Body Composition and Bone Characteristics Data in NCAA D1 League Football Team by DXA and HRpQCT

by

Yufei Hua

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This thesis was presented

by

Yufei Hua

It was defended on

March 14, 2023

and approved by

Thesis Co-Advisor: Bradley Nindl, PhD, FACSM, Vice Chair of Research, Professor and Director, Neuromuscular Research Laboratory, Department of Sports Medicine and Nutrition

Kristen Koltun, PhD, Postdoctoral Fellow, Department of Sports Medicine and Nutrition

Mita Lovalekar, MBBS, PhD, MPH, Associate Professor, Vice Chair for Academic Affairs, Department of Sports Medicine and Nutrition

Thesis Advisor: Katelyn Allison, PhD, ACSM-EP, Associate Professor, Department of Sports Medicine and Nutrition

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Yufei Hua, M.S.

University of Pittsburgh, 2023

Body composition plays an important role in an athlete's health and performance. While there have been abundant studies examining body composition using Dual-energy X-ray absorptiometry (DXA) with football players, high-resolution peripheral quantitative computed tomography (HRpQCT) is relatively a new technology that has not been examined in this population. Therefore, the purpose of this study was to provide normative ranges of body composition and bone characteristic data of football players, compare position differences of their bone characteristics and detect changes between their two visits. The first visit was in January 2022 and the second visit was in July and August 2022. 96 players of the University of Pittsburgh football team were measured in the first visit and 79 players were measured in the second visit. They were divided into Line, Skill and Combination groups by different functional movements on the field. Body composition and bone characteristics were measured by DXA and HRpQCT. There were 3 DXA scans of their total body, non-dominant femur and AP spine, and 2 HRpQCT scans at 4% and 30% tibia sites in each visit. Appropriate descriptive analysis was used for providing normative range of data. One-way ANOVA was used to compare position differences. Paired t tests or Wilcoxon signed ranks tests were used to assess changes between the two visits, as appropriate. The results provided normative ranges of players' body composition and bone characteristics. For position differences, players in Line group had significantly higher BMD variables ($p < 0.05$). For HRpQCT data, players in Skill group had

significantly lower bone geometry data ($p < 0.05$) and some trabecular bone microarchitecture variables ($p < 0.05$) than other two groups. For changes between the two visits, Lean mass, Fat-free mass, Spine BMD, Trunk BMD and most HRpQCT variables were significantly increased. This study did present position differences and changes for bone characteristic data, while the biggest limitation is that there is lack of knowledge about these data related to football athletes. Future research should focus on why bone data is different between players from different positions and how bone data changes can improve players' health and performance.

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Preface

I want to thank BoBCAT study team providing me the chance working in the project, and also my co-investigators (Nicole M. Sekel[1], Kristen J. Koltun[1], Adam J. Sterczala[1], Evan D. Feigel[1], David N. Mowery[1], Mita Lovalekar[1], Pouneh K. Fazeli[1], Jane A. Cauley[1], Sophie L. Wardle[2], Thomas J. O'Leary[2], Julie P. Greeves[2], Bradley C. Nindl[2]).

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1.0 Introduction

Body composition is an important indicator of an athlete's athletic performance and health. Different sports have different requirements for the body composition of the athlete due to specific movement patterns and functional movements. In team sports like basketball, volleyball and football, there is a corresponding difference in the body composition of the athletes due to the different positions on the field.[14,15] Managing the body composition of athletes is key for sports teams to prevent injuries and achieve good performance.

There are many ways to assess body composition like skinfold and bioelectrical impedance assessment. However, Dual Energy X-Ray Absorptiometry (DXA) is the gold standard for determining body composition for team athletes.[3] There are an increasing number of studies using DXA to measure body composition for football players.[4,5,6,15,16] However, few studies have focused on the body composition of football players in different positions.[17,19] Raymond et al. illustrated that different positions of football players can significantly affect the results of different body composition testing methods.[17] Malvin et al. suggested that players on the offensive line have a higher lean mass, body fat and fat mass than players at other positions and suggests that elite athletes may have differences on muscle quality regardless differences of body composition, but this study did not use DXA. Therefore, it is important to test body composition differences of football players by DXA.

Bone is an extraordinary biological material both in strength and flexibility. In particular, the structure, size and strength of bone depend on and respond to the conventional physiological and mechanical demands placed on it.[12,13] Bone mass accounts for 50-70% of bone strength.[10] The amount of trabecular bone and cortical bone at a given site of bone affect bone strength independently.[11] High Resolution Peripheral Quantitative Computed Tomography (HR-pQCT) is a method which provides a non-invasive, in-vivo way to exam bone microarchitecture. HR-pQCT scans can be used to construct micro-finite element (mFE) models of bone strength, which correlate well with biomechanically measured bone strength.[7,8] Nevertheless, to achieve this resolution, scanning is only available at peripheral skeletal sites (such as distal radius and tibia).[7] Several studies have used HR-pQCT to test bony characteristics of athletes.[20,22,23,24] Schipilow et al. compared bone data of athletes at different levels of competition and concluded that the higher the level of competition the higher the bone quality.[20] Moreover, Sada et al. included baseball pitchers to compare the skeletal structure of their dominant and non-dominant elbows.[23] He found the dominant elbow had higher bone mineral density, bone volume fraction and trabecular thickness.[23] However, these studies did not investigate football players, and these studies are more focused on female players whose bone quality can be affected by amenorrhea.[69] Bone and Body Composition Adaptations to Training (BoBCAT) study aims to have a better understanding of the mechanisms through which exercise influences bone and the optimal training parameters to maximize beneficial osteogenic adaptations especially to military population. Therefore, the BoBCAT study analyzes training induced changes in bone density, geometry and strength as well as novel bone-muscle crosswalk factors, bone marrow adiposity, and extracellular vesicle cargo in a laboratory training group and a military group. Additionally, the BoBCAT also takes several Pitt sports teams into research over the season. The BoBCAT study is one of the first to assess HR-

pQCT in football players. External loading is an important factor in bone remodeling.[12] Different movement patterns and body composition may cause differences in the microscopic biological structure of bone.[18]

The purpose of this study is to evaluate body composition and bone characteristics of players playing different positions on the field and investigate their body composition and bone characteristics changes off-season.

1.1 Body Composition

1.1.1 Body Composition Related to Sports

Body composition is a term used in the fitness and health community to refer to the percentage of fat, water, bone, muscle, skin, and other lean tissues that make up the body.[25] Body composition can be approached through five levels of increasing complexity, with body mass defined as the total of atoms, molecules, cells, tissues, or distinct body segments.[29] The proper approach for analyzing body composition should take into account the characteristics generated at each level independently, with the sum of the values within each level determining body mass. Thus, (i) the atomic level considers the number of hydrogen, carbon, oxygen, and other atoms, (ii) the molecular level takes into account fat mass and fat-free mass buried in total body water and bone mineral content, and (iii) the cellular level takes into account adipocytes. (iv) the tissue

level analyses the quantity of fat and lean soft tissue and skeletal muscle mass, and (v) the whole-body level summarizes the mass of different bodily sections.[30,31,32]

For the general population, body composition can reflect the risk of developing some diseases, such as obesity and cardiovascular disease. Sports-related professionals are interested to know how and which body components are important for boosting performance, reducing injury risk, and monitoring sports health.[26] Indeed, modifying an athlete's body mass or body composition traits can give them an advantage in numerous sports. In sports that require physical aesthetics such as gymnastics and synchronized swimming, it is important for athletes to keep their body in good shape and proportion for their performance in competitive events.[27] But, for example, boxing and wrestling, which require weight to divide the group, control of body composition also plays a vital role in the athletes' competitiveness.[28] Unhealthy body composition can be a risk factor for injury. In some studies, reduced muscle strength, excessive BMI, position played on the field, growing height, and hormonal fluctuations are all risk factors for ACL injuries.[33,34,35] Meanwhile, there are difference in body composition between players playing different positions.[17,19] Therefore, managing an athlete's body composition can be useful for athletes and coaching teams to help athletes improve their competitiveness and prevent injuries.

1.1.2 Body Composition and Football Players

The body composition of football players is critical to their athletic performance including speed, strength, agility and flexibility. Previous research has shown that various indices of body size and composition correlate with outcomes in football-related performance tests evaluating speed,

strength, and power, even in-game performance. Gains in body mass or height have been linked to increases in playing time and income in the National Football League (NFL).[57] Aside from performance, interest in football athletes' body composition is expanding due to its impact on health.[57,58] Nearly all position groups prioritize the accretion of lean mass, based on the established links between lean body mass, strength, and power development.[58] Lean mass accretion is an even higher priority for athletes in positions that specifically emphasize size and strength, such as linemen, tight ends, and linebackers.[60] Body fat percentage is also an important performance correlate, with evidence showing higher body fat to adversely influence measured of speed and endurance. However, a recent study of college football players showed offensive liners had a relative higher body fat percentage than their opposite positions because they might have less moving distance.[60] This is an example for football players requiring differential body composition for different positions because of different tasks on the field. Nearly none of studies except Bosch et al. used DXA on college football players.[60] Bosch et al. measured 467 football players from four universities giving normative data of body composition for all positions.[60] The current study was designed to test the consistency with Bosch's study.

In addition to performance, interest in body composition of football athletes is growing because of its effect on health. Researchers using body mass index (BMI) as a measure of obesity have suggested that up to 56% of football players are obese.[61] All positions were classified as overweight or obese based on BMI (>25 kg/m^2), yet other than offensive and defensive linemen, all positions had healthy percent body fat (13–20%) and low visceral fat mass (<500 g) in a DXA study.[60] Although the inaccuracy of associating a high BMI with increased risk of mortality has been reported, the link between obesity in football players and cardiovascular risk has been

shown consistently in numerous recent studies.[62,63] Therefore, it is important to identify a valid method to evaluate football players fat tissue. Oliver et al. developed a set of equations to accurately access fat mass for college football players.[64] From a player health perspective, even though all positions had relatively high BMI values, most positions had relatively low body fat and visceral fat, which is important for the health of players during and after their playing career.

1.2 Body Composition Measurements

Because performance is so heavily influenced by body shape and composition, the ability to track these changes in an athlete over time is critical for both coaches and players. Its evaluation is critical in determining the efficacy of a diet or features connected to the athlete's nutritional state. As it mentioned before, there are five levels to describe body composition. To assess body composition, the various methods available are based on two-compartment (2C), three-compartment (3C), four-compartment (4C) or multi-compartment models.[42](fig.1) In clinical and research contexts, the four-compartment molecular model and the three-compartment tissue model are largely used to assess body composition.[36] Once the model has been selected, each parameter should be evaluated using its reference method to obtain maximum accuracy. The following will talk about the measurement of body composition in clinical and laboratory practice.

