J Musculoskelet Neuronal Interact 2020; 20(1):62-76

Journal of Musculoskeletal
and Neuronal Interactions

Original Article

Sex differences in bone density, geometry, and bone strength of competitive soccer players

Breanne S. Baker, Zhaojing Chen, Rebecca D. Larson, Michael G. Bemben, Debra A. Bemben

Department of Health and Exercise Science, University of Oklahoma, Norman, USA

Abstract

Objectives: To examine sex differences in bone characteristics in competitive soccer players. **Methods**: 43 soccer players (male, n=23; female, n=20), and 43 matched controls (males, n=23; females, n=20), completed the study. Areal BMD (aBMD) of the total body, lumbar spine, and dual femur and tibiae volumetric BMD (vBMD), bone geometry, and bone strength variables (pQCT) were measured. Bone-specific physical activity and training history were assessed. **Results**: Male soccer players had significantly greater ($p \leq 0.05$) total body and hip aBMD, hip strength indices and 4% and 38% tibia variables than females. Regression analyses determined that BFLBM, not sex, was the strongest predictor of bone variables. Female soccer players exhibited significantly greater percent differences from controls for tibiae variables than males ($p \leq 0.05$). Soccer players had greater aBMD and hip strength indices than controls ($p \leq 0.040$). Soccer-specific asymmetries were found for 38% total area (2.1%) and pSSI (3.8%), favoring the non-dominant leg (both $p \leq 0.017$). **Conclusion**: Bone characteristics adjusted for body size were greater in male versus female soccer players. However, body composition variables were more important predictors of bone characteristics than sex. There were no sex differences in the magnitude of limb asymmetries, suggesting skeletal responsiveness to mechanical loading was similar in males and females.

Keywords: Volumetric BMD, Bone Quality, Competitive Soccer, Limb Asymmetry

Introduction

Peak bone mineral density (BMD), achieved typically by the end of the second decade, is an important determinant of osteoporosis risk later in life[1]. Many factors influence the early development of bone geometry and the accumulation of bone mass, including genetics, endocrine function, nutrition, body size, and mechanical forces[1,2]. Physical activity (PA) is a critical source of mechanical loading for the skeleton, but not all types of exercise exert osteogenic effects. Based on animal studies, dynamic, high magnitude, and odd impact loading exercises provide the maximal stimulus for bone formation[3,4]. These concepts are supported by human athlete studies documenting greater areal BMD (aBMD) measured by dual energy x-ray absorptiometry (DXA) in high impact (e.g., volleyball, hurdling, jump sports) and odd impact (e.g., soccer, racket sports) athletes compared to low impact (endurance running) athletes[5-7].

Sex differences exist in osteoporotic fracture rates, aBMD, bone geometry, and bone strength, with males having more favorable values than females[8]. Potential causes of these sex differences include body size and composition, hormonal mechanisms, and responsiveness to mechanical loading. Sex-specific bone adaptations to mechanical stimuli are discussed in the literature[9-11]; these sex-dependent responses may be linked to the effects of sex steroid receptor signaling on the bone response to loading[12-14]. Typically, the female skeleton is reported to be less responsive to mechanical loading than the male skeleton based on rodent models documenting greater improvements in BMC, bone area, and fracture load in males compared to females[15,16]. Human data on sex differences in bone responses to exercise are sparse. In a cross-sectional study, Kriemler et al.[10] found that boys in the highest physical activity tertile had significantly greater total hip BMC than the girls, suggesting a sex difference in bone sensitivity to

---

The authors have no conflict of interest. This study was funded in part by the University of Oklahoma Graduate College.

