

Please cite the Published Version

Varley, Ian, Ward, Marcus, Thorpe, Chris, Beardsley, Nathan, Greeves, Julie, Sale, Craig and Saward, Chris (2022) Modelling changes in bone and body composition over a season in elite male footballers. International Journal of Sports Medicine, 43 (8). pp. 729-739. ISSN 0172-4622

DOI: https://doi.org/10.1055/a-1810-6774

Publisher: Thieme Publishing

Version: Accepted Version

Downloaded from: https://e-space.mmu.ac.uk/630593/

Additional Information: This is an Accepted Manuscript of an article which appeared in International Journal of Sports Medicine, published by Thieme Publishing

Enquiries:

If you have questions about this document, contact openresearch@mmu.ac.uk. Please include the URL of the record in e-space. If you believe that your, or a third party's rights have been compromised through this document please see our Take Down policy (available from https://www.mmu.ac.uk/library/using-the-library/policies-and-guidelines)

1 Modelling Changes in Bone and Body Composition over a Season in Elite Male

2 Footballers.

3

4 Abstract

This study investigated the change in bone and body composition characteristics of elite
football players and recreationally active control participants across the course of a season.
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

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

Long-term weight bearing exercise has a positive effect on bone accrual [1, 2]. The physiological benefits of football participation on bone health are wide-ranging [3]. Habitual football participation has been associated with a greater whole-body bone mineral density (BMD) [4] and BMD at specific anatomical locations, such as the proximal femur and the femoral shaft [5], when compared to participation in other sports and to untrained control participants. Others have reported similar associations for bone size and bone strength [6].. The
osteogenic effect of football participation is likely to be due to the high magnitude of loading
that takes place during football training and match-play [7], which stimulates the bone
remodelling cycle through mechotransductive-related mechanisms [8].

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

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

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

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

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

93

94 Methods

95 Participants

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

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

114

115 Design

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

128 $(42 \pm 4 \text{ weeks})$. 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*

Participants were tested for body composition and bone characteristics using DXA and pQCT. 133 Each participant completed a health status questionnaire prior to each testing session. Height 134 (Stadiometer, Seca, Hamburg, Germany) and body mass (Seca, Birmingham, U.K.) were 135 recorded with participants wearing minimal clothing. DXA scans assessed participant BMD 136 (g/cm²), Bone Mineral Content (BMC, g), lean mass (g) and fat mass (g). pQCT assessed the 137 following tibial measures: mass (4%, 14%, 38%, g), polar, Y and X stress strain index (14%, 138 38%, mm³), trabecular Area (4%), trabecular density (4%, mg·cm³), cortical area (14%, 38%, 139 mg·cm³), cortical density (14%, 38%, 66%, mg·cm³) cortical thickness (14%, 38%, mm), 140 periosteal circumference (14%, 38%, mm) and total area (14%, 38%, 66%, mm²). A 141 manufacturer-trained operator performed all scans consistent with the manufacturer's 142 guidelines. Calibration of the DXA and pQCT was completed prior to scanning using a 143 phantom of a known density. Participants were asked to wear minimal clothing or a cotton 144 examination gown and remove any jewellery or metal prior to the scan to avoid measurement 145 distortion. Participants fasted for at least 2 hours, emptied their bladder immediately before and 146 were asked to be euhydrated prior to the scan. 147

148

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

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

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

scan was performed in the frontal plane to confirm the location of the middle of the distal end 174 plate, which would act as a positioning line. Sectional images were then obtained at distal sites 175 (4%, 14%) and the diaphysis of the tibia (38% and 66%) from the positioning line, with a voxel 176 size set at 0.5mm and a slice thickness of 2.5mm for all measurements. A contour mode, with 177 a threshold of 180mg·cm³, was used to separate soft tissue and bone. To analyse trabecular 178 bone, a constant default threshold of $711 \text{mg} \cdot \text{cm}^3$ was used to identify and remove cortical bone. 179 The integral XCT2000L software (version 6.20A) was used to analyse the pQCT images. If 180 any movement artefacts were present following the scan, the image was classed as invalid, and 181 182 a repeat scan was performed. If an artefact was present in the second image, the participant was removed from the study in line with the radiation exposure guidelines. In the present study, no 183 participants were removed from the analysis due to artefacts. 184