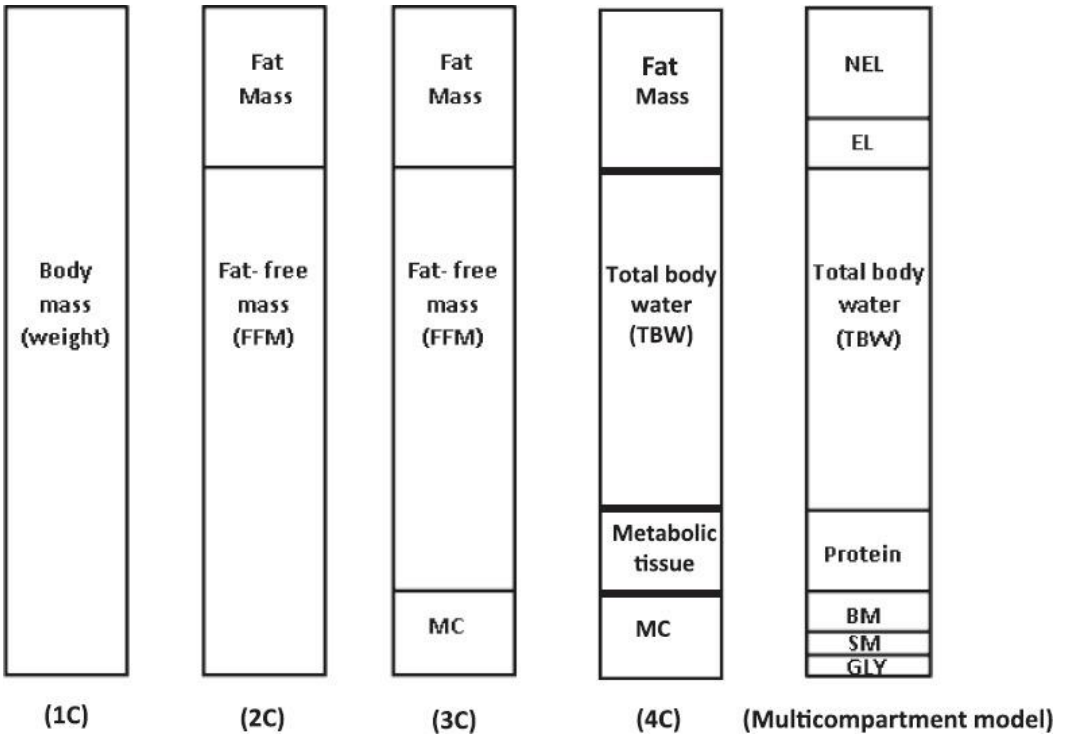

Figure 1: Different types of body composition models. 1C, one-compartment; 2C, two-compartment; 3C, three-compartment; 4C, four-compartment; MC, mineral content; NEL, non-essential lipid; EL, essential lipid; BM, bone mineral; SM, soft-tissue mineral; GLY, glycogen.[42]

1.2.1.1 Double Indirect Methods

Anthropometry: Anthropometric measurements are non-invasive and help in assessing the nutritional status, identifying individuals at risk, monitoring the efficacy of a nutrition intervention and providing information about the body's stores of fat and muscle.[37] Since these are relatively simple to measure, inexpensive and do not require high level of technical skill, anthropometric measurements are used widely in clinical situations and large epidemiological studies.[37] The body mass index (BMI) is widely used to estimate body fat as it is simple and inexpensive. The WHO classification is commonly used to categorize BMI.

Skinfold (SKF): The skinfold (SKF) technique is a measure of subcutaneous fat, by estimating body density (Db) to derive per cent body fat (BF). The commonly used calipers are Holtain, Lange and Harpenden, which measure to the nearest 0.2 mm. Measurements are made at sites such as biceps, triceps, subscapular and suprailiac, which are used in age- and gender-specific equations, to arrive at values of body density.[38] Body fat is obtained from Db using a population specific conversion formula.[37]

Waist circumference and Waist-hip ratio: Waist circumference is utilized as an indicator of intra-abdominal fat in both children and adults. In a standing position during end-tidal expiration, the waist circumference is measured using a non-stretchable tape to the nearest 0.1 cm at the midpoint of the lowest rib cage and the iliac crest.[37]

The waist-hip ratio (WHR) is a proxy measure of lower and upper body fat distribution that measures where fat is stored in the body. Android, or excess upper body fat, is more common in men, whereas gynoid, or excess lower body fat, is more common in women.[37] A high WHR indicates an increased risk of obesity-related health issues.[37]

Bioelectrical impedance analysis (BIA): Bioelectrical impedance analysis technique is used to predict body composition based on the electrical conductive properties of the body and involves measuring the impedance (Z) to the flow of a low-electrical current (800 μA), at a fixed frequency (50 kHz).[40] The principle of BIA is that Lean Tissue (LT), consisting of water and electrolytes, is a good electrical conductor, while fat, which does not have water, is a poor conductor. Hydration factor of 73 per cent is used to predict fat-free mass (FFM) from total body water (TBW).[40] The possible sources of error in BIA are differences in limb length, physical

activity, nutrition status, hydration level, blood chemistry, ovulation, and placement of electrodes.[39] The BIA can offer quick, straightforward, and generally inexpensive estimates of FFM and TBW in healthy populations and obese persons with adequate standardization of procedures, instruments, and individual preparation.[37] The BIA instrument is portable, safe, and simple to use, as well as relatively cheap in cost and participant burden, making it a suitable tool for large studies. However, BIA is population-specific and cannot measure segmental body fat.[41]

1.2.1.2 Indirect Methods

Hydrodensitometry [underwater weighing (UWW)]: When the body is entirely submerged, the approach measures the water displaced by it, and when combined with residual lung volume measurements, it can offer an accurate measure of body volume (BV), from which Db can be determined.[37] A person with a higher percentage of FFM will weigh more in water and have a lower percentage of BF since bone and muscle are denser than water and fat floats.[37] A high percentage of FM makes the body lighter in the water, and the individual has a high %BF.[37] The individual's underwater weight is utilized to calculate weight loss. The UWW method is a valid method for measuring BV and Db and the estimates of %BF from UWW had average errors ranging from −2.8 to 1.8 %BF when compared to 4C method.[43] The UWW method is relatively fast to other lab methods and non-invasive, but it cannot provide regional evaluation and high-cost.[41]

Air displacement plethysmography (ADP): The ADP which is similar in principle to UWW measures the Db and hence total body fat and lean tissue (LT). The subject is sat in an enclosed chamber, and by varying the capacity of the chamber, the volume of displaced air may be

calculated based on the change in air pressure. BV is calculated by subtracting the volume of air in an empty chamber from the volume of air in the chamber after a person has seated in it.[37]

Dual-energy X-ray absorptiometry (DXA): DXA is a two-dimensional imaging technique that uses X-rays with two different energies. The attenuation of an X-ray is dependent on the thickness of the tissue and the tissue's attenuation coefficient, which is dependent on the X-ray energy.[44] By using two different energy levels, the images can be separated into two components (e.g., bone and soft tissue). DXA has been found to be more accurate than density-based methods for estimating total body fat. The DXA is quick, has low radiation exposure and needs little technical skill and preparation by the individual. The images can be split into the components of bone and soft tissue using two different energy levels. While DXA is the gold standard for bone mineral density measurements, it is also used to estimate total and regional body fat and lean tissue mass (LTM).[45]

Computed tomography (CT): CT gives a three-dimensional high-resolution image volume of the complete or selected parts of the body, computed from a large number of X-ray projections of the body from different angles.[45] The known differences in attenuations of X-rays between lean soft tissue and adipose tissue (AT) can then be used to separate these tissues, as well as to determine mixtures between them. As opposed to the previously described techniques, CT can accurately determine fat in skeletal muscle tissue.[46] CT, as a three-dimensional imaging technology, has the capability of providing direct volumetric measurements of organs and AT depots. However, in most situations, CT-based body composition measurement is confined to two-dimensional analysis of one or a limited number of axial slices of the body, resulting in the

use of the area measured as a proxy for the volume.[45] This method, however, has limitations in terms of precision because the precise placements of slices in relation to internal organs cannot be specified in advance and will thus vary across scans.[45]

Magnetic resonance imaging (MRI): Images of soft tissue in the body can be produced by MRI, which uses the different magnetic properties of the nuclei of elements in the cell, usually hydrogen in water and fat.[37] Diffuse fat infiltration in organs and precise regional measurements of AT and LT are estimated using 'quantitative fat water imaging', which is based on Dixon imaging.[47] In this technique, the separation of the signals into water and fat image is made using the magnetic resonance frequencies of protons in fat and water.[37] Since the MRI does not use ionizing radiation, it can be used for three-dimensional volumetric imaging even in neonate and infants. However, due to the limited availability of efficient tools for analyzing three-dimensional image segmentation, body composition using MRI is restricted to one- or two-dimensional slices.[37]

1.2.1.3 Direct Method

Whole-body potassium counter (WBKC): The cellular 4C model partitions the body into fat, body cell mass (BCM), ECF, and extra cellular solids (ECSs). The WBKC is the gold standard to accurately measure the BCM. However, it is highly cost and difficult to use.[37]

1.2.2 DXA in Athletic Population

Athletes and coaches aware that skeletal muscle mass and body fat are related to competitive performance.[48] Skeletal muscle mass, historically measured as FFM and more recently as lean soft tissue mass (LSTM), reflects functional mass and adds to strength and force generation, hence boosting sport performance.[6] Conversely, FM is considered non-functional mass, with increasing amounts of FM mechanically and metabolically hindering sport performance and adversely affecting thermoregulation.[50,51]

Although surface anthropometry protocols remain the primary source of information on body composition in athletes, the increasing availability and popularity of new techniques for physique assessment have allowed sports scientists to consider them as additional tools for use in the everyday monitoring of athletes. According to a review based on the data on PubMed, DXA is considered as the golden standard method to assess whole-body composition, regional fat and FFM.[52] DXA shows its advantages:1) Suitable for most athletes, 2) Fast and repeatable, 3) Able to provide regional body composition, 4) Low radiation dose and 5) Nonintrusive.[53] While, DXA also has disadvantages for using: 1) Expensive equipment; 2) Not portable, 3) Scanning bed is smaller than typical physique of many larger athletes, 4) Trained technician required, 5) Unable to directly compare results between different DXA machines.[53] DXA has shown validity to estimate body composition in general or obese population. However, fewer of these indirect studies of the validity of DXA technology in physique assessment exist in athletic populations.[53,54,55,56] Bilsborough et al. claim DXA is suitable for assessing body composition in lean team sports athletes.[55] For taller individuals who might excess scanning bed, Santos et al.

prove DXA also shows great validity and reliability.[54] For muscular athletes or obese non-athletic individuals, reflection scanning allows DXA body composition assessment. Although this procedure introduces error, it may be minimized through manual adjustment of ROIs and consistency of analysis methods.[84]

This study chooses DXA to measure body composition of male football players because studies mentioned before shown its validity and reliability. The DXA can be the fast, repeatable, safe and precise method for the target population.

1.3 Bone Biomechanics

Bone is a remarkable and exquisite biomaterial. It is highly adaptive, structurally dynamic, and metabolically active, and is superior to all other biomaterials in terms of strength and toughness.[65] In particular, bone structure, size and strength are reliant upon and responsive to the routine physiological and mechanical demands placed upon it.[66] For athletic population, bone physiology is vital for their health and related to performance. Having a thorough view of athletes' bone biomechanics is quite important for researchers, coaches and athletic trainers.

1.3.1 Bone Strength and Adaptation

Bone strength explicitly refers to the ability of bone to withstand force prior to catastrophic failure and is inextricably linked with fatigue resistance to repetitive loads.[65] Given the complex

and multidimensional nature of bone, its strength is ultimately determined by the interaction and adjustment of its material and structural properties evident at macroscopic, microscopic and nanoscopic levels.[65]

The adaptability, modulation, and regulation of bone to mechanical and non-mechanical stimuli provides practitioners with the ability to directly influence and target bone strength through numerous interdependent mechanisms.[12] As all forms of bone adaptation collaboratively determine structural integrity and mechanical competency, it is desirable to optimize and preserve bone strength during growth, development, maturity and advanced age through multi-disciplinary and holistic approaches which importantly address all bone strength determinants.[12] Bone mass accounts for 50 to 70% of bone strength.[10] Bone geometry and composition are important, however, because larger bones are stronger than smaller bones, even with equivalent bone mineral density. As bone diameter expands radially, the strength of bone increases by the radius of the involved bone raised to the fourth power. The amount and proportion of trabecular and cortical bone at a given skeletal site affect bone strength independently. Bone material properties are important for bone strength. The biological basis of bone strength is determined by its structure and function through its anatomy and physiology.