Corresponding author: Debra A. Bemben, PhD ORCHID iD 0000-0003-4397-553X, Professor and Director, Bone Density Research Laboratory, Department of Health and Exercise Science University of Oklahoma, 1401 Asp Avenue, Norman OK, USA 73019
E-mail: dbemben@ou.edu

Edited by: G. Lyritis
Accepted 17 July 2019

loading. In contrast, another cross-sectional study of tibia bone characteristics found no interaction between sex, maturity level, and loading history[17]. The assessment of limb asymmetries by peripheral quantitative computed tomography (pQCT) is used to examine skeletal adaptations to sports participation; thus, it may be a useful model to shed light on sex differences in bone responses to loading. Ireland et al.[18] reported significant sex differences in the magnitude of bone asymmetries between the racket and non-racket arms in adolescent tennis players. Males tennis players had greater percent differences in bone traits between arms than females suggesting that males may be more responsive to loading. It is currently unclear if males and females develop similar skeletal asymmetries in response to lower body sports activities.

Soccer is an odd impact loading sport that has potential to induce positive skeletal adaptations resulting from the dynamic, multidirectional loads placed on the body[19-21]. Previous studies have reported greater total body, lumbar spine, and femoral neck (FN) aBMD[20-22] and hip bone strength indices[19] in soccer players compared to controls. Anliker et al.[23] reported lower limb skeletal asymmetries in young male soccer players (12-18 years), which may be a result of performing side specific movements such as jumping, passing, and shooting. However, Fousekis et al.,[24] reported the magnitude of lower limb muscular strength asymmetries decreased as players gained more years of experience when competing at the professional level, which may be the result of more skilled players being more ambidextrous.

Since no studies to date have compared sex differences in bone characteristics of competitive soccer players, we examined whether males and females have similar bone adaptations to loading associated with soccer participation. We also compared hip and leg asymmetries in male and female soccer players to gain insight into sex differences in loading adaptations. The primary purpose of this study was to compare sex differences in bone density, bone geometry, bone strength indices, and limb asymmetries in competitive collegiate soccer players. The bone characteristics of the soccer players also were compared to their sex, age, and body mass matched controls. It was hypothesized that male soccer players would have greater aBMD and vBMD compared to female soccer players, and these players would also have greater aBMD and vBMD compared their respective matched control groups. Additionally, we hypothesized that male soccer players would exhibit greater lower limb asymmetries than female soccer players.

Methods

Participants

In total, 104 participants were enrolled in the study; however, only 86 participants were used in the analysis. Twenty-three male and 20 female competitive soccer players were matched with sex, age (±2 yr), and body mass (±2.2 kg) controls. All female soccer players were currently competing at the National Collegiate Athletic Association (NCAA) Division I level while male soccer players were currently competing on NCAA Division II or club teams. Soccer player inclusion criteria were: 1. 18-30 years old; 2. practicing and/or playing soccer at least four hours per week for a minimum of two months prior to the study; 3. be part of a competitive soccer program for at least five years, with no more than six months off. Exclusion criteria were: 1. if they had been diagnosed with a recent leg injury/stress fracture; 2. had a history of musculoskeletal diseases; 3. current smokers; 4. had lower body metal implants; 5. taking medications that affect BMD (e.g., corticosteroids, testosterone); and 6. had participated in competitive swimming, cycling, or rowing in the past ten years. Controls were included in the study if they: 1. matched a soccer player for sex, age (±2 yr), and weight (±2.2 kg); and 2. were not participating in regular exercise more than 3 times per week. The control exclusion criteria were the same as those for the soccer players. Additionally, females were excluded if they were pregnant or had amenorrhea, defined as having no menses for more than three consecutive months in the past year not due to contraceptive use. Enrolled participants were excluded for the following reasons: voluntary termination (n=8), controls that were too physically active (n=5), soccer players who reported too much time off from practice and/or competition (n=3), amenorrhea (n=1), and metal implants (n=1). Participants gave written informed consent prior to beginning the study. All procedures were approved by the Institutional Review Board at the University of Oklahoma Health Sciences Center.

