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

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 dual-energy x-ray absorptiometry and peripheral Quantitative Computed Tomography for a range of bone and body composition characteristics at four points over the course of a competitive season. Multilevel modelling was used to examine changes.

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

Whole-body bone mineral density, leg and whole-body bone mineral content, 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 a method of identifying those with abnormal bone response to football training and match-play.

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 stress-related bone conditions that result in long-term absence from training and match-play, 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 a method to assess an individual's risk of stress fracture injury [10], there is some debate over the efficacy of this with some studies showing positive associations between bone structural 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 response to mechanical loading could lead to stress-related bone injury [15], rather than 'low' 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. Furthermore, associations between bone characteristics and body composition have been demonstrated due to the interaction between adipose tissue [16] and skeletal muscle [17] with bone. Muscle size has been implicated in bone adaptation [18], with a larger muscle size and a 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 adaptation (for review see Avin et al.,[19]), furthermore muscle-driven biochemical and endocrine stimuli are also known to mediate bone adaptation (for review seeBrotto and Bonewald [20]).

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 characteristics, such as BMD, bone size and bone strength, over the course of a competitive football season is yet to be established. Previous studies have used a cross-sectional study designs [14,21] or a limited number of scans (2 scans, [22]; 3 scans, [23]). Cortical area and cortical thickness have been shown to increase following a collegiate football season, although it is difficult to truly determine seasonal changes from these data, as the study only scanned 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 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 only utilised DXA for the measurement of bone characteristics [11,14,21], meaning that no measures of volumetric bone characteristics, which are vital in accurately determining bone strength [24], have been made. A prospective longitudinal study design, with multiple measurement points over the course of a season, and the attainment of both volumetric and areal bone density measurements is required to provide accurate data on bone structural characteristics. Within such a design, it is also important to have a comparison group to examine whether changes across a season are particular to elite footballers or are common to the more recreationally active population. Most studies examining bone characteristics in elite athletes have not, however, employed an active comparison group [11,14,21], and so season-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 ANOVA-based 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, and can be overly restrictive in their assumptions. Conversely, longitudinal multilevel 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 (random part) [27]. Thus, as well as mean group effects, normal variation between individuals in terms of levels and changes in body composition and bone across time can be estimated, 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 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 susceptibility to stress-related bone injuries. Therefore, the aim of the present study was to describe changes in bone and body composition characteristics over the course of a competitive season in elite football players and active controls, using multilevel modelling.

Methods

Participants

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

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

Design

This was a prospective longitudinal study. Participants underwent DXA (iDXA, GE Healthcare, United Kingdom) and peripheral Quantitative Computed Tomography (pQCT) (XCT2000L, Stratec Medizintechnik) scans on four occasions across the study period. Prior to the commencement of the study (visit 1), the footballers had 7 weeks between the end of the 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 consisted of running, cycling and strength maintenance activities. Visit 1 / baseline (0 weeks) coincided with the start of the footballers' pre-season training period. At baseline, 46 participants were assessed. Visit 2 (8 ± 6 weeks) coincided with the end of footballers' pre-season training period / the start of competitive matches. At visit 2, 46 participants were 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

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

Procedures

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

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].

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

Peripheral Quantitative Computed Tomography pQCT

pQCT scans were taken of the dominant lower leg (defined as the leg that the participant most 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 known density in accordance with manufacturer guidelines. pQCT has previously been shown to provide a reliable measurement of bone characteristics in humans (Intraclass correlation coefficient, CC: 0.76-0.99; [30]). Each participant's tibial length was measured to the nearest 1 mm, determined as the midpoint of the medial malleolus to the medial aspect of the tibial plateau. The participant's leg was then placed in the scanner with their foot secured in a purpose-built attachment. The leg was aligned with use of an integral laser and a clamp was placed to the knee to reduce movement, with the participant instructed to remain as still as possible for the duration of the scan. Initially, a preliminary reference point locating scout-view

scan was performed in the frontal plane to confirm the location of the middle of the distal end plate, which would act as a positioning line. Sectional images were then obtained at distal sites (4%, 14%) and the diaphysis of the tibia (38% and 66%) from the positioning line, with a voxel size set at 0.5mm and a slice thickness of 2.5mm for all measurements. A contour mode, with a threshold of 180mg·cm^3, was used to separate soft tissue and bone. To analyse trabecular bone, a constant default threshold of 711mg·cm^3 was used to identify and remove cortical bone. The integral XCT2000L software (version 6.20A) was used to analyse the pQCT images. If any movement artefacts were present following the scan, the image was classed as invalid, and 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 participants were removed from the analysis due to artefacts.