1.3.1.1 Bone Anatomy

At macroscopic view, bone must be rigid and stiff to withstand forces and accommodate loading, yet be flexible and elastic to deform and absorb energy.[12] To manage these contradictory and paradoxical requirements, the skeleton contains two macroscopic osseous tissues (trabecular and cortical bone) which are architecturally and functionally different.[67]

Trabecular bone, also known as cancellous bone, is encapsulated beneath cortical bone. It is most prominently found in weight-bearing skeletal structures, specifically the proximal and distal ends of long-bones (epiphyseal and metaphyseal regions), the carpals and tarsals of the extremities, and vertebrae.[11] The three-dimensional lattice-like structure of trabecular bone is primarily organized in the direction from which the greatest stresses are most commonly experienced, a design best suited for the mechanical loading of bone.[68] The spongy and porous architecture of trabecular bone enables it to store large amounts of energy prior to yielding, thus allowing it to routinely tolerate cyclical low-grade forces.[67]

Cortical bone, also known as compact bone, forms the thin superficial layer of all bones, though is most prominently found in the thick central cortex (diaphysis) of long bones through-out the appendicular skeleton.[11] In long bones, cortical tissue is arranged in a cylindrical fashion with concentric layers across two primary surfaces: the periosteum (a dense fibrous membrane forming the outside layer) and endosteum (a thin membrane forming the inner layer) of the diaphyseal shaft. Both surfaces contain important cells (osteoclasts, osteoblasts and osteocytes) responsible for modeling and remodeling processes essential to bone adaptation and osteogenesis.[70] Structurally, cortical bone is highly organized, densely packed, rigid, and texturally smooth, with mineralized lamellar bone and collagen fiber matrix most prominently arranged in the direction of routine mechanical stress.[69] This provides cortical bone with an increased capability to tolerate sudden, high impact forces i.e. a sample of cortical bone is ~25% stronger than a sample of trabecular bone.[12,65,70]

Bone also has microscopic and sub-microscopic levels which, together with the macroscopic level, form a multidimensional architectural biomaterial with a deliberate mass (size, geometry and density) aimed at achieving optimal structural strength.[12]

1.3.1.2 Bone Remodeling

In particular, the skeleton is able to construct (model) and reconstruct (remodel) itself through cellular processes in response to developmental and mechanical loading demands through tightly controlled cellular activities.[71]

Modeling is the process by which bones change their overall shape in response to physiologic influences or mechanical forces, leading to gradual adjustment of the skeleton to the forces that it encounters.[11] Bones may widen or change axis by removal or addition of bone to the appropriate surfaces by independent action of osteoblasts and osteoclasts in response to biomechanical forces.[11] Bones normally widen with aging in response to periosteal apposition of new bone and endosteal resorption of old bone.[11] Wolff's law describes the observation that long bones change shape to accommodate stresses placed on them. During bone modeling, bone formation and resorption are not tightly coupled.[11]

Bone modeling is less frequent than remodeling in adults.[72] Bone remodeling is the process by which bone is renewed to maintain bone strength and mineral homeostasis.[11] Remodeling involves continuous removal of discrete packets of old bone, replacement of these packets with newly synthesized proteinaceous matrix, and subsequent mineralization of the matrix to form new bone. (Fig.2)[11,73] The remodeling process resorbs old bone and forms new bone to prevent

accumulation of bone microdamage.[11] Remodeling begins before birth and continues until death. The bone remodeling unit is composed of a tightly coupled group of osteoclasts and osteoblasts that sequentially carry out resorption of old bone and formation of new bone.[11] Bone remodeling increases in perimenopausal and early postmenopausal women and then slows with further aging but continues at a faster rate than in premenopausal women. Bone remodeling is thought to increase mildly in aging men.[11]

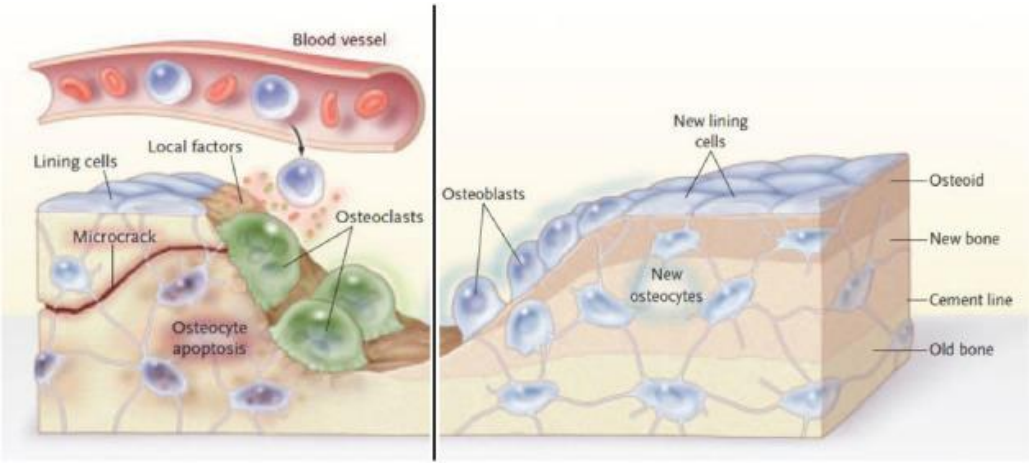

Figure 2: A graphical representation of the remodeling cycle.[73] Bone resorption (left) is stimulated by a micro-crack which severs canaliculi channels between osteocytes leading to osteocytic apoptosis. Lining cells and osteocytes release signals attracting cells from blood and marrow reservoirs into the damaged area leading to osteoclastogenesis. Bone formation (right) commences with successive streams of osteoblastic activity depositing new lamellar bone. Osteoblasts then transform into new lining cells (extra-cellular layer) or osteocytes (embedded in osteoid and bone matrix).

1.3.2 Bone Measurements

The accessibility of bone in-vivo has traditionally made it difficult for scientists to study bone adaptation. With help of modern technology, several devices can detect bone density, structure

and strength non-invasively. (Fig.3)[65] Due to relatively low-cost and availability, DXA and HRpQCT are commonly used for bone measurement.[65]

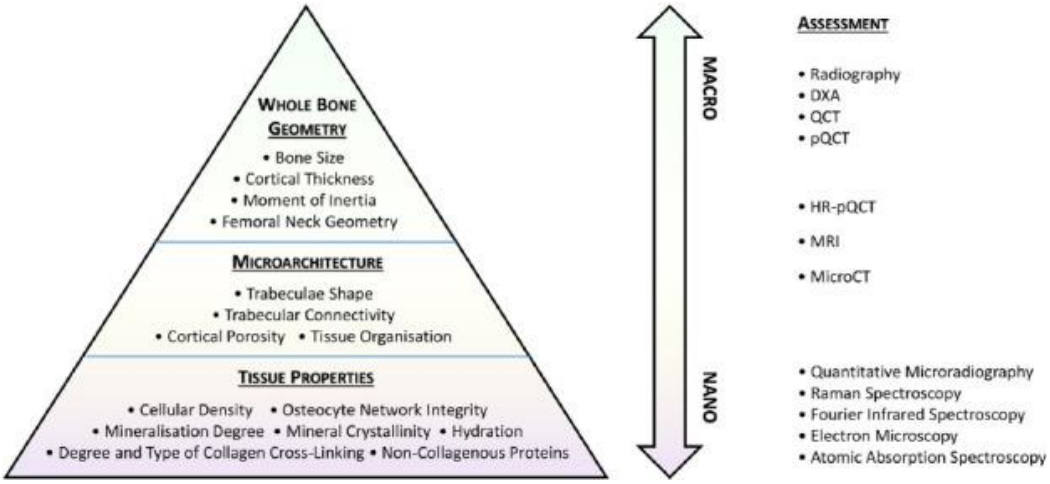

Figure 3: Material and structural determinants of bone strength or fragility (left) with associated technologies required to examine bone properties (right); along the macroscopic, microscopic and nanoscopic continuum [top to bottom].[65]

Dual-energy X-ray absorptiometry (DXA): As mentioned before, DXA uses X-rays with two different energies; the attenuation coefficients and ratios of which differentiate hard tissue from soft tissue, and fat mass from lean mass in an expedient and effective manner.[44] DXA quantifies areal bone mineral density (aBMD) and its derivatives (bone area and bone mineral content) in order to examine bone quality.[74] aBMD T-score from DXA is used for bone health and skeletal fragility diagnoses of bone disorders defined by the World Health Organization from population-based values. However, the bone architecture, size and shape cannot be measured by DXA.[74,75] Specifically, DXA's uniplanar, low-resolution images restrict clinicians to descriptions of whole bone mass, which only partially explains bone strength variation.[75]

Quantitative Computed Tomography (QCT, axial; pQCT, peripheral): QCT is a multi-planar, three-dimensional bone densitometry imaging device which measures the material and structural properties of bone at macroscopic depth, providing clinicians with more accurate descriptions of bone shape, size and quality.[76,79] pQCT devices are able to provide unobstructed circumferential measures of hard- and soft- tissue masses, generating volumetric measures of area, content and density for trabecular bone, cortical bone, marrow, muscle and fat compartments; bone strength indices and fracture loads; periosteal and endosteal size; cortical thickness; and bone mass.[77,78] Bone quality and skeletal fragility examinations using pQCT are superior to those provided by DXA.[77,78] Importantly, applications of mechanical assumptions to quantified material and structural properties across numerous cross-sections allow indices of bone strength to be established, providing better predictive accuracy of fracture risk beyond generic aBMD and vBMD measures.[80,81] Despite the advantageous diagnostic power afforded to clinicians using pQCT, complexity arises as normative and comparative data for general, specific and special populations scarcely exist at present, owing to its emerging status as an alternate imaging device in clinical and research environments.[76] Some forms of pQCT are limited to macroscopic depth, however the emerging use of micro-scanners (HRpQCT) provides higher resolution images that are capable of detecting critically important microarchitectural features including trabecular thickness, connectivity, and number; cortical porosity; volume fraction; and arterial calcification.[80,81]

1.3.3 Bone Characteristics in Athletic Population

Sports training characterized by impacts or weight-bearing activity is well known to induce osteogenic effects on the skeleton. Few studies have linked skeletal strength and skeletal geometry to athletic performance, but some studies have given them description and shown them associated with injury risk.[82,83] Because of characteristics of pQCT, these studies used pQCT to measure bone geometry in athletic population.[82,83] For soccer players, both male and female athletes had greater BMD at all sites , cortical and trabecular area at tibial sites and higher bone strength than their comparison controls.[82] Male soccer players also showed greater bone variables than female players.[82] As it mentioned before, in macroscopic level, bone geometry can be affected by load bearing in sports training and competition in different sports. For artistic gymnastics, gymnastic activity applies impact loads that involve the total body mass, imparting high muscular loads and mass inertia to both upper and lower extremities.[83] Therefore, bone adaptation at both upper and lower extremities are necessary to be considered. Dowthwaite et al claimed that gymnastic loading during growth appears to yield significant enlargement of total and cortical bone geometry (+10 to 30%) and elevation of trabecular density (+20%) in the forearm, yielding elevated indices of skeletal strength (+20 to +50%).[83] Furthermore, bone parameters were more linked with body weight or muscle, but this trend seems to be reflected only in leaner athletes.

There does not seem to be much experimentation linking bone geometry to performance. A study aimed to investigate the relationship between body composition, bone geometry and plantar pressure. They found an association between muscle mass bone density and plantar pressure, but

no significant association between bone geometry and plantar pressure. As of yet, researchers do not have a good way to explore the association between bone geometry and motor performance. For the time being, bone geometry measurements are more often used for screening for injuries and for the study of injury risk factors.