Research design

This cross-sectional study required two visits. Participants completed the informed consent and multiple questionnaires at the first visit. During the second visit, participants underwent anthropometric measures (height, body mass, tibiae lengths), DXA scans (total body, lumbar spine, dual proximal femur) and pQCT scans (4%, 38%, 66% dual tibiae).

A power analysis using G*Power (version 3.1.9.2) was conducted to estimate sample size for soccer player versus control comparisons. Effect sizes for male soccer players ranged from 0.91 (spine) to 1.72 (femoral neck)[25] therefore, sample size ranged from 6-16 for 80% power for aBMD variables. Effect sizes for pQCT variables ranged from 0.35 for total vBMD to 1.42 for cortical vBMD[26], requiring sample sizes between 5 and 53 for 80% power. Our sample size of 86 was adequate for 80% power for the primary bone variables.

Questionnaires

All participants completed the following questionnaires during the first visit: health status, training logs, bone-specific physical activity (BPAQ)[27] and calcium intake[28]. The BPAQ was administered to estimate past, current and total levels of physical activity. Also, females completed an in house menstrual history questionnaire to obtain information on menstrual cycle characteristics such as past and current hormonal contraceptive use, age at menarche, and symptoms of menstrual cycle disturbances.

Dual energy X-ray absorptiometry (DXA)

DXA (GE Lunar Prodigy, enCORE software, version 13.31.016, GE Healthcare, Madison, WI) was used to measure total body composition and aBMD. Measures of total fat mass (FM) (g), % body fat, bone free lean body mass (BFLBM) (g), and bone mineral content (BMC) (g) were obtained from the whole body scan. aBMD was measured using specific scans of the total body, lumbar spine (L1-L4), and dual proximal femur (total hip, femoral neck, trochanter). For the total body scan, participants were asked to lie in the supine position, centered within the scan field. The hands were placed on the sides of the legs in the prone position, while the legs were straight and strapped together. Participants remained centered and placed their legs on a foam block so the lumbar spine was completely flat for L1-L4 scans. Lastly, for both proximal femur scans the feet were strapped to an angled brace to create internal rotation of the femur. Hip Structural Analysis (HSA) uses the proximal femur scans to measure both the aBMD of the hip and the structural geometry of the cross-sections traversing the proximal femur allowing for the determination of the hip strength index, buckling ratio, cross-sectional moment of inertia (CSMI, mm^4), and section modulus (mm^3)[29]. Short term precision (RMS CV%) for FM, BFLBM, and % body fat are 2.74%, 1.39%, and 2.5%, respectively. *In vivo* precision is 0.6% for total body aBMD, 0.9% for L1-L4 aBMD, and 0.4-0.8% for the proximal femur sites. The same two qualified and trained technicians performed all quality assurance tests, scans and analysis for each DXA and pQCT measurement.

Peripheral quantitative computed tomography (pQCT)

A pQCT scanner (XCT 3000, Software v.6.00, Stratec Medizintechnik GmbH, Pforzheim, Germany) was used to measure tibiae vBMD and bone geometry characteristics. Tibia lengths were measured from the medial malleolus to the tibial plateau. Leg dominance was defined as the participant's self-reported preferred kicking leg. Participants were seated with their leg supported horizontally and centered in the gantry. Tibia scans were obtained at 4%, 38%, and 66% of tibia length proximal to the reference line. A voxel size of 0.4 mm was used for all sites at the scout view speed of 40 mm/sec and CT speed of 20 mm/sec. At the distal tibia (4%), contour mode 3 at 169 mg/cm^3 and peel mode 4 at 650 mg/cm^3 with a 10% peel were used to determine total vBMD (mg/cm^3), total bone area (mm^2), trabecular vBMD (mg/cm^3), trabecular area (mm^2), periosteal circumference (mm) (Peri C), endosteal circumference (mm) (Endo C), and bone strength index (mg/mm^4) (BSI). For 38% and 66% tibia, cort mode 2 at 710 mg/cm^3 was used to define total vBMD (mg/cm^3), total bone area (mm^2), cortical density (mg/cm^3), cortical area (mm^2), cortical thickness (mm), Peri C (mm), while cort mode 2 at 480 mg/cm^3 was used to obtain torsional polar strength for strength-strain index (pSSI) (mm^3) and resistance to torsional deformation polar moment of inertia (Ipolar) (mm^4). Muscle cross-sectional area (mm^2) (MCSA) was also calculated for the 66% tibia site. The RMS CV% for the pQCT bone measurements ranged from 0.31-1.21% for all sites. All scans were visually rated as a two or below and the average pMovement was 45.3 mm^2. Scans with pMovement values less than 50 mm^2 are considered to be scans with little to no movement as described by Blew et al.[30].