Data analysis

The mixed-longitudinal sample represented a hierarchically structured data set, with measurement occasion nested within participant. Thus, multilevel models were developed using MLwiN (v 3.05, Bristol, U.K.) to investigate changes in DXA and pQCT variables across time, in controls and football players. Longitudinal multilevel modelling does not require the same number of measurement occasions per individual, meaning all data can be included within the analysis. Following Rasbash et al. [27], a two-level multilevel structure was defined, with measurement occasion (level 1) nested within participant (level 2), with a given DXA or pQCT variable as the continuous response variable for each model. For each model, relevant 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 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 ± SD were used to
214 describe the average and variability of data, unless stated otherwise.

215

216 **Results**

217 The average values (mean ± 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

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 ($p>0.05$, $d=0.23$).

For DXA variables, modelling revealed that at baseline footballers had lower total fat 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 Table 3). For pQCT, modelling revealed that at baseline, footballers had higher estimates for 17 out of 21 variables (all $p<0.05$, $d>0.19$) compared to controls. Exceptions were that there were no differences between groups in bone density at the 4% and 14% sites, and in endosteal circumference at the 14% and 38% sites (see Table 4). Allowing the intercepts to randomly vary for controls and footballers, improved the fit of every model. This allows variation in baseline values for each group to be estimated using a 95% coverage range for each variable 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 and footballers, and changes in total BMD, Legs BMC, Total BMC, Tibial Mass (38%), and Tibial Area (38%) in footballers. We also allowed slopes to randomly vary. This improved model fit for footballers for legs BMC and total BMC. This allows variation in the rate of change to be estimated using a 95% coverage range using the standard deviations from the random part of models displayed in Table 3.

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=8.17 kg) 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 ± (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 ± (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 versus controls ($p<0.05$, d=0.90) (Table 3). Total lean mass was predicted to be 58.01 kg, 95% CR [43.68, 72.34 kg] for controls, and 66.59 kg, 95% CR [54.89, 78.29 kg] for footballers. On average, total lean mass was predicted to increase in both groups (controls = 0.30 kg per visit, $p<0.05$; footballers = 0.35 kg per visit, $p<0.05$). Thus, footballers' total lean mass was predicted to increase from 66.59 kg in pre-season to 67.64 kg at the end of the season and controls' total lean mass was predicted to increase from 58.01 kg to 58.91 kg. There was no random inter-individual variation in the rate of change (slope) for either group.

On average, total BMD at baseline was predicted to be 0.106 g/cm^2 higher in footballers versus controls ($p<0.05$, d=0.71). Total BMD was predicted to be 1.309 g/cm^2, 95% CR [1.078, 1.540 g/cm^2] for controls and 1.415 g/cm^2, 95% CR [1.241, 1.589 g/cm^2] 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/cm^2 per visit, $p<0.05$) from 1.415 g/cm^2 in pre-season to 1.427 g/cm^2 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 each participant across the study period, and also the average predicted changes across the 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 predicted to be 3315 g, 95% CR [2411, 4219g] for controls and 3801g, 95% CR [2954, 4648g] for footballers. Total BMC was predicted to increase in footballers but not in controls. On average, footballers increased total BMC by 54 g (18 g per visit, $p<0.05$) from 3801 g in pre-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. Footballers were predicted to increase total BMC by 18 g per visit, 95% CR[-25, 61g]. 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 to increase total BMC more between the start and end of the season (see figure 1).

On average, leg BMC at baseline was predicted to be 274 g higher in footballers versus controls ($p<0.05$, d=0.99). At baseline leg BMC was predicted to be 1286 g, 95% CR [894, 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 BMC by 12 g (4 g per visit, $p<0.05$) from 1560g in pre-season to 1572g at the end of the season. 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 BMC by 4 g per visit, but with a random intercept SD of 7 g, the coverage range within which 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^2 higher in footballers than
308 in controls ($p<0.05$, $d=0.55$). Area at the 38% site was predicted to be 489 mm^2, 95% CR [364,
309 614 mm^2] for controls and 532 mm^2, 95% CR [454, 610 mm^2] 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^2 (4 mm^2 per visit, $p<0.05$) from 532 mm^2 in pre-season to 536
312 mm^2 at the end of the season. There was no random inter-individual variation in the rate of
313 change (slope).