However, bone geometry of football players requires further investigation. Football players have different body composition and specific functional movements due to the different positions on the field. These differences expose their bone under different load and intensity. Adaptations of these differences have not been studied. It is important for sports team to have these notes to be better known about their players. The newest description of football players' bone geometry can provide normative data to be compared with other sports, which might be helpful to indicate how functional movements or specific body composition affect bone geometry to athletic population.

1.4 Problem Statement

Body composition is always a significant factor of athletic performance and related to injury prevention. For football players, there are few research about their body composition by using DXA the "Gold Standard". Due to the variation of positions on football court, how the body composition differs between positions is necessary to measure. Moreover, description of bone architecture for football players remains unknown. There is a necessity to have a description of bone geometry for football population. How football training affect bone geometry is worth to be

investigated. Therefore, coaches, athletic trainers and performance trainers can have a better knowledge and modify players' training program for sake of their bone health.

1.5 Study Purpose

DXA is the golden standard measurement of body composition, and HRpQCT can give a structural view of bone in football players. The purpose of this study is to compare body composition and bone characteristics differences between football players playing different positions on the court, changes of body composition and bone characteristics during January to August, and to get a description of bone data for a college football team.

1.6 Specific Aims

Specific Aim 1: To describe normative values of body composition variables including BMI, Fat Mass, BMD etc. of UPITT football players.

Specific Aim 2: To describe normative values of bone geometry data including vBMD, trabecular/cortical area, trabecular/cortical thickness, stiffness, failure load etc. of UPITT football players.

Specific Aim 3: To describe differences in bone characteristic data (vBMD, trabecular/cortical area, trabecular/cortical thickness, stiffness, failure load etc.) between players play different positions on the field.

Specific Aim 4: To investigate body composition (BMI, Fat Mass, BMD etc.) and bone characteristic variables (vBMD, trabecular/cortical area, trabecular/cortical thickness, stiffness, failure load etc.) changes from January to August in football players.

1.7 Study Significance

With the use of DXA, this study could provide detailed and accurate information of body composition of collegiate football players. Therefore, coaches and trainers can make more specific regulation on players' body composition and injury prevention program. Moreover, having these data can help players to clarify their own position and move forward to break their limitation. Due to football team of University of Pittsburgh winning the ACC championship in 2021, players' body composition data could give other collegiate football team as a reference. Meanwhile, it was the first time to test football players by HRpQCT, and it is vital to investigate how football training affect bone adaptation. This study could give a first glance at bone data of such population. This information could make sports teams better know their athletes and give further recommendation to improve their bone health.

2.0 Methods

2.1 Experimental Design

This study utilized a prospective cohort, within-subject controlled study design. Subjects came to NMRL and went through DXA and HRpQCT scan in January, 2022 and July or August, 2022. The purpose of this study was to compare body composition and bone geometry differences between football players playing different positions on the court, and to get a description of bone data for a college football team.

Variables in this study were body composition characteristics, bone geometry characteristics and positions on football field.

Body composition variables: Weight(kg), BMI (kg/m^2), Region %Fat (%), Lean Mass (kg), Fat Mass (kg), Fat Free Mass(kg); Total BMD (g/cm^2), Trunk BMD(g/cm^2), Leg BMD (g/cm^2), Spine BMD (g/cm^2), Trunk BMD (g/cm^2), Total Z-score; Femur Neck BMD (g/cm^2), Femur BMD (g/cm^2), Femur Neck Z-score and Femur Z-score.

Bone characteristc variables: total vBMD (mg HA/cm3), trabecular vBMD (mg HA/cm3), cortical vBMD (mg HA/cm3), trabecular area (mm2), trabecular bone volume fraction (%), cortical area (mm2), cortical thickness (mm), trabecular thickness (mm), trabecular number (1·mm), trabecular separation (mm), cortical porosity (%), and cortical pore diameter (mm).

Micro–finite element analysis was performed to calculate stiffness (kN/mm) and failure load (kN) under uniaxial compression.

Potential participants would then contact the NMRL to participate. Participants were grouped into positions as follows:

- Line group: offensive and defensive linemen
- Skill group: defensive backs, wide receivers, and running backs
- Combination group: linebackers, tight ends, quarterbacks, and special teams [87,89]

2.2 Subject Recruitment

Athletics medical and performance staff introduced the PI and other study team members to the UPITT Division I Football team on multiple occasions so that all athletes were given the opportunity to participate; athletes were not pre-selected by Athletics personnel. The study team then spoke to the athletics team, informing the potential participants in detail of the research project. The PI and medical/performance staff emphasized emphatically that participation was voluntary. The study team would answer any questions the potential participants may have regarding the proposed project. Potential participants would be given a copy of the informed consent for them to review.

2.2.1 Inclusion Criteria

1. NCAA Division I Athletes enrolled at the University of Pittsburgh
2. Medically eligible to compete
3. Agrees to adhere to study requirements
4. Aged 18 years or older

2.2.2 Exclusion Criteria

Children (under 18) demonstrate significant physiological differences from adults including differences in endocrine values, lean body mass, and bone composition.

2.3 Instruments

2.3.1 DXA

DXA is used to assess body composition and bone mineral density; subjects will be asked to lay still on a table as a series of body images are captured. DXA (GE Healthcare) is the most accurate technology to perform body composition scans and provides both segmented and full body information regarding body composition and bone density. Using the DXA is the most

reliable and valid measurement of BMD and body composition, and the only valid measurement for segmental body composition assessment. DXA is considered as the golden standard method to assess whole-body composition, regional fat and FFM.[52] DXA shows its advantages:1) Suitable for most athletes, 2) Fast and repeatable, 3) Able to provide regional body composition, 4) Low radiation dose and 5) Nonintrusive.[53] Meanwhile, DXA is suitable for assessing body composition in lean team sports athletes.[55] For taller individuals who might excess scanning bed, Santos et al. prove DXA also shows great validity and reliability.[54] For muscular athletes or obese non-athletic individuals, reflection scanning allows DXA body composition assessment.[84]

2.3.2 HRpQCT

HRpQCT is a noninvasive imaging modality for assessing volumetric bone mineral density (vBMD) and microarchitecture of cancellous and cortical bone.[85] Currently, the gold standard for clinical imaging of bone mass is dual-energy X-ray absorptiometry (DXA).[85] DXA-derived areal BMD (aBMD) is a significant predictor of fracture risk; however, its predictive value is limited because (1) the aBMD in most older individuals that experience a fracture is outside the osteoporotic range (T-score < -2.5); (2) it does not differentiate between the cortical and cancellous bone compartment; and (3) additional information on bone microarchitecture—which is an indicator of bone quality and predictor of fracture—cannot be determined.[85] HRpQCT provides higher resolution images that are capable of detecting critically important microarchitectural features including trabecular thickness, connectivity, and number; cortical porosity; volume fraction; and arterial calcification which are more detailed information for bone adaptation for athletic population..

2.4 Procedures

Informed consent would be completed prior to the completion of any research related activities. The initial consent would occur at the Neuromuscular Research Laboratory. One of the listed co-investigators named on the IRB would complete the consent process, which included all testing procedures, directly with the prospective participant. Tests may not be in this following order, but in a “round robin” fashion in order to test as many subjects as quickly as possible.

2.4.1 DXA

The scan was administered with subjects positioned supine in accordance with manufacturer recommendations. Upon lying on the open DXA table, non-invasive measurements of body composition (total body) and bone mineral density (total body, lumbar spine, hip) will be taken. 6 DXA scans were performed across the entire study. Three areas (total body, non-dominant femur, lumbar spine) were scanned during visit 1 (in January 2022) and those same three areas were scanned again during visit 2 (in July and August 2022). Subjects would be asked to take off all metal materials with them, and then laid down in the scan region with a strip holding them ankle and palms towards their body. Subjects with long hair were asked to life their hair up out of scan region. For football players, some of them exceed the DXA scan region. These athletes would be move part of their left body out of the scan region, and their right body was scanned. For tall subjects, their heads were moved out of scan region to keep their feet inside. Then, the device could calculate their whole-body data by reflection scanning.

2.4.2 HRpQCT

This test (Scanco, Switzerland) takes 3 minutes per scan and measures three-dimensional bone microarchitecture and volumetric bone mineral density, in vivo. Subjects will be asked to sit comfortably in a chair, with the non-dominant leg outstretched. Once in place, the leg will be placed into the HRpQCT machine, and non-invasive measurements of the tibia bone (distal 4% and 30% sites) will be taken. 4 HRpQCT scans will be performed across the entire study. The tibia will be scanned at 2 sites during visit 1 (pre-training) and at 2 sites during visit 2 (post-training). Reconstructed images were analyzed according to the manufacturer's standard protocol. The outer periosteal and inner endosteal surfaces of the bone were identified automatically, and segmentations were checked for accuracy and manually modified when needed. μFE analysis (Scanco Medical FE software version 1.13) was used to estimate stiffness ($kN \cdot mm^{-1}$) and failure load (kN) at both the metaphyseal and diaphyseal sites.

2.5 Data Reduction

For DXA, each scan would be checking Region of Interest (ROI) and artifacts and adjusting as needed by an investigator. Scans which could not be analyzed by computer would not be used. Then reports would be exported and checked for outlier values.

For HRpQCT, scans went through auto-contour by computer program. An investigator would manually check contours and run standard evaluation. Due to many tall subjects in football team,

30% tibia site were unable to be measured because of long tibia. Therefore, only 4% tibia data was used in this study. Finite element analysis (FEA) would be run after standard evaluation checked. At last, exported data would be checked for outliers. Due to comparability at 4% tibia, common region less than 80% would not be analyzed.

In order to include more data, position difference analysis would use Visit 1 data.

2.6 Data Analysis

Descriptive statistics (mean, standard deviation, median, interquartile range, proportion, as appropriate) will be calculated for all variables.

Specific aims 1 and 2: These aims are descriptive. Appropriate descriptive statistics will be estimated.

Specific aim 3: Differences in body composition and bone characteristic data between groups will be assessed using one-way ANOVA or Kruskal-Wallis test, as appropriate. Significant omnibus tests were followed by Bonferroni adjusted pair-wise comparison, as necessary.

Hypothesis 3: Line Group subjects might have highest body composition and bone characteristic data due to their big size, while skill group might have the lowest.

Specific aim 4: Body composition and bone characteristics changes from Visit 1 to Visit 2 will be assessed using paired t tests or Wilcoxon signed ranks tests, as appropriate.

Hypothesis 4: Body composition and bone characteristics data might increase from Visit 1 to Visit 2 because of many training programs during this period.

Statistical significance will be set a priori at alpha = 0.05, two-sided. Statistical analysis will be conducted using IBM SPSS Statistics (IBM Corp.; Armonk, NY).

3.0 Results

The purpose of this study was to compare bone characteristics differences between football players playing different positions on the field, changes in body composition and bone characteristics during January to August, and to describe of bone data for a college football team. The following sections display the analyzed results of data collected during the study. Further interpretation of the results and the conclusions they provide are detailed in the following chapter.

3.1 Demographic Information

A total of 96 subjects volunteered to participate in this study. All subjects met the required inclusion and exclusion criteria.

As mentioned before, participants were divided in to 3 groups by different positions on the football field. First is Line Group including offensive and defensive linemen (OL, DL) (N=36). Line position players are all similar in stature and generally stay near the line of scrimmage where strength is a premium with reduced requirements for speed.[87,88] Second is Skill Group including defensive backs, wide receivers, and running backs (DB, WR, RB) (N=30). Skill Group players are traditionally the faster players on the team playing positions that require high-end speed and acceleration as well as cutting, jumping, and quick changes in direction.[87,88] Last

is Combination Group including linebackers, tight ends, quarterbacks, and special teams (LB, TE, QB and SP) (N=26). They are not generally the fastest or the strongest players on the team but use combination of size, strength, and speed.[87,88] There were 4 participants not on the team roaster, so they would not be included in the position analysis.