Statistical analysis

All statistical procedures were performed using IBM SPSS (v24, Armonk, New York), and significance was set at $p \leq 0.05$. Data were tested for normality using the Kolmogorov-Smirnov test and reported as means ± standard deviation (SD) unless otherwise stated. Sex differences in dependent variables within soccer players were analyzed by two approaches. First, potential covariates were identified by: 1. comparing sex differences in body size and body composition, BPAQ scores, training characteristics, and calcium intakes using independent t-tests; and 2. using these variables as independent variables in univariate regression analysis to determine the significant predictors of bone outcomes. There were significant sex differences in height, body mass, body composition, calcium intake, and current BPAQ. In the univariate regression, height, body mass, BFLBM, and regional lean mass were significant predictors for the majority of bone variables. The primary sex difference analysis for bone variables was performed by ANCOVA adjusting for body mass and height. This was followed by stepwise regression analyses to determine the best predictors of bone variables using sex, BFLBM, FM, calcium intake, and current BPAQ scores as independent variables. Pearson correlation coefficients were used to determine relationships between training variables and bone strength qualities in soccer players. Relationships between muscle size (MCSA) and bone variables at the 66% tibia site for soccer players also were determined using Pearson correlation coefficients.

Sex differences in bone variable asymmetries within soccer players were analyzed by two-way repeated measures ANCOVA (sex × side) adjusted for body mass and height for proximal femur, HSA, and pQCT dependent variables. The second approach was to calculate a symmetry index variable for the pQCT variables using the following formula: Symmetry Index (SI) % = (Non-Dominant (support) leg – Dominant (kicking) leg / [(Non-Dominant + Dominant)/2]) × 100[31]. Two-way ANCOVA (sex × group) was used to examine sex and group differences in SI adjusted for body mass and height. Stepwise regression analyses to determine the best predictors of SI variables using sex, BFLBM, FM, calcium intake, and current BPAQ scores as independent variables.

Independent t-tests were used to compare soccer players to their respective control group for physical characteristics, physical activity, calcium intake, body composition, and bone characteristics. Percent differences

Table 1. Participant characteristics (unadjusted means ± SD).

	Males		Females	
	Soccer (n=23)	**Control (n=23)**	**Soccer (n=20)**	**Control (n=20)**
Age (yrs)	20.8±2.3	21.9±1.9	20.5±1.5	19.9±1.2
Height (cm)	175.4±7.0 ††	176.1±7.3	167.5±6.2	166.6±5.3
Body Mass (kg)	74.2±10.6 ††	73.6±12.2	65.8±6.9	64.9±1.4
Total Fat Mass (kg)	11.4±1.2 ††	14.6±1.4	17.1±5.2 **	22.1±1.2
BFLBM (kg)	59.7±1.5 ††	56.0±1.6	44.2±3.9 **	37.2±0.9
% Body Fat	15.0±5.5 *††	19.0±6.7	26.3±5.6 **	35.7±5.7
Arm BFLBM (kg)	7.2±1.3 ††	6.9±1.3	4.9±0.5 **	4.2±0.6
Leg BFLBM (kg)	20.7±2.9 *††	19.0±2.6	15.3±2.8 **	13.1±1.4
Calcium Intake (mg/day)	1651.1±770.6 ††	1347.9±824.0	856.3±272.7 **	616.6±226.4
Total BPAQ	45.2±23.9 *	30.0±19.5	53.6±15.6 **	29.4±25.6
Current BPAQ	21.5±7.6 **†	5.5±5.0	16.2±5.5 **	4.4±12.0
Past BPAQ	69.0±47.8	54.5±37.6	91.1±29.8 **	54.5±47.3
Soccer Participation (yrs)	14.0±3.7		15.6±1.6	
Soccer Training (hrs/wk)	12.4±3.6		13.1±6.4	
Weight Lifting (months)	6.3±4.3 †		9.3±4.5	

