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Full Length Article

SNPs in the vicinity of P2X7R, RANK/RANKL/OPG and Wnt signalling pathways and their association with bone phenotypes in academy footballers

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ABSTRACT

Context: Genotype plays an important role in influencing bone phenotypes, such as bone mineral density, but the role of genotype in determining responses of bone to exercise has yet to be elucidated.
Objective: To determine whether 10 SNPs associated with genes in the vicinity of P2X7R, RANK/RANKL/OPG and Wnt Signalling Pathways are associated with bone phenotypes in elite academy footballers (Soccer players) and to determine whether these genotypes are associated with training induced changes in bone.
Design, participants, and methods: 99 elite academy footballers volunteered to participate. Peripheral computed tomography of the tibia (4%, 14%, 38% and 66% sites) was performed immediately before and 12 weeks after an increase in football training volume. Genotypes were determined using proprietary fluorescence-based competitive allele-specific PCR assays.
Results: No significant genotype by time interactions were shown for any of the SNPs analysed ($P > .05$). A main effect of genotype was shown. SOST SNP rs1877632 (trabecular density), P2X7R SNPs rs1718119 (cortical thickness and CSA), rs3751143 (SSI, CSA, cortical CSA and periosteal circumference) RANK/RANKL/OPG SNPs rs9594738 (periosteal circumference), rs1021188 (cortical thickness and CSA) and rs9594759 (cortical density) were associated with bone phenotypes ($P < .05$).
Conclusions: No association was shown between P2X7R, RANK/RANKL/OPG and Wnt Signalling SNPs and a change in bone phenotypes following 12 weeks of increased training volume in elite academy footballers. However, SNPs were associated with bone phenotypes pre training. These data highlight the complexity of the interaction between SNPs in the vicinity of the RANK/RANKL/OPG, P2X7R and Wnt metabolic regulatory pathways and bone phenotypes in elite academy footballers.

1. Introduction

Attaining a heightened bone mass in early adulthood is important for long-term bone health and the prevention of osteoporosis [1], which makes the adolescent population highly relevant for investigating how bone responds to exercise. The osteogenic effects of football are greater than in other sports [2, 3], most likely due to the high magnitude, frequency and multi-directional nature of the movements that football training and match play necessitate [4]. Bone Mineral Content (BMC) [3, 5], areal Bone Mineral Density (BMD) [6] and cortical cross sectional area (CSA), circumference and thickness [7], as well as bone strength [8], have all been shown to be increased in recreational football players compared to sedentary control populations. Bone adaptions have also been shown in the same cohort of adolescent elite footballers used in the present study after only 12 weeks of increased volume of football training [9].

Despite this, negative bone related responses to exercise have been shown in football players. Participation in unaccustomed exercise and rapid increases in training volume, for example, have been implicated in the development of stress fracture injury [10]. The reasons for exercise eliciting both positive and negative changes to bone structural

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properties are multi-faceted and are likely to involve the mode, intensity and volume of exercise, as well as various intrinsic and extrinsic factors [11].

There is a lack of information relating to the mechanisms that may regulate the individual adaptations that are caused by exercise participation. Genotype has been associated with osteoporosis [12], stress fracture injury [9, 13] and bone turnover [14, 15]. It has been suggested that genotype may mediate the bone response to exercise and may explain some of the variability observed in bone adaptations [16]. Despite evidence of genetic factors being associated with bone phenotypes, little is known about how genotype mediates the bone response to training volume.

The aim of the present study was to investigate whether a genotype dependent change in bone phenotypes is evident in adolescent academy footballers following 12 weeks of increased football-specific training.

2. Method

2.1. Participants

First year, full-time male academy footballers (n = 117) were recruited through previously established relationships with Nottingham Trent University and by word of mouth from five full-time football academies to form the Bone Adaptation in Academy Footballers cohort. Participants were deemed eligible for the study if they were aged ≥16 y, not currently taking any medication that influenced bone metabolism and had not received a joint replacement or prostheses. After reading the participant information sheet and being fully briefed and having the opportunity to ask questions, participants signed a statement of informed consent, completed a pre-scan screening form and completed a health screen questionnaire, which was scrutinised in order to confirm that they met the inclusion/exclusion criteria. Participants detailed their playing position, the age at which they first played competitive football and the amount of hours they spent training prior to full-time academy enrolment.