185

186 Data analysis

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

the control group and the football player group were considered. The effect of allowing the 198 control group intercept and football player group intercept to randomly vary was then 199 examined. This allows the inter-individual variation in the response variable to be modelled 200 separately for the two groups. Subsequently, the fixed effect of 'visit number' (centred at 201 baseline / time point 1) was considered for each group, to examine whether the response 202 variable changed across time for each group. The effect of allowing the control group slope for 203 204 time and football player group slope for time to randomly vary was then examined. This allows the inter-individual variation in the rate of change in the response variable to be modelled 205 206 separately for the two groups. The fixed effect of 'group' was also considered, to examine differences between controls and football players in relation to the response variable. The size 207 of the effects when comparing controls and football players were examined using Cohen's d208 209 adapted for multilevel modelling by Feingold [31]. Effect sizes were evaluated based upon Cohen's guidance using the following boundaries: <0.20 (trivial), 0.20-0.49 (small), 0.50-0.79 210 (medium), and >0.79 (large) [32]. The assumption that variance in random effects followed a 211 normal distribution with a mean of zero, was checked following each analysis [27]. Statistical 212 significance was accepted at the 95% confidence level (p < 0.05). Mean \pm SD were used to 213 describe the average and variability of data, unless stated otherwise. 214

215

216 **Results**

The average values (mean \pm SD) for controls and footballers at each visit for DXA variables and pQCT variables are displayed in Tables 1 and 2. The multilevel models predicting changes across the study period in controls and footballers are displayed in Table 3 for DXA outcome variables and Table 4 for pQCT outcome variables. For stature and body mass, modelling revealed that controls were significantly shorter than footballers at the start of the study (1.78 m vs. 1.82 m, p<0.05, d=0.52) and that stature did not significantly change across the study period (p>0.05). Furthermore, there were no significant differences between controls and footballers in body mass at the start of the study (77.85 kg vs. 81.25 kg, p>0.05, 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 legs), bone mineral content (total and legs), and area (total and legs) (all p<0.05, d>0.49) (see 228 Table 3). For pOCT, modelling revealed that at baseline, footballers had higher estimates for 229 17 out of 21 variables (all p<0.05, d>0.19) compared to controls. Exceptions were that there 230 were no differences between groups in bone density at the 4% and 14% sites, and in endosteal 231 circumference at the 14% and 38% sites (see Table 4). Allowing the intercepts to randomly 232 vary for controls and footballers, improved the fit of every model. This allows variation in 233 234 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. There were changes across the study period in total fat mass and total lean mass in both controls 236 and footballers, and changes in total BMD, Legs BMC, Total BMC, Tibial Mass (38%), and 237 Tibial Area (38%) in footballers. We also allowed slopes to randomly vary. This improved 238 model fit for footballers for legs BMC and total BMC. This allows variation in the rate of 239 240 change to be estimated using a 95% coverage range using the standard deviations from the random part of models displayed in Table 3. 241

On average, total fat mass at baseline was predicted to be 11.13 kg for footballers, 5.34 kg lower than controls (p<0.05, d=0.62) (Table 3). However, modelling also revealed that there was significant random inter-individual variation in total fat mass for both controls (SD=8.17kg) and footballers (SD=2.38kg) at baseline (Table 3). This information can be used to construct a 95% coverage range (CR) for predicted total fat mass for the two groups. Controls were predicted to have a total fat mass of 16.47 kg, but with a random intercept SD of 8.17 kg, the coverage range within which 95% of controls are expected to lie can be estimated as 0.46 kg to 32.48 kg (16.47 kg \pm (1.96*8.17 kg)). Conversely, footballers were predicted to have a total fat mass of 11.13 kg, but with a random intercept SD of 2.38 kg, the coverage range within which 95% of footballers are expected to lie can be estimated as 6.47 kg to 15.80 kg (11.13 kg \pm (1.96*2.38 kg)).

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

On average, total lean mass at baseline was predicted to be 8.58 kg higher in footballers 259 versus controls (p<0.05, d=0.90) (Table 3). Total lean mass was predicted to be 58.01 kg, 95% 260 CR [43.68, 72.34 kg] for controls, and 66.59 kg, 95% CR [54.89, 78.29 kg] for footballers. On 261 average, total lean mass was predicted to increase in both groups (controls = 0.30 kg per visit, 262 p<0.05; footballers = 0.35 kg per visit, p<0.05). Thus, footballers' total lean mass was predicted 263 264 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 interindividual variation in the rate of change (slope) for either group. 266

On average, total BMD at baseline was predicted to be 0.106 g/cm² higher in footballers
versus controls (p<0.05, *d*=0.71). Total BMD was predicted to be 1.309 g/cm², 95% CR [1.078,
1.540 g/cm²] for controls and 1.415 g/cm², 95% CR [1.241, 1.589 g/cm²] for footballers. Total
BMD was predicted to increase in footballers but not in controls. On average, footballers

increased total BMD by 0.012 g/cm^2 (0.004 g/cm² per visit, p<0.05) from 1.415 g/cm² in preseason to 1.427 g/cm² at the end of the season. There was no random inter-individual variation in the rate of change (slope).