Demographic data are presented in Table 1 and 2 for Visit 1 and Visit 2. There were 17 participants dropped out in Visit 2.

Table 1: Visit 1 Demographic Information of Participants

	N	Mean	SD	Median
Height (cm)	96	186.57	7.09	187.45
Weight (kg)	96	106.10	23.10	100.38
BMI ($kg \cdot m^{-2}$)	96	30.25	5.08	29.05

Table 2: Visit 2 Demographic Information of Participants

	N	Mean	SD	Median
Height (cm)	79	187.80	6.42	188.47
Weight (kg)	79	107.67	22.39	103.24
BMI ($kg \cdot m^{-2}$)	79	30.33	5.03	29.40

3.2 Description of Body Composition and Bone Characteristics

Descriptive normative data of participants' body composition by DXA in Visit 1 are shown in Table 3.

Table 3: Visit 1 Body Composition by Dual-energy X-ray Absorptiometry of Participants

	N	Mean	SD	Median	25th	75th	Min.	Max.
BMI	96	30.25	5.08	29.05	25.94	34.69	22.23	42.18
Fat Mass (kg)	96	23.18	13.64	18.85	11.24	31.91	6.12	61.84
Lean Mass (kg)	96	78.77	10.00	77.66	69.59	88.63	63.45	101.52
Fat-Free Mass (kg)	96	83.10	10.37	82.05	73.75	93.39	67.14	106.53
Region %Fat	96	20.35%	8.03%	19.55%	12.58%	26.98%	7.80%	38.10%
Total Tissue %Fat	96	21.19%	8.25%	20.45%	13.25%	27.98%	8.30%	39.50%

The average BMI of subjects could be classified as Class I Obese (30 ~ 35) by WHO, while the normal range is (18.5 ~ 24.9). The average Total Tissue %Fat is a little higher than normal range for men aged 20-39 (8% ~ 19%). However, Total Tissue %Fat of subjects vary between individuals. The lowest Total Tissue %Fat is 8.30% while the highest is 39.50%.

Table 4 shows normative total bone data from DXA total body and femur scans in Visit 1.

Table 4: Visit 1 Bone Data by Dual-energy X-ray Absorptiometry of Participants

	N	Mean	SD	Median	25th	75th	Min.	Max.
Total BMD (g/cm^2)	96	1.60	0.11	1.58	1.52	1.67	1.30	1.87
Legs BMD (g/cm^2)	96	1.73	0.13	1.71	1.66	1.81	1.36	2.09
Spine BMD (g/cm^2)	96	1.49	0.13	1.48	1.41	1.58	1.20	1.83
Trunk BMD (g/cm^2)	96	1.43	0.11	1.42	1.35	1.51	1.14	1.73
Total Z-Score	94	2.58	0.82	2.50	2.08	3.03	0.20	4.60
Femur Neck BMD (g/cm^2)	84	1.54	0.17	1.55	1.42	1.65	1.05	2.00
Femur Neck Z-Score	83	2.34	1.16	2.40	1.50	3.10	-0.70	5.30

Table 5 shows 4% tibial bone characteristics by HRpQCT in Visit 1. Variables from HRpQCT were divided into Geometry: total bone area (Tt.Ar), cortical bone area (Ct.Ar), trabecular bone area (Tb.Ar) and cortical perimeter (Ct.Pm); Volumetric BMD (vBMD): total vBMD (Tt.vBMD), cortical vBMD (Ct.vBMD) and trabecular vBMD (Tb.vBMD); Microarchitecture: trabecular bone volume fraction (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N), inhomogeneity of network (Tb.1/N.SD), trabecular separation (Tb.Sp), cortical thickness (Ct.Th) and intra-cortical porosity (Ct.Po).

Table 5: Visit 1 4% Tibial Bone Characteristics by High-resolution Peripheral Quantitative Computed Tomography of Participants

	N	Mean	SD	Median	25th	75th	Min.	Max.
Geometry								
Tt.Ar (mm^2)	74	1437.17	170.81	1416.65	1316.25	1554.33	1066.30	1887.80
Ct.Ar (mm^2)	74	155.99	35.28	149.35	132.10	172.10	92.20	265.10
Tb.Ar (mm^2)	74	1295.84	177.29	1273.35	1169.38	1432.75	924.50	1779.10
Ct.Pm (mm)	74	151.77	10.13	152.25	145.10	159.50	127.20	176.20
Volumetric BMD								
Tt.vBMD ($mg*HA/cm^3$)	74	321.83	34.35	316.70	294.75	341.43	260.70	412.30
Tb.vBMD ($mg*HA/cm^3$)	74	266.25	25.94	264.60	244.75	281.65	223.40	331.30
Ct.vBMD ($mg*HA/cm^3$)	74	785.05	40.22	787.20	761.13	814.53	679.00	867.30
Microarchitecture								
BV/TV	74	0.40	0.04	0.40	0.36	0.42	0.33	0.49
Tb.Th (mm)	74	0.26	0.02	0.26	0.25	0.27	0.23	0.30
Tb.N (1/mm)	74	2.10	0.22	2.06	1.95	2.26	1.58	2.64
Tb.1/N.SD	74	0.17	0.03	0.17	0.15	0.18	0.12	0.27
Tb.Sp (mm)	74	0.43	0.05	0.43	0.39	0.45	0.33	0.59
Ct.Po (%)	74	26.17	1.62	26.00	25.00	27.23	23.10	30.40
Ct.Th (mm)	74	1.23	0.35	1.15	0.98	1.38	0.63	2.60

Normative data of 4% tibial stiffness and failure load from FEA of all subjects in Visit 1 were shown in Table 6.

Table 6: Visit 1 Finite Element Analysis of Participants

	N	Mean	SD	Median	25th	75th	Min.	Max.
Stiffness (kN*mm)	70	423.66	73.63	413.62	373.40	459.17	251.08	653.60
Failure load (kN)	70	-22.45	3.76	-21.83	-24.14	-20.00	-34.77	-14.01

3.3 Differences of Body Composition and Bone Characteristics Between Positions on Football Court

Differences in bone characteristics between groups in Visit 1 were assessed using one-way ANOVA.

3.3.1 Position Difference in Body Composition Measured by DXA

Table 7 presented results from one-way ANOVA test for body composition between groups. Table 8 presented p-value from Bonferroni post hoc test. Variables include BMI, Lean Mass, Fat-Free Mass, Region %Fat and Tissue %Fat.

Table 7: Position Difference in Body Composition, Measured by Dual-energy X-ray Absorptiometry in Visit 1

	Line Group				Skill Group				Combination Group				A Group comparison p-value
	N	Mean	SD	Median	N	Mean	SD	Median	N	Mean	SD	Median	
BMI ($kg \cdot m^{-2}$)	36	35.06	3.60	35.96	31	25.80	2.53	25.65	26	28.83	2.15	28.95	**<0.001**
Fat Mass (kg)	36	35.39	11.31	38.86	31	11.29	4.41	10.13	26	19.82	5.59	18.64	**<0.001**
Lean Mass (kg)	36	87.36	6.91	89.32	31	69.84	4.54	68.91	26	77.26	7.38	76.25	**<0.001**
Fat-Free Mass (kg)	36	92.00	7.14	94.06	31	73.87	4.81	73.11	26	81.49	7.66	80.51	**<0.001**
Region %Fat	36	27.20 %	6.26 %	28.45 %	31	13.07 %	3.93 %	11.90 %	26	19.45 %	4.58 %	19.95 %	**<0.001**
Total Tissue %Fat	36	28.22 %	6.41 %	29.50 %	31	13.72 %	4.07 %	12.50 %	26	20.30 %	4.74 %	20.80 %	**<0.001**

Table 8: Bonferroni-adjusted Pair-wise Comparisons, of Body Composition Measured by Dual-energy X-ray Absorptiometry between Groups

	Line Group vs Skill Group	Line Group vs Combination Group	Skill Group vs Combination Group
	p-value		
BMI ($kg \cdot m^{-2}$)	**<0.001**	**<0.001**	**<0.001**
Fat Mass (kg)	**<0.001**	**<0.001**	**<0.001**
Lean Mass (kg)	**<0.001**	**<0.001**	**<0.001**
Fat-Free Mass (kg)	**<0.001**	**<0.001**	**<0.001**
Region %Fat	**<0.001**	**<0.001**	**<0.001**
Total Tissue %Fat	**<0.001**	**<0.001**	**<0.001**

There was a significant difference in BMI among positions, $F(2, 90) = 88.643$, $p = < 0.001$, $\eta^2 = 0.663$. The participants in Line Group (Mean = 35.06, SD = 3.60) had significantly higher BMI than the participants in Skill Group (Mean = 25.80, SD = 2.53), $p < 0.001$. The participants in Line Group had significantly higher BMI than the participants in Combination Group (Mean =

28.84, SD = 2.15), $p < 0.001$. The participants in Combination Group had significantly higher BMI than the participants in Skill Group, $p < 0.001$.

There was a significant difference in Fat Mass among positions, $F(2, 90) = 77.399$, $p = < 0.001$, $\eta^2 = 0.632$. The participants in Line Group (Mean = 35.39, SD = 11.31kg) had significantly higher Lean Mass than the participants in Skill Group (Mean = 11.29, SD = 4.41kg), $p < 0.001$. The participants in Line Group had significantly higher Lean Mass than the participants in Combination Group (Mean = 19.82, SD = 5.59kg), $p < 0.001$. The participants in Combination Group had significantly higher Lean Mass than the participants in Skill Group, $p < 0.001$.

There was a significant difference in Lean Mass among positions, $F(2, 90) = 63.915$, $p = < 0.001$, $\eta^2 = 0.587$. The participants in Line Group (Mean = 87.36, SD = 6.91kg) had significantly higher Lean Mass than the participants in Skill Group (Mean = 69.84, SD = 4.54kg), $p < 0.001$. The participants in Line Group had significantly higher Lean Mass than the participants in Combination Group (Mean = 77.26, SD = 7.38kg), $p < 0.001$. The participants in Combination Group had significantly higher Lean Mass than the participants in Skill Group, $p < 0.001$.

There was a significant difference in Fat-Free Mass among positions, $F(2, 90) = 63.499$, $p = < 0.001$, $\eta^2 = 0.585$. The participants in Line Group (Mean = 92.00, SD = 7.14kg) had significantly higher Fat-Free Mass than the participants in Skill Group (Mean = 73.87, SD = 4.81kg), $p < 0.001$. The participants in Line Group had significantly higher Fat-Free Mass than the participants in Combination Group (Mean = 81.49, SD = 7.66kg), $p < 0.001$. The participants in

Combination Group had significantly higher Fat-Free Mass than the participants in Skill Group, $p < 0.001$.

There was a significant difference in Region %Fat among positions, $F(2, 90) = 63.889$, $p = < 0.001$, $\eta^2 = 0.587$. The participants in Line Group (Mean = 27.20, SD = 6.26%) had significantly higher Region %Fat than the participants in Skill Group (Mean = 13.07, SD = 3.93%), $p < 0.001$. The participants in Line Group had significantly higher Region %Fat than the participants in Combination Group (Mean = 19.45, SD = 4.58%), $p < 0.001$. The participants in Combination Group had significantly higher Region %Fat than the participants in Skill Group, $p < 0.001$. There was a significant difference in Tissue %Fat among positions, $F(2, 90) = 63.594$, $p = < 0.001$, $\eta^2 = 0.586$. The participants in Line Group (Mean = 28.22, SD = 6.41%) had significantly higher Tissue %Fat than the participants in Skill Group (Mean = 13.72, SD = 4.07%), $p < 0.001$. The participants in Line Group had significantly higher Tissue %Fat than the participants in Combination Group (Mean = 20.30, SD = 4.74%), $p < 0.001$. The participants in Combination Group had significantly higher Tissue %Fat than the participants in Skill Group, $p < 0.001$.