Following study completion, the respective coach and/or physiotherapist of the football club provided information related to each individual's training time, which included time missed as a result of injury for the previous 12 weeks. Fourteen players who received an initial scan were lost to the follow-up scan for a variety of reasons (The cohort is described elsewhere, [17]) leaving a cohort of n = 99 who completed both scans (Fig. 1). The study conformed to Ionising Radiation (Medical Exposure) Regulations and was approved by the National Health Service Research Ethics Committee (reference 12/EM/0183).

2.2. Experimental design

All participants were recently enrolled full-time academy football players. Participants were tested before an increase in training volume during the first week of pre-season training including height, body mass and bone phenotypes using pQCT. Participants then conducted 12 weeks of football specific training with their respective clubs, followed by a repeat of the inital measurements.

2.3. Procedures

2.3.1. Training intervention

Academy footballers that were deemed of a suitable standard graduated through the academy to become first year scholars. All footballers were habitually accustomed to football training and match-play, as part of their representation of the academy in younger age groups. The start of the study was timed to coincide with their first experience of full-time training. Football specific training (including, high intensity running drills, small-sided games and technique based drills) and match play was organised by qualified coaches at the respective clubs.

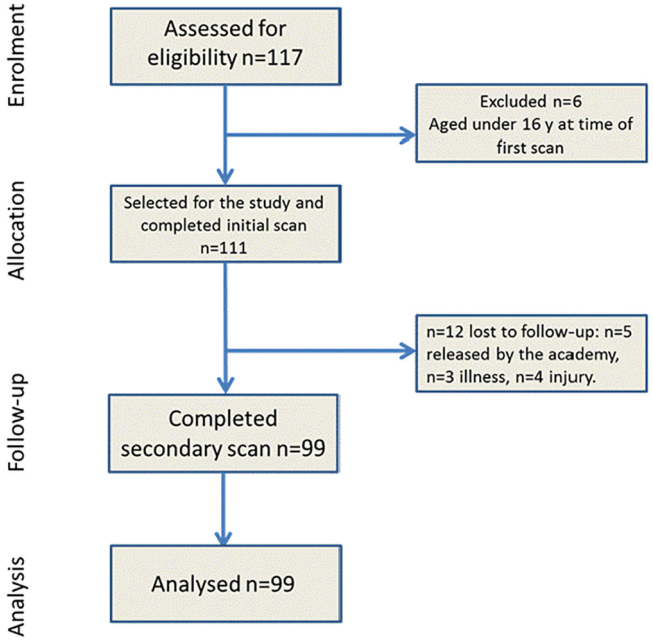

Fig. 1. Academy footballers assessed and analysed.

2.3.2. pQCT

pQCT scans were conducted using an XCT 2000 (Stratec Medizintechnik, Pforzheim, Germany) to assess the bone phenotypes of the tibia of the dominant leg (the leg the participant most comfortably kicked a ball with). Before scanning commenced, the scanner was calibrated using a phantom of known density in accordance with manufacturer guidelines. pQCT has previously been shown to provide a reliable measurement of bone characteristics in humans by our wider research group (CV < 2% for total and Tb.Dn, and CV < 1% for Ct.Dn) [18]. The participant's tibial length was measured to the nearest 1 mm; defined as the midpoint of the medial malleolus to the medial aspect of the tibial plateau. The participants leg was then placed in the scanner with their foot secured in a purpose built attachment. The leg was aligned and a clamp was placed to the knee to reduce the possibility of artefacts by minimising any movement of the limb. The participant was 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, 2 mm thick were then obtained at the 4%, 14%, 38% and 66% sites of the tibia from this reference line with a voxel size set at 0.5 mm for all measurements. These sites are typically used to analyse trabecular and cortical phenotypes of the tibia. A contour mode, with a threshold of 180 $mg \cdot cm^{-3}$, was used to separate soft tissue and bone. To analyse trabecular bone, a constant default threshold of 711 $mg \cdot cm^{-3}$ was used to identify and remove cortical bone. The integral XCT 2000 software (version 6.20A) was used to analyse the pQCT images.

2.3.3. Bone phenotypes

The following measures were analysed at each site of the tibia:

4%: total cross sectional area (Tot CSA, mm^2) and trabecular mineral density ($mg \cdot cm^3$).14% and 38%: Tot CSA, (mm^2), cortical CSA (mm^2), cortical mineral density ($mg \cdot cm^3$), cortical thickness (mm), periosteal circumference (mm) and stress strain index (SSI, mm^3). 66%: Tot CSA, (mm^2) and cortical mineral density ($mg \cdot cm^3$).

The same operator performed all pQCT measurements. If any movement artefacts (inaccuracies in the measurement caused by motion) were present following the scan, the image was classed as invalid and