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

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

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

For pQCT variables, the only significant changes across time were in tibial mass and 299 area at the 38% site in footballers, which will be discussed in detail henceforth. On average, 300 tibial mass at the 38% site at baseline was predicted to be 0.65 g higher in footballers than in 301 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] 302 g] for controls and 5.10 g, 95% CR [4.22, 5.98 g] for footballers. Mass at the 38% site was 303 predicted to increase in footballers but not controls. On average, footballers increased mass at 304 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 305 of the season. There was no random inter-individual variation in the rate of change (slope). On 306 average, area at the 38% site at baseline was predicted to be 43 mm² higher in footballers than 307 in controls (p<0.05, d=0.55). Area at the 38% site was predicted to be 489 mm², 95% CR [364, 308 614 mm²] for controls and 532 mm², 95% CR [454, 610 mm²] for footballers. Area at the 38% 309 site was predicted to increase in footballers but not controls. On average, footballers increased 310 area at the 38% site by 0.09 mm² (4 mm² per visit, p<0.05) from 532 mm² in pre-season to 536 311 mm² at the end of the season. There was no random inter-individual variation in the rate of 312 change (slope). 313

315 **Discussion**

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

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

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

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

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

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

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

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

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

421

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

start of a new season in order to investigate the impact of the off-season on bone characteristicsand subsequent bone injury risk.

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

447 <u>Perspective</u>

Accurate information quantifying the expected change in bone characteristics, such as BMD, 448 bone size and bone strength, as a result of exercise is yet to be established. Previous findings 449 450 have shown football to be osteogenic [4,5], however the expected change in bone characteristics is not known. The findings from the present study demonstrate the bone and 451 body composition adaptions that occur across the course of a season in professional footballers 452 and a healthy active population. Furthermore, by going beyond mean effects of change across 453 time and estimating individual variation in response to a competitive season, the findings show 454 455 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 reporting of the 'normal' range of bone adaptation in the present study allows for those 457 458 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 and can be used as a benchmark for similar populations. 460

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.

- 2) Nilsson M, Ohlsson C, Odén A, Et al. Increased physical activity is associated with enhanced
 development of peak bone mass in men: A five-year longitudinal study. JBMR
 2012:27(5),1206-1214.
- 3) Creighton DL, Morgan AL, Boardley D, Et al. Weight-bearing exercise and markers of bone
 turnover in female athletes. J Appl Physiol 2001;90(2),565-570.
- 469 4) Krustrup 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.
- 5)Hagman M, Helge EW, Hornstrup T. Bone mineral density in lifelong trained male football
 players compared with young and elderly untrained men. Journal of Sport and Health Science
 2018; 7(2), 159-168
- 6)Varley I, Hughes DC, Greeves JP, Et al. Increased training volume improves bone density
 and cortical area in adolescent football players. Int J Sports Med 2017; 38(5),341-346. doi:
 10.1055/s-0042-124510
- 7)Vicente-Rodriguez G, Jimenez-Ramirez J, Ara I, Et al. Enhanced bone mass and physical
 fitness in prepubescent footballers. Bone 2003;33(5),853-859. doi:
 10.1016/j.bone.2003.08.003.
- 8) Scott A, Khan KM, Duronio V, Hart DA. Mechanotransduction in human bone: in vitro
 cellular physiology that underpins bone changes with exercise. Sports Med 2008;38(2),139160.
- 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.
- 12) Duckham RL, Brooke-Wavell K, Summers GD, Et al. Stress Fracture Injury in Female
 Endurance Athletes in the United Kingdom: A 12-month Prospective Study. Scand J Med Sci
 Sports 2015;25:854-859.
- 13) Kraus E, Tenforde AS, Nattiv A, Et al. Bone Stress Injuries in Male Distance Runners:
- Higher Modified Female Athlete Triad Cumulative Risk Assessment Scores Predict Increased
 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,
- Nutrient-Poor Dietary Pattern Is Inversely Associated With Bone Health in Women. J Nutr2011;141:1516-1523.
- 17) Tagliaferri C, Wittrant Y, Davicco MJ, Et al. Muscle and bone, two interconnected
 tissues. Ageing Res Rev 2015;21:55-70.
- 18) Popp KL, Hughes JM, Smock AJ, Et al. Bone geometry, strength, and muscle size in
 runners with a history of stress fracture Med Sci Sports Exerc 2009;41(12):2145-2150. doi:
 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
- Bone Mineral Density Are Associated With Bone Stress Injuries and Triad Risk Factors in
 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),1219521 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
- 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
- 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
- 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 and541 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:
- A Product of Limb Function over Time. Med Sci Sports Exerc. 2016;48(7):1379-1387. doi:
- 546 10.1249/MSS.00000000000897
- 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.
- 35) Scott JP, Sale C, Greeves JP, Et al. The role of exercise intensity in the bone metabolic
 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
- rugby league players during a super league season, measured by dual-energy X-ray
- 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-0767558 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:
- 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-
- 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
- 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, Krustrup 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