clinical Reasoning. *J*  
500 *Orthop Sports Phys Ther* 2021;51(7):322-330. doi: 10.2519/jospt.2021.9982..
- 501 16) McNaughton SA, Wattanapenpaiboon N, Wark JD, Nowson CA. An Energy-Dense,  
502 Nutrient-Poor Dietary Pattern Is Inversely Associated With Bone Health in Women. *J Nutr*  
503 2011;141:1516-1523.
- 504 17) Tagliaferri C, Wittrant Y, Davicco MJ, Et al. Muscle and bone, two interconnected  
505 tissues. *Ageing Res Rev* 2015;21:55-70.
- 506 18) Popp KL, Hughes JM, Smock AJ, Et al. Bone geometry, strength, and muscle size in  
507 runners with a history of stress fracture *Med Sci Sports Exerc* 2009;41(12):2145-2150. doi:  
508 10.1249/MSS.0b013e3181a9e772.
- 509 19) Avin KG, Bloomfield SA, Gross TS, Warden SJ. Biomechanical Aspects of the Muscle-  
510 Bone Interaction. *Curr Osteoporos Rep.* 2015; 13(1): 1–8. doi: 10.1007/s11914-014-0244-x
- 511 20) Brotto M, Bonewald L (2015) Bone and muscle: Interactions beyond mechanical. *Bone*,  
512 80 109-114
- 513 21) Tenforde AS, Carlson JL, Sainani KL, Et al. Lower Trabecular Bone Score and Spine  
514 Bone Mineral Density Are Associated With Bone Stress Injuries and Triad Risk Factors in  
515 Collegiate Athletes. *PM R.* 2020;10. doi: 10.1002/pmrj.12510.
- 516 22) Weidauer L, Minett M, Negus C, Et al. Odd-impact loading results in increased cortical  
517 area and moments of inertia in collegiate athletes. *Eur J Appl Physiol* 2014;114(7),1429-  
518 1438. doi: 10.1007/s00421-014-2870-5
- 519 23) Milanese C, Cavedon V, Corradini G, Et al. Seasonal DXA-measured body composition  
520 changes in professional male soccer players. *Journal of Sports Sciences* 2015;33(12),1219-  
521 1228. doi: 10.1080/02640414.2015.1022573
- 522 24) Ammann P, Rizzoli R. Bone strength and its determinants. *Osteoporos Int.* 2003;14 Suppl  
523 3:S13-28. doi: 10.1007/s00198-002-1345-4.
- 524 25) Minett MM, Binkley TB, Weidauer LA, Specker BL. Changes in body composition and  
525 bone of female collegiate soccer players through the competitive season and off-season. *J*  
526 *Musculoskelet Neuronal Interact* 2017;17(1), 386-398.
- 527 26) Milanese C, Cavedon V, Corradini G, Et al. Long-Term Patterns of Bone Mineral  
528 Density in an Elite Soccer Player. *Front Physiol* 2021;12:631543. doi:  
529 10.3389/fphys.2021.631543.
- 530 27) Rasbash J, Steele F, Browne WJ, Goldstein H, Charlton C. *A User's Guide to MLwiN.*  
531 Bristol, UK. 2017

- 532 28) Norcross J, Van Loan MD. Validation of Fan Beam Dual Energy X Ray Absorptiometry  
533 for Body Composition Assessment in Adults Aged 18-45 Years. *Br J Sports Med*  
534 2004;38:472-476.
- 535 29) Ward LC, Dyer JM, Byrne NM, Et al. Validation of a three-frequency bioimpedance  
536 spectroscopic method for body composition analysis. *Nutrition* 2007;23:657-664.
- 537 30) Jenkins MA, Hart NH, Rantalainen T, Et al. Reliability of upper-limb diaphyseal mineral  
538 and soft-tissue measurements using peripheral Quantitative Computed Tomography (pQCT) *J*  
539 *Musculoskelet Neuronal Interact* 2018;1;18(4):438-445.
- 540 31) Feingold A. Confidence interval estimation for standardized effect sizes in multilevel and  
541 latent growth modeling. *J Consult Clin Psychol* 2015;83(1), 157.
- 542 32) Cohen J. *Statistical Power Analysis for the Behavioral Sciences*. New York, NY:  
543 Routledge Academic. 1988.
- 544 33) Hart NH, Nimphius S, Weber J, ET al. Musculoskeletal Asymmetry in Football Athletes:  
545 A Product of Limb Function over Time. *Med Sci Sports Exerc*. 2016;48(7):1379-1387. doi:  
546 10.1249/MSS.0000000000000897
- 547 34) Hart NH, Nimphius S, Rantalainen T, Et al. Mechanical basis of bone strength: influence  
548 of bone material, bone structure and muscle action. *J Musculoskelet Neuronal Interact*  
549 2017;17(3): 114–139.
- 550 35) Scott JP, Sale C, Greeves JP, Et al. The role of exercise intensity in the bone metabolic  
551 response to an acute bout of weight-bearing exercise. *J Appl Physiol* 2011;110(2),423-432.
- 552 36) Harley JA, Hind K, O'hara JP. Three-compartment body composition changes in elite  
553 rugby league players during a super league season, measured by dual-energy X-ray  
554 absorptiometry *J Strength Cond Res* 2011;25(4):1024-1029. doi:  
555 10.1519/JSC.0b013e3181cc21fb.
- 556 37) Varley I, Greeves JP, Sale C. Seasonal Difference in Bone Characteristics and Body  
557 Composition of Elite Speed Skaters. *Int J Sports Med*. 2019;40(1):9-15. doi: 10.1055/a-0767-  
558 6924.
- 559 38) Coady CM, Micheli LJ. Stress fractures in the pediatric athlete. *Clin Sports Med*  
560 1997;16:225–238.
- 561 39) Riggs BL, Melton LJ, Robb RA, Et al. Population-based study of age and sex differences  
562 in bone volumetric density, size, geometry, and structure at different skeletal sites. *J Bone*  
563 *Miner Res* 2004;19(12):1945–1954. doi: 10.1359/jbmr.040916.
- 564 40) Ranson CA, Burnett AF, Kerslake RW. Injuries to the lower back in elite fast bowlers:  
565 acute stress changes on MRI predict stress fracture. *J Bone Joint Surg Br* 2010;12, 1664-  
566 1668.

- 567 41) Carling C, Orhant E. Variation in body composition in professional soccer players: inter-  
568 seasonal and intra-seasonal changes and the effects of exposure time and player position. *J*  
569 *Strength Cond Res* 2010;24(5),1332-1339. doi: 10.1519/JSC.0b013e3181cc6154
- 570 42) Lago-Peñas C, Rey E, Lago-Ballesteros J, Et al. Seasonal variations in body composition  
571 and fitness parameters according to individual percentage of training completion in  
572 professional soccer players. *Int. J. Sports Med* 2013;14(4), 205-215.
- 573 43) Oliva-Lozano JM, Fortes V, Krstrup P, Muyor JM. Acceleration and sprint profiles of  
574 professional male football players in relation to playing position. *PLos* 2020;  
575 doi.org/10.1371/journal.pone.0236959

576