3.3.2 Position Difference in Bone Characteristics Measured by DXA

Table 9 presented one-way ANOVA results of bone data by DXA among groups. Table 10 listed Bonferroni hoc post test p-value for groups. Variables include Total BMD, Legs BMD, Spine BMD, Trunk BMD, Total Z-Score, Femur Neck BMD and Femur Neck Z-Score.

Table 9: Position Differences of Bone Data, Measured by Dual-energy X-ray Absorptiometry in Visit 1

	Line Group				Skill Group				Combination Group				Group comparison p-value
	N	Mean	SD	Median	N	Mean	SD	Median	N	Mean	SD	Median	
Total BMD (g/cm^2)	36	1.64	0.10	1.62	31	1.57	0.10	1.55	26	1.57	0.11	1.55	**0.015**
Legs BMD (g/cm^2)	36	1.78	0.12	1.77	31	1.69	0.10	1.69	26	1.69	0.13	1.67	**0.003**
Spine BMD (g/cm^2)	36	1.55	0.13	1.53	31	1.44	0.11	1.44	26	1.47	0.13	1.48	**0.003**
Trunk BMD (g/cm^2)	36	1.47	0.11	1.45	31	1.40	0.10	1.39	26	1.41	0.13	1.40	**0.020**
Total Z-Score	34	2.74	0.90	2.75	31	2.47	0.76	2.30	26	2.48	0.82	2.50	0.331
Femur Neck BMD (g/cm^2)	30	1.60	0.16	1.58	28	1.53	0.14	1.57	23	1.46	0.20	1.43	**0.014**
Femur Neck Z-Score	29	2.73	1.22	2.90	28	2.25	0.95	2.50	23	1.96	1.27	2.00	0.064

Table 10: Bonferroni-adjusted Pair-wise Comparisons, of Bone Data by Dual-energy X-ray Absorptiometry between Groups

	Line Group vs Skill Group	Line Group vs Combination Group	Skill Group vs Combination Group
	p-value		
Total BMD (g/cm^2)	**0.035**	**0.047**	1.000
Legs BMD (g/cm^2)	**0.009**	**0.013**	1.000
Spine BMD (g/cm^2)	**0.003**	0.076	1.000
Trunk BMD (g/cm^2)	**0.024**	0.145	1.000
Femur Neck BMD (g/cm^2)	0.331	**0.011**	0.453

There was a significant difference in Total BMD among positions, $F(2, 90) = 4.400$, $p = 0.015$, $\eta^2 = 0.089$. The participants in Line Group (Mean = 1.64, SD = 0.10 g/cm^2) had significantly higher Total BMD than the participants in Skill Group (Mean = 1.57, SD = 0.10 g/cm^2), $p = 0.035$. The participants in Line Group had significantly higher Total BMD than the participants in Combination Group (Mean = 1.57, SD = 0.11 g/cm^2), $p < 0.001$. There was no significant difference in Total BMD among participants in Skill Group and Combination Group, $p = 1.000$.

There was a significant difference in Legs BMD among positions, $F(2, 90) = 6.259$, $p = 0.003$, $\eta^2 = 0.122$. The participants in Line Group (Mean = 1.78, SD = 0.12 g/cm^2) had significantly higher Legs BMD than the participants in Skill Group (Mean = 1.69, SD = 0.10 g/cm^2), $p = 0.009$. The participants in Line Group had significantly higher Legs BMD than the participants in Combination Group (Mean = 1.69, SD = 0.13 g/cm^2), $p = 0.013$. There was no significant difference in Legs BMD among participants in Skill Group and Combination Group, $p = 1.000$.

There was a significant difference in Spine BMD among positions, $F(2, 90) = 6.133$, $p = 0.003$, $\eta^2 = 0.120$. The participants in Line Group (Mean = 1.55, SD = 0.13 g/cm^2) had significantly higher Spine BMD than the participants in Skill Group (Mean = 1.44, SD = 0.11 g/cm^2), $p = 0.003$. There was no significant difference in Spine BMD among participants in Line Group and Combination Group, $p = 0.076$. There was no significant difference in Spine BMD among participants in Skill Group and Combination Group, $p = 1.000$.

There was a significant difference in Trunk BMD among positions, $F(2, 90) = 4.098$, $p = 0.020$, $\eta^2 = 0.084$. The participants in Line Group (Mean = 1.47, SD = 0.11 g/cm^2) had significantly

higher Trunk BMD than the participants in Skill Group (Mean = 1.40, SD = 0.10 g/cm^2), p = 0.024. There was no significant difference in Trunk BMD among participants in Line Group and Combination Group, p = 0.145. There was no significant difference in Spine BMD among participants in Skill Group and Combination Group, p = 1.000.

There was a significant difference in Femur Neck BMD among positions, F(2, 78) = 4.548, p = 0.014, η^2 = 0.104. The participants in Skill Group (Mean = 1.53, SD = 0.14 g/cm^2) had significantly higher Femur Neck BMD than the participants in Combination Group (Mean = 1.46, SD = 0.20 g/cm^2), p = 0.011. There was no significant difference in Trunk BMD among participants in Line Group and Skill Group, p = 0.331. There was no significant difference in Spine BMD among participants in Line Group and Combination Group, p = 0.453.

Total Z-Score and Femur Neck Z-Score were not significantly different among positions.

3.3.3 Position Differences in Bone Characteristics Measured by HRpQCT

For bone characteristics data by HRpQCT, variables include Tt.Ar, Ct.Ar, TbAr, Ct.Pm, Tt.vBMD, Ct.vBMD, Tb.vBMD, BV/TV, Tb.Th, Tb.N, Tb.1/N.SD, Tb.Sp, Ct.Po and Ct.Th.

3.3.3.1 Bone Geometry Position Differences

Table 11 presented results from one-way ANOVA test for bone geometry between groups. Table 12 presented p-value from Bonferroni-adjusted pair-wise comparisons.

Table 11: Position Difference in Bone Geometry, Measured by High-resolution Peripheral Quantitative Computed Tomography in Visit 1

	Line Group				Skill Group				Combination Group				Group comparison p-value
	N	Mean	SD	Median	N	Mean	SD	Median	N	Mean	SD	Median	
Geometry													
Tt.Ar (mm^2)	26	1528.29	168.10	1518.50	25	1327.28	125.97	1345.60	21	1461.64	153.71	1528.90	**<0.001**
Ct.Ar (mm^2)	26	172.01	38.94	158.60	25	149.03	28.18	148.20	21	144.41	33.81	133.90	**0.013**
Tb.Ar (mm^2)	26	1364.52	177.49	1330.05	25	1185.74	134.16	1196.80	21	1349.08	164.46	1399.30	**<0.001**
Ct.Pm (mm)	26	157.05	10.35	154.15	25	144.89	7.36	146.60	21	153.74	8.30	154.90	**<0.001**

Table 12: Bonferroni-adjusted Pair-wise Comparisons, of Bone Geometery Measured by High-resolution Peripheral Quantitative Computed Tomography between Groups

	Line Group vs Skill Group	Line Group vs Combination Group	Skill Group vs Combination Group
Geometry			
	p-value		
Tt.Ar (mm^2)	**<0.001**	0.406	**0.011**
Ct.Ar (mm^2)	0.056	**0.022**	1.000
Tb.Ar (mm^2)	**<0.001**	1.000	**0.003**
Ct.Pm (mm)	**<0.001**	0.613	**0.003**

There was a significant difference in Tt.Ar among positions, $F(2, 69) = 11.724$, $p < 0.001$, $\eta^2 = 0.253$. The participants in Line Group (Mean = 1528.29, SD = 168.10 mm^2) had significantly higher Tt.Ar than the participants in Skill Group (Mean = 1327.28, SD = 125.97 mm^2), $p < 0.001$. The participants in Combination Group (Mean = 1461.64, SD = 153.71 mm^2) had significantly higher Tt.Ar than the participants in Skill Group, $p = 0.011$. There was no

significant difference in Tt.Ar among participants in Line Group and Combination Group, $p = 0.406$.

There was a significant difference in Ct.Ar among positions, $F(2, 69) = 4.625$, $p = 0.013$, $\eta^2 = 0.118$. The participants in Line Group (Mean = 172.01, SD = 38.94 mm^2) had significantly higher Ct.Ar than the participants in Combination Group (Mean = 144.41, SD = 33.81 mm^2), $p = 0.022$. There was no significant difference in Ct.Ar among participants in Line Group and Skill Group (Mean = 149.03, SD = 28.18 mm^2), $p = 0.056$. There was no significant difference in Ct.Ar among participants in Skill Group and Combination Group, $p = 1.000$.

There was a significant difference in Tb.Ar among positions, $F(2, 69) = 9.502$, $p < 0.001$, $\eta^2 = 0.216$. The participants in Line Group (Mean = 1364.52, SD = 177.49 mm^2) had significantly higher Tb.Ar than the participants in Skill Group (Mean = 1185.74, SD = 134.16 mm^2), $p < 0.001$. The participants in Combination Group (Mean = 1349.08, SD = 164.46 mm^2) had significantly higher Tb.Ar than the participants in Skill Group, $p = 0.003$. There was no significant difference in Tb.Ar among participants in Line Group and Combination Group, $p = 1.000$.

There was a significant difference in Ct.Pm among positions, $F(2, 69) = 12.827$, $p < 0.001$, $\eta^2 = 0.271$. The participants in Line Group (Mean = 157.05, SD = 10.35 mm) had significantly higher Ct.Pm than the participants in Skill Group (Mean = 144.89, SD = 7.36 mm), $p < 0.001$. The participants in Combination Group (Mean = 1349.83, SD = 160.53 mm^2) had significantly higher

Ct.Pm than the participants in Skill Group, p = 0.001. There was no significant difference in Ct.Pm among participants in Line Group and Combination Group, p = 1.000.

3.3.3.2 Position Differences in Volumetric BMD

Table 13 presented results from one-way ANOVA test for volumetric BMD between groups. There were no significant differences in Tt.vBMD, Ct.vBMD and Tb.vBMD among positions.

Table 13: Position Difference of Volumetric BMD by High-resolution Peripheral Quantitative Computed Tomography in Visit 1

	Line Group				Skill Group				Combination Group				Group comparison p-value
	N	Mean	SD	Median	N	Mean	SD	Median	N	Mean	SD	Median	
Volumetric BMD													
Tt.vBMD (g/cm^2)	26	321.92	34.32	316.40	25	327.15	28.18	328.30	21	315.23	40.93	304.60	0.550
Ct.vBMD (g/cm^2)	26	791.99	42.54	790.10	25	793.07	33.34	787.40	21	767.12	41.50	762.20	0.051
Tb.vBMD (g/cm^2)	26	263.13	27.37	255.55	25	268.97	20.93	271.20	21	267.60	30.10	265.75	0.711

3.3.3.3 Microarchitecture Position Differences

Table 14 presented results from one-way ANOVA test for bone geometry between groups. Table 15 presented p-value from Bonferroni post hoc test.