*$p \leq 0.05$ significant vs. control group, **$p \leq 0.01$ significant vs. control group. †$p \leq 0.05$ significant vs. female soccer players, ††$p \leq 0.01$ significant vs. female soccer players. BFLBM: Bone Free Lean Body Mass. BPAQ: Bone-specific Physical Activity Questionnaire.

Table 2. Areal BMD variables (unadjusted means ± SD).

	Males		Females	
	Soccer (n=23)	**Control (n=23)**	**Soccer (n=20)**	**Control (n=20)**
Total Body aBMD (g/cm²)	1.373±0.100 **††	1.286±0.089	1.235±0.111 **	1.104±0.072
L1-L4 aBMD (g/cm²)	1.338±0.142 *	1.236±0.147	1.272±0.114 *	1.203±0.084
Dominant Hip aBMD (g/cm²)				
Total Hip	1.390±0.171 **†	1.150±0.123	1.214±0.140 **	1.041±0.097
Femoral Neck	1.365±0.192 **	1.169±0.141	1.212±0.126 **	1.060±0.099
Trochanter	1.172±0.140 **††	0.929±0.107	0.989±0.123**	0.814±0.095
Non-Dominant Hip aBMD (g/cm²)				
Total Hip	1.395 ±0.167 **††	1.184±0.148	1.200±0.128 **	0.986±0.227
Femoral Neck	1.376±0.182 **†	1.185±0.170	1.199±0.116 **	1.055±0.095
Trochanter	1.176±0.146 **††	0.969±0.137	0.971±0.130 **	0.818±0.108

*$p \leq 0.05$ significant vs. control group; **$p \leq 0.004$ significant vs. control group; †$p \leq 0.05$ significant vs. female soccer players adjusted for height, and body mass; ††$p \leq 0.01$ significant vs. female soccer players adjusted for height, and body mass. aBMD: areal Bone Mineral Density (g/cm²). L1-L4: Lumbar Spine 1-4.

and the 95% confidence intervals (95% CI) for dominant tibia bone variables were calculated by subtracting the control group mean (referent mean) from the value for each soccer athlete. The magnitude of mean percent differences for each soccer group was assessed by one sample t-tests with a population mean of 0. When the 95% CI did not include 0 (control group mean), the between group percent difference was statistically significant. Independent t-tests were used for sex comparisons of the magnitude of the percent difference variables.

Results

Participants

Participant characteristics are shown in Table 1. Twelve (60%) female soccer players and 13 (65%) female controls reported current use of hormonal contraceptives and all females were eumenorrheic. Within soccer players, significant sex differences were observed as males were taller, heavier, had lower fat mass, % body fat, and greater total, arm, and leg BFLBM than females (all $p \leq 0.004$). There were significant

Table 3. Hip structural analysis variables (unadjusted means ± SD).