314

Discussion

The findings from the present study show the modelling of changes in bone and body composition characteristics derived from both DXA and pQCT over the course of a competitive 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, 33]. Until now, the specific effects of a competitive season on body composition and bone characteristics in male professional footballers had yet to be fully determined. Lean mass 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 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 variation within and between footballers and controls. By going beyond mean effects of change in body composition and bone characteristics across time and estimating individual variation in response to a competitive season, the current study provides novel insight into the osteogenic effect of football participation.

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 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 have resulted in mechonstrasductive mechanisms being stimulated, ultimately leading to a greater BMD and BMC [7, 34]. Although on average footballers' increase their BMC across the season, it is important to note that some players may respond differently. A major strength 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, results suggest that some players may decrease BMC across the season. For both controls and

elite football players, there was significant between-person variation in levels of all body composition and bone characteristics. For example, despite footballers' average BMC being 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 composition and bone characteristics in professional footballers at the start of pre-season, given that values within range could be considered normal, yet values outside this range might be a cause for concern from a bone health perspective in elite football environments and warrants further investigation.

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

Tibial area and tibial mass at the 38% site were also shown to increase over the course of the season in elite footballers, and not controls. The tibial shaft is likely to subjected to a 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 seasonal change at other tibial sites may be reflective of a bone that is already adapted to football training and match-play. The increase in tibial area and mass suggests that a 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 implications for injury prevention [38]. Knowledge of the expected changes in body composition and bone characteristics, particularly at bone sites where stress fracture commonly occurs, may assist with the identification of abnormal adaptation in response to exercise. 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 strength, density and size characteristics alone. Recent data has shown that DXA derived bone measurements were not associated with stress fracture history [14], whilst the changes in bone characteristics across the lifespan in the general population are well characterised [39], until now, no such information for bone characteristics derived from DXA and pQCT is known in an elite footballer population. Due to the debilitating nature of stress fracture injury [9, 40], the findings from the present study could potentially be used as a benchmark for practitioners and clinicians as part of a multifaceted approach in the identification of individuals with a heighten risk of stress fracture injury.

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 magnitude loading and physical strength, both of which are known to be osteogenic [7,17] 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 over the course of the season. This suggests that although the footballers were accustomed to the football specific training undertaken, however football participation still generated an 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 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 has implications for those using football specific training to improve bone health in a range of populations.

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 characteristics to have been missed. Furthermore, previous studies have also only implemented measurement points at two [22] or three [23] time points during the season. As a professional football season typically lasts ≥ 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 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 vigorous nature of professional football training and match-play, the changes in the control 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 to have been greater. Previous research has shown that lean mass in footballers increases during pre-season and then be maintained for the rest of the season [23,41,42]. While fat mass has

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.

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 to involvement in elite football. The greater lean mass and lower body fat characteristics may have contributed to them becoming an elite football player. Playing position wasn't 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 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 measures were not applied. Therefore, habitual diet and lifestyle preferences, such as sleep quantity and quality, alcohol consumption and use of anti-inflammation drugs could have influenced the findings. However, prescribing control measures to elite athletes is not possible 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 characteristics across a season. Future research is warranted to examine the factors that may be 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 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

start of a new season in order to investigate the impact of the off-season on bone characteristics and 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.

Perspective

Accurate information quantifying the expected change in bone characteristics, such as BMD, bone size and bone strength, as a result of exercise is yet to be established. Previous findings 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 body composition adaptions that occur across the course of a season in professional footballers and a healthy active population. Furthermore, by going beyond mean effects of change across time and estimating individual variation in response to a competitive season, the findings show that although bone characteristics, such as BMC, increased across the season in professional 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 responding outside of this range to be assessed from a bone health perspective. The results provide insight for practitioners and health professionals into changes in bone characteristics and can be used as a benchmark for similar populations.

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