Table 14: Position Difference of Bone Characteristics by High-resolution Peripheral Quantitative Computed Tomography in Visit 1

	Line Group				Skill Group				Combination Group				Group comparison p-value
	N	Mean	SD	Median	N	Mean	SD	Median	N	Mean	SD	Median	
Microarchitecture													
BV/TV	26	0.392	0.040	0.382	25	0.400	0.032	0.401	21	0.398	0.050	0.399	0.783
Tb.Th (mm)	26	0.261	0.016	0.261	25	0.264	0.015	0.264	21	0.261	0.018	0.257	0.779
Tb.N	26	2.174	0.220	2.159	25	1.984	0.214	1.991	21	2.173	0.164	2.176	**0.002**
Tb.1/N.SD	26	0.162	0.024	0.161	25	0.178	0.031	0.177	21	0.160	0.017	0.160	**0.021**
Tb.Sp (mm)	26	0.417	0.049	0.422	25	0.447	0.058	0.440	21	0.409	0.037	0.407	**0.025**
Ct.Po (mm)	26	0.027	0.016	0.024	25	0.027	0.014	0.023	21	0.027	0.013	0.025	0.984
Ct.Th (mm)	26	1.338	0.416	1.204	25	1.208	0.260	1.261	21	1.135	0.350	1.049	0.132

Table 15: Bonferroni Multiple Comparisons of Microarchitecture by High-resolution Peripheral Quantitative Computed Tomography between Groups

	Line Group vs Skill Group	Line Group vs Combination Group	Skill Group vs Combination Group
Microarchitecture			
	p-value		
Tb.N	**0.004**	1.000	**0.008**
Tb.1/N.SD	0.063	1.000	**0.039**
Tb.Sp (mm)	0.109	1.000	**0.034**

There was a significant difference in Tb.N among positions, $F(2, 69) = 7.101$, $p = 0.002$, $\eta^2 = 0.171$. The participants in Line Group (Mean = 2.174, SD = 0.220) had significantly higher Tb.N than the participants in Skill Group (Mean = 1.984, SD = 0.214), $p = 0.004$. The participants in Combination Group (Mean = 2.173, SD = 0.164) had significantly higher Tb.N than the participants in Skill Group, $p = 0.008$. There was no significant difference in Tb.N among participants in Line Group and Combination Group, $p = 1.000$.

There was a significant difference in Tb.1/N.SD among positions, $F(2, 69) = 4.086$, $p = 0.021$, $\eta^2 = 0.104$. The participants in Skill Group (Mean = 0.178, SD = 0.031) had significantly lower Tb.1/N.SD than the participants in Combination Group (Mean = 0.160, SD = 0.017), $p = 0.039$. There was no significant difference in Tb.1/N.SD among participants in Line Group (Mean = 0.162, SD = 0.024) and Skill Group, $p = 0.063$. There was no significant difference in Tb.1/N.SD among participants in Line Group and Combination Group, $p = 1.000$.

There was a significant difference in Tb.Sp among positions, $F(2, 69) = 3.885$, $p = 0.025$, $\eta^2 = 0.102$. The participants in Skill Group (Mean = 0.447, SD = 0.058) had significantly higher Tb.Sp than the participants in Combination Group (Mean = 0.409, SD = 0.037), $p = 0.024$. There was no significant difference in Tb.Sp among participants in Line Group (Mean = 0.417, SD = 0.049) and Skill Group, $p = 0.109$. There was no significant difference in Tb.Sp among participants in Line Group and Combination Group, $p = 1.000$.

There were no significant differences in BV/TV, Tb.Th, Ct.Po and Ct.Th among positions.

3.3.4 Position Differences of FEA Data in Visit 1

For Bone FEA data, variables include Stiffness and Failure load. Position differences were presented in Table 16.

There was no significant difference in Stiffness and Failure load between groups.

Table 16: Position Differences of FEA Data between Groups

	Line Group				Skill Group				Combination Group				Group comparison p-value
	N	Mean	SD	Median	N	Mean	SD	Median	N	Mean	SD	Median	
Stiffness (kN*mm)	24	440.65	85.16	421.50	24	412.29	59.61	408.92	20	417.88	77.43	420.23	0.389
Failure load (kN)	24	-23.49	4.38	-22.19	24	-21.71	3.06	-21.50	20	-22.13	3.83	-22.45	0.246

3.4 Changes in Body Composition and Bone Characteristics from Visit 1 to 2

Changes in body composition and bone characteristics were assessed using a pre-post comparison of variables measured at participants' Visit 1 and 2.

3.4.1 Changes in Body Composition Measured by DXA

For body composition by DXA, variables include BMI, Fat Mass, Lean Mass, Fat-Free Mass, Region %Fat and Total Tissue %Fat. Changes in these variables are presented in Table 17.

Table 17: Body Composition Differences from Visit 1 to 2 Measured by Dual-energy X-ray Absorptiometry of Participants

		Visit 1			Visit 2			
	N	Mean	SD	Median	Mean	SD	Median	p-value
BMI ($kg \cdot m^{-2}$)	79	30.54	5.15	29.17	30.33	5.03	29.40	0.082
Fat Mass (kg)	79	23.58	13.62	18.40	23.48	13.26	19.28	0.738
Lean Mass (kg)	79	78.98	10.03	79.30	79.86	10.12	80.27	**<0.001**
Fat-Free Mass (kg)	79	83.38	10.35	83.99	84.21	10.49	84.72	**0.001**

Region %Fat	79	20.60%	8.08%	19.50%	20.44%	8.07%	19.60%	0.396
Total Tissue %Fat	79	21.46%	8.30%	20.40%	21.28%	8.30%	20.40%	0.378

Lean mass of participants in Visit 2 (79.86 ± 10.12, 80.27kg) was significantly higher than Lean Mass in Visit 1 (78.98 ± 10.03, 79.30kg, $p < 0.001$). Fat-Free Mass of participants in Visit 2 (79.86 ± 10.12, 80.27kg) was significantly higher than Fat-Free Mass in Visit 1 (78.98 ± 10.03, 79.30kg, $p < 0.001$).

3.4.2 Changes in Bone Data by DXA

For bone data by DXA, variables were presented in Table 18 including Total BMD, Legs BMD, Spine BMD, Trunk BMD, Total Z-score, Femur Neck BMD and Femur Neck Z-score.

Table 18: Bone Data Differences between Visit 1 and 2 Measured by Dual-energy X-ray Absorptiometry of Participants

		Visit 1			Visit 2			
	N	Mean	SD	Median	Mean	SD	Median	p-value
Total BMD (g/cm^2)	79	1.59	0.10	1.58	1.60	0.10	1.58	0.614
Legs BMD (g/cm^2)	79	1.72	0.13	1.71	1.73	0.12	1.72	0.482
Spine BMD (g/cm^2)	79	1.49	0.13	1.48	1.51	0.13	1.49	**0.029**
Trunk BMD (g/cm^2)	79	1.428	0.111	1.409	1.434	0.109	1.422	**0.015**
Total Z-Score	76	2.59	0.81	2.50	2.58	0.83	2.60	0.778
Femur Neck BMD (g/cm^2)	71	1.53	0.18	1.55	1.53	0.19	1.53	0.827
Femur Neck Z-Score	69	2.30	1.22	2.40	2.30	1.32	2.20	0.844

Spine BMD of participants in Visit 2 (1.51 ± 0.13, 1.49g/cm^2) was significantly higher than Spine BMD in Visit 1 (1.49 ± 0.13, 1.48g/cm^2, p = 0.029). Trunk BMD of participants in Visit 2 (1.43 ± 0.11, 1.42g/cm^2) was significantly higher than Trunk BMD in Visit 1 (1.43 ± 0.11, 1.41g/cm^2, p = 0.015).

There were no significant changes for Total BMD, Legs BMD, Total Z-Score, Femur Neck BMD and Femur Neck Z-Score.

3.4.3 Changes of Bone Characteristics at 4% Tibia by HRpQCT

For bone characteristic data by HRpQCT at 4% tibia, variables were presented in Table 19 including Ct.Ar, Tb.Ar, Ct.Pm, Tt.vBMD, Tb.vBMD, Ct.vBMD, BV/TV, Tb.Th, Tb.N, Tb.1/N.SD, Tb.Sp, Ct.Po and Ct.Th. There were 5 participants having less than 80% common region of the second scan at 4% tibia, therefore they were removed in comparison.

Table 19: Bone Characteristics Differences between Visit 1 and 2 by High-resolution Peripheral Quantitative Computed Tomography of Participants

		Visit 1			Visit 2			
	N	Mean	SD	Median	Mean	SD	Median	p-value
Geometry								
Ct.Ar (mm^2)	52	157.85	36.82	149.75	160.63	37.96	151.10	**0.003**
Tb.Ar (mm^2)	52	1283.20	180.65	1242.20	1280.36	181.28	1239.15	**<0.001***
Ct.Pm (mm)	52	151.30	10.31	151.65	151.09	10.48	151.60	0.301
Volumetric BMD								

Tt.vBMD (mg*HA/cm^3)	52	323.65	33.57	318.70	326.97	33.62	322.40	**<0.001**
Tb.vBMD (mg*HA/cm^3)	52	267.59	26.21	264.90	269.83	25.82	267.50	**<0.001***
Ct.vBMD (mg*HA/cm^3)	52	782.98	38.78	784.20	786.32	41.43	780.50	**0.034**
Microarchitecture								
BV/TV	52	0.399	0.039	0.396	0.402	0.039	0.399	**<0.001**
Tb.Th (mm)	52	0.262	0.016	0.262	0.265	0.016	0.263	**<0.001**
Tb.N (1/mm)	52	2.099	0.229	2.083	2.137	0.252	2.155	**0.039**
Tb.1/N.SD	52	0.167	0.025	0.166	0.165	0.028	0.160	0.117
Tb.Sp (mm)	52	0.427	0.052	0.425	0.420	0.057	0.413	**0.047***
Ct.Po (%)	52	2.81	1.56	2.25	2.79	1.66	2.50	0.893
Ct.Th (mm)	52	1.26	0.37	1.16	1.28	0.37	1.21	**0.013***

*Nonparametric test

For Geometry aspect, Ct.Ar of participants in Visit 2 (160.63 ± 37.96, 151.10mm^2) was significantly higher than Ct.Ar in Visit 1 (157.85 ± 10.31, 149.75mm^2, p = 0.003). Tb.Ar of participants in Visit 2 (1283.36 ± 181.28, 1239.15mm^2) was significantly higher than Tb.Ar in Visit 1 (1283.20 ± 180.65, 1242.20mm^2, p < 0.001).

For vBMD aspect, Tt.vBMD of participants in Visit 2 (326.97 ± 33.62, 322.40 mg*HA/cm^3) was significantly higher than Tt.vBMD in Visit 1 (323.65 ± 33.57, 318.70 mg*HA/cm^3, p < 0.001). Tb.vBMD of participants in Visit 2 (267.59 ± 26.21, 264.90mg*HA/cm^3) was significantly higher than Tb.vBMD in Visit 1 (268.83 ± 25.82, 267.50mg*HA/cm^3, p < 0.001).

For microarchitecture aspect, BV/TV of participants in Visit 2 (0.402 ± 0.039, 0.399) was significantly higher than BV/TV in Visit 1 (0.399 ± 0.039, 0.396, p < 0.001). Tb.Th of participants in Visit 2 (0.265 ± 0.016, 0.263mm, p < 0.001) was significantly higher than Tb.Th

in Visit 1 (0.262 ± 0.039, 0.262mm, $p < 0.001$). Tb.N of participants in Visit 2 (2.137 ± 0.252, 2.155 1/mm) was significantly higher than Tb.N in Visit 1 (2.099 ± 0.229, 2.083 1/mm, $p < 0.001$). Tb.Sp of participants in Visit 2 (0.420 ± 0.057, 0.413mm) were significantly higher than Tb.Sp in Visit 1 (0.427 ± 0.052, 0.425mm, $p < 0.001$). Ct.Th of participants in Visit 2 (1.28 ± 0.37, 1.21mm) were significantly higher than Ct.Th in Visit 1 (1.26 ± 0.37, 1.16mm, $p = 0.013$).