	Males		Females	
	Soccer (n=23)	**Control (n=23)**	**Soccer (n=20)**	**Control (n=20)**
Dominant Hip				
Strength Index	2.1 ± 0.6 **††	1.7 ± 0.4	1.7 ± 0.2 **	1.4 ± 0.3
Buckling Ratio	2.8 ± 0.9 †	3.6 ± 1.6	2.4 ± 0.7	2.5 ± 0.9
Section Modulus (mm^3)	1111.0 ± 185.5 **††	894.4 ± 153.4	741.6 ± 138.3 **	610.6 ± 94.6
CSMI (mm^4)	19109 ± 4101 *††	16046 ± 4501	11048 ± 2765 **	8927 ± 1727
Non-Dominant Hip				
Strength Index	2.1 ± 0.4 **††	1.7 ± 0.4	1.7 ± 0.2 *	1.5 ± 0.3
Buckling Ratio	2.8 ± 1.8 ††	3.5 ± 1.6	2.3 ± 0.7	2.5 ± 0.9
Section Modulus (mm^3)	1118.6 ± 185.5 *††	959.4 ± 244.2	725.5 ± 122.1**	618.4 ± 94.9
CSMI (mm^4)	19243 ± 2391 ††	16898 ± 5695	10759 ± 2390 *	9050 ± 1543

*p≤0.05 significant vs. control group; **p≤0.006 significant vs. control group; †p≤0.05 significant vs. female soccer players adjusted for height, and body mass; ††p≤0.001 significant vs. female soccer players adjusted for height, and body mass; CSMI: Cross-sectional Moment of Inertia.

Table 4. Stepwise regression models for aBMD and HSA variables in soccer players (n=43).

Dependent Variable	Independent Variables	β	SEE	R^2	p
L1-L4 aBMD	BFLBM	0.866	3.0^{-6}	0.335	0.0003
	Sex	-0.440	0.056		
Femoral Neck aBMD (D)	BFLBM	0.573	2.0^{-6}	0.328	6.0^{-5}
Trochanter aBMD (D)	BFLBM	0.667	2.0^{-6}	0.445	1.0^{-6}
Total Hip aBMD (D)	BFLBM	0.608	2.0^{-6}	0.369	2.0^{-5}
Total Body aBMD	BFLBM	0.843	1.0^{-6}	0.662	3.8^{-10}
	FM	0.203	2.0^{-6}		
Strength Index	FM	-0.369	6.0^{-6}	0.280	1.0^{-6}
	Current BPAQ	0.304	0.004		
Buckling Ratio	Sex	0.581	0.395	0.140	0.002
	BFLBM	-0.335	1.8^{-4}		
Section Modulus	BFLBM	0.786	0.001	0.715	2.2^{-23}
	Current BPAQ	0.178	1.339		
CSMI	BFLBM	0.855	0.028	0.732	1.0^{-25}

Predictors used: Sex, BFLBM, FM, calcium intake and Current BPAQ. D: Dominant hip sites, BFLBM: Bone Free Lean Body Mass, FM: Fat Mass, BPAQ: Bone-Specific Physical Activity Questionnaire, CSMI: Cross-sectional Moment of Inertia.

sex and group differences for calcium intake, which were higher in male versus female soccer players (p<0.001), and in female soccer players compared to their controls (p=0.004). Male soccer players had significantly greater current BPAQ scores (p=0.012) than female soccer players. Soccer training characteristics were similar for males and females, however, female soccer players reported weight lifting more months (p=0.028) in the previous year than male soccer players.

Age, height, and body mass were not significantly different between soccer players and their respective control groups (all p≥0.079). Female soccer players had significantly lower fat mass, % body fat, and greater total body, arm, and leg BFLBM compared to controls (all p≤0.004). Male soccer players had significantly lower % body fat, and greater leg BFLBM versus controls (all p≤0.043). Total, past, and current BPAQ scores were significantly greater (all p≤0.006) in female soccer players compared to controls, but only total and current BPAQ scores were significantly greater (both p≤0.023) in male soccer players versus controls. There were no significant differences in BPAQ scores between male and female controls.

Table 5. Dominant tibia bone variables in male and female soccer players.