There was no significant changes for Ct.Pm, Tb.1/N.SD and Ct.Po.

3.4.4 Changes of FEA Data at 4% Tibia

For FEA data at 4% tibia, variables were presented in Table 20 including Stiffness and Failure Load. There were no significant changes for Stiffness and Failure Load.

Table 20: Finite Element Analysis Difference between Visit 1 and 2 of Participats

		Visit 1			Visit 2			
	N	Mean	SD	Median	Mean	SD	Median	p-value
Stiffness (kN)	55	422.82	75.00	410.01	421.62	76.93	409.00	0.727
Failure Load (kN)	55	-22.38	3.85	-21.77	-22.34	3.97	-21.69	0.818

4.0 Discussion

Body composition and bone characteristics play an important role on football players' health and performance. Previous studies presented comparison of body composition and some bone data of similar positions and body composition adaptations to training programs or over a certain period of training.[4,15,17,19,49,60] However, there are limited studies looking into bone geometry and bone microarchitecture. The BoBCAT study recruited different sports teams of the University of Pittsburgh to see how specific sports exercise affect bone adaptation. It provided us a chance to have a first view of bone characteristics of colligate football players. Although there were limitations the purpose of this study was to provide normative ranges of body composition and bone characteristics data of a colligate football team and investigate position differences and changes between two visits. The results gave us ranges of body composition and bone characteristics. Moreover, the results revealed position differences of bone data. Skill group was found to be the most unique group compared to other two groups. For changes between two visits, specific area BMD, bone geometry and some of bone microarchitecture did change.

4.1.1 Provide Normative Ranges of Body Composition and Bone Characteristics Data of a Division-1 Colligative Football Team

The results from this study show normative ranges of body composition and characteristics of a D1 champion colligative football team which could give other teams a referential standard for their players.

Football players usually have higher BMI. Results revealed participants in this study had a mean BMI (30.25 ± 5.08 kg/m^2) which can be classified as Class I obese (30.0-34.9 kg/m^2). It is consistent with other studies.[2,4,60] Total Tissue %Fat (21.19 ± 8.25%) was a little higher than previous report.[89] It might be caused by not giving them a positional description. In a previous study, offensive and defensive linemen usually have much higher %body fat, which could increase the mean total tissue %fat of the whole team.[60,89] However, lean mass is not considered in BMI, which can mistakenly categorize many football players who have relatively low %body fat as obese.

4.1.2 Position Differences of Body Composition and Bone Characteristics

For position differences in body composition, Line Group subjects had the highest variables like both fat mass and lean mass followed by Combination Group subjects. Subjects in Skill Group had the lowest variables. Results are similar to the study by Bosch et. al., according to standard BMI classifications, the linemen position group would be classified as severely obese (BMI >35 kg·m^{-2}), the LB/TE/RB position group would be classified as moderately obese (BMI, 30–34.9 kg·m^{-2}), and the WR/DB position group would be classified as overweight (BMI, 25–29.9 kg·m^{-2}).[99] Unlike the BMI classifications, only the linemen are classified as obese (>24%) using standard percent body fat classifications. The other 2 position groups would be classified as acceptable (15–20%) or healthy (11–14%).[99] However in this study, Combination Group players would be classified as overweight as well. Only Line Group players would be classified as obese.

As what was hypothesized, Line Group had the highest BMD totally and regionally. In adolescence, Free-Fat Mass and grip strength were revealed positively associated with BMD both in boys and girls.[100] Therefore, Line Group players had higher BMD than other two groups of players. It is consistent with their larger body size and weight than other two groups which had been described by Bosch et al.[60] Athletes in the Skill and Combination group have similar BMD data.

However, when it comes to bone geometry, the Skill group has the smallest Tt.Ar, Tb.Ar and Ct.Pm among 3 groups, while Ct.Ar has no significant difference among groups. Previous study revealed link between bone cross-sectional properties and body composition.[90] People with larger lean mass could have larger bone cross-sectional properties such as Ct.Ar.[90] Due to position difference, football players have different on-court movement and functional movements in training. Skill Group players have relativly more running tasks, while Line Group Players have more pushing or contact movements. In a study by Ward et al., running backs and wide receivers had the most running distance in training while defense and offense linemen had less high-speed running distance but a higher amount of nonrunning activities.[98] Skill group players have many sprinting or changing direction movements which requires their bone to be stronger in those sudden speed-changing movements. As it mentioned before, cortical bone is stronger than trabecular bone and it can increase bone's capability to tolerate sudden high-impact forces.[12] Therefore, although the Skill group players have smallest Tt.Ar and Tb.Ar, they have similar Ct.Ar to other groups.

In microarchitecture comparison, the Skill group had the lowest Tb.N but highest Tb.Sp and Tb.1/N.SD. Due to limited research about body composition and body size, these differences might relate to body mass of players. A study by Sode et al. presented regional trabecular distribution related to age and sex.[95] However, most studies focus on bone architecture with various diseases, because trabecular bone also takes account for part of bone strength.[12] Future research is needed to investigate reasons for differences of bone microarchitecture among athletes. Since there was no significance difference between bone strength and failure load, and z-score of these players (2.58 ± 0.82) is much greater than 1, clinicians might not be concerned about their bone health or risk for bone stress injury. However, there is lack of research to discover relations between these number to plays' performance.

4.1.3 Changes of Body Composition and Bone Characteristics between 2 Visits

It was hypothesized that components of body composition like lean mass and bone characteristics data would increase between the two visits.

The results showed lean mass and fat free mass in Visit 2 were significantly larger than those in Visit 1, which is consistent with a previous study measuring data during a similar period.[49,91] In a study by Trexler et al., football players' Lean mass increased from May to pre-season. Spine and trunk BMD increased in Visit 2 which is similar to a previous study's results as well. BMD increased incrementally over time in the Trexler et al. study.[91] In another study, high intensity functional training with a minimum twice a week for 16-weeks was revealed could improve BMC, and women were favored to have this improvement than men.[96]

For bone geometry at the 4% tibia site, this study presented increases in Ct.Ar and Tb.Ar from Visit 1 and Visit 2. For volumetric BMD, Tt.vBMD, Ct.vBMD and Tb.BMD all increased significantly. For bone microarchitecture, BV/TV, Tb.Th, Tb.N, Tb.Sp and Ct.Th increased significantly from Visit 1 to Visit 2. Due to this study being the first to utilize HRpQCT with football players, it is difficult to explain these changes. This was found similar to gymnasts after training in BoBCAT study. Most application of HRpQCT with an athletic population has studies females athletes, especially female adolescent and amenorrhea athletes.[92,93] However, in a study by Best et al., runners showed greater bone mineral density and some higher trabecular bone variables than nonrunners, and demonstrated a difference in trabecular bone of the calcaneus between forefoot strikers and rearfoot strikers.[94] Therefore, the accumulation of physical exercise and different functional movements can cause difference and changes in bone characteristics, which as it was showed in this study of football players. Another study using pQCT to detect bone adaptation under increased training volume of soccer players.[97] Increased density of trabecular and cortical compartments and cortical thickening were shown following an increased volume of training for adolescent subjects.[97] In O'Leary et. al. study, researchers tracked macrostructure and microstructure tibial bone adaptation in young female warfighters receiving long period military training.[101] They measured 4% and 30% tibial site by HRpQCT in 1, 14, 28 and 44 week. They found temporal decrease in trabecular area in 1 to 14 week, and increase in trabecular bone volume fraction, cortical area and cortical thickness at 4% tibia site in 1 to 44 week.[101] Fail Load increased in 1 to 44 week.[101] Results of their study are similar to data from football players. Decrease in trabecular bone area could be a sign for bone remodeling.

However, bone stiffness and failure load had no significant change from Visit 1 to Visit 2. This data can provide insight to how long or what training program can induce increase in bone stiffness and failure load.

4.2 Limitation and Future Study Directions

The first limitation of this study is that the data collection was done in a vague period time. Visit 1 data was collected in January 2022 when the football season was just finished, and Visit 2 data was collected in July and August 2022 in which the pre-season practice had already begun. Commonly, a complete season is break down into off-season, pre-season, in-season and post-season. However, Visit 1 and 2 included off-season and pre-season. It was difficult to determine when these changes happened. It is more reasonable to collect these data in a particular period listed above. Another limitation is that there was only the University of Pittsburgh football participating in this study. There were not enough players to be divided into more specific position group. All the players available were grouped into a Line, Skill and Combination Group, unlike in the study by Bosch et al. study, where there were over 400 players from 4 D1 university football teams which was a large enough sample to be more specific divided.[60] One more limitation is that the study did not analyze data changes of Visit 1 and 2 by positions but as a whole team. It will be more practical for not only coaches, trainers and physicians but also sports scientists to know how bone data change by position over a period of time. Another issue is that DXA and HRpQCT did not well fit football players' body size. Football players playing some specific positions are too tall and wide for these devices. Therefore, some data had to be

excluded and investigators had to use some methods to make up unmeasurable data. For example, in this study, HRpQCT scans at 30% tibia site were excluded, because some tall individuals have too long tibias to fit in HRpQCT device, while they are an important part in the football team.

This study is a first glance at football players' bone characteristics by HRpQCT. More studies are needed to be done on planning to collect certain period of bone data in a season to see how their bone characteristics change and explain the reason why some particular variables change. Moreover, how trabecular bone and cortical bone being formed, resorbed or transformed during training remains unknown. This could indicate physiology changes relating to some injuries or sports performance which are significant for sports teams to monitor players' health. However, this needs more study to be done to know what each bone characteristic variable presents for. Limited research elaborated clearly how these variables affect bone physiology condition not to mention sports performance. Future research should investigate a larger sample of football players from different competition levels to have a thorough view of bone physiology of football players. When a series of normative ranges for body composition and bone characteristics being investigated, it will be efficiency to assess a player's physical abilities to determine if he can play in a certain competition level.

4.3 Conclusion

This study is a first application of HRpQCT on NCAA Division I football players, some of whom might go on to play in professionally. This study provides researchers a new direction to have more various population scanned by HRpQCT. Current HRpQCT research is more focused on some metabolism diseases, elder population and athletes with high risk of stress fractures. For football players, this study did present a normative data of a collegiate football team. Moreover, there were significant differences among different position groups, and bone architecture variables did have significant changes during off-season and preseason period. These differences and changes could not be clearly explained because of limited information about how bone variables are related to athletic population with different body composition. However, this study could still guide future research in bone characteristics of athletic population and how bone characteristics relate to sports performance.

Appendix A Participants and Position

Subject ID	Position	Subject ID	Position	Subject ID	Position
94	LB	126	QB	158	P
95	TE	127	QB	159	LS
96	DL	128	DL	160	QB
97	DL	129	DL	161	DL
98	DL	130	OL	162	*
99	DL	131	OL	163	DL
100	LB	132	OL	164	WR
101	OL	133	*	165	LS
102	DL	134	WR	166	WR
103	TE	135	DB	167	WR
104	OL	136	DB	168	OL
105	OL	137	OL	169	PK/P
106	OL	138	DB	170	LB
107	OL	139	TE	171	P
108	RB	140	WR	172	DB
109	RB	141	WR	173	WR
110	LB	142	TE	174	WR
111	LB	143	TE	175	WR
112	LB	144	LB	176	DB
113	LB	145	LB	177	WR
114	LB	146	DB	178	DL
115	LB	147	*	179	DL
116	DB	148	OL	180	DL
117	DL	149	DL	181	PK
118	DB	150	DB	182	DB
119	DL	151	DB	183	DB
120	DB	152	DL	184	OL
121	DL	153	RB	185	OL
122	DB	154	LB	186	OL
123	DB	155	RB	187	OL
124	DB	156	OL	188	DL
125	QB	157	PK	189	WR

*Subjects not on 2022 roaster

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