	Males (n=23)	Females (n=20)
4% Tibia		
Total vBMD (g/cm^3)	388.6±34.5 ††	342.9±37.4
Total BMC (g)	457.7±49.4 ††	397.8±53.7
Total Area (mm^2)	1176.7±109.8	1158.7±119.4
Trab vBMD (g/cm^3)	327.7±30.7 ††	290.6±33.1
Trab BMC (g)	321.3±43.2 ††	276.5±47.0
Trab Area (mm^2)	977.1±107.9	946.5±117.0
Peri C (mm)	121.2±5.7	120.3±6.2
38% Tibia		
Total vBMD (g/cm^3)	942.9±70.5	953.8±56.1
Total BMC (g)	414.9±33.1	395.1±36.0
Total Area (mm^2)	441.4±36.0 †	415.5±39.3
Cort vBMD (g/cm^3)	1160.8±20.1	1169.3±22.1
Cort BMC (g)	400.7±33.1	381.5±36.0
Cort Area (mm^2)	345.6±30.2	326.3±33.1
Cort Thickness (mm)	6.4±0.5	6.2±0.5
Peri C (mm)	74.3±2.9 †	72.1±3.4
66% Tibia		
Total vBMD (g/cm^3)	757.4±71.0	753.2±77.2
Total BMC (g)	468.1±42.7	442.9±46.0
Total Area (mm^2)	620.0±59.0	591.6±63.8
Cort vBMD (g/cm^3)	1126.7±19.2	1138.3±21.1
Cort BMC (g)	426.9±43.6	407.4±47.5
Cort Area (mm^2)	379.3±38.8	357.8±42.2
Cort Thickness (mm)	5.3±0.5	5.1±1.0
Peri C (mm)	88.0±4.3	86.0±4.3
MCSA (mm^2)	8154.1±1070.4	7658.4±1160.6

†p≤0.05, ††p≤0.01 significant vs. female soccer group (means ± SD adjusted for height and body mass); vBMD: Volumetric Bone Mineral Density, BMC: Bone Mineral Content, Trab: Trabecular, Peri C: Periosteal Circumference, Cort: Cortical, MCSA: Muscle Cross Sectional Area.

Areal BMD

Table 2 shows the DXA bone variables. Body mass and height adjusted sex comparisons within soccer players showed that males had significantly greater (all p<0.05) aBMD than females at all sites except for lumbar spine and dominant femoral neck (Table 2). HSA variables (hip strength index, section modulus, buckling ratio, CSMI) were significantly greater in male versus female soccer players for both hips (all p≤0.001) (Table 3). Table 4 shows the significant regression models (all p≤0.002) for aBMD and HSA dependent variables using sex, BFLBM, FM, calcium intake, and current BPAQ as independent variables performed within soccer players. Sex was a significant predictor for lumbar spine aBMD (p=0.046) and buckling ratio (p=0.001). BFLBM was a significant predictor for all aBMD sites (all p≤0.0002), buckling ratio (p=0.049), section modulus (p≤0.0001) and CSMI (p≤ 0.0001). Current BPAQ was a significant predictor for section modulus (p=0.002) and hip strength index (p=0.004). Fat mass predicted total body aBMD (p=0.039) and hip strength index (p=0.0002).

Both male and female soccer players had significantly greater aBMD at the total body (both p≤0.004), lumbar spine (both p≤0.037), and dual proximal femur sites (all p≤0.001) compared to their respective control groups (Table 2). The dominant (all p≤0.006) and non-dominant (all p≤0.05) hip strength index, section modulus, CSMI variables were significantly greater in female soccer players compared to female controls (Table 3). Similarly, male soccer players had significantly greater (all p≤0.02) hip strength index, section modulus, and CSMI than controls for the dominant hip, and greater hip strength index (p=0.001) and section modulus (p=0.017) than controls for the non-dominant hip. Buckling ratio was not different from controls for either soccer group.

pQCT variables

Table 5 shows the 4%, 38%, and 66% dominant tibia bone variables for male and female soccer players adjusted for height and body mass; unadjusted sex, group, and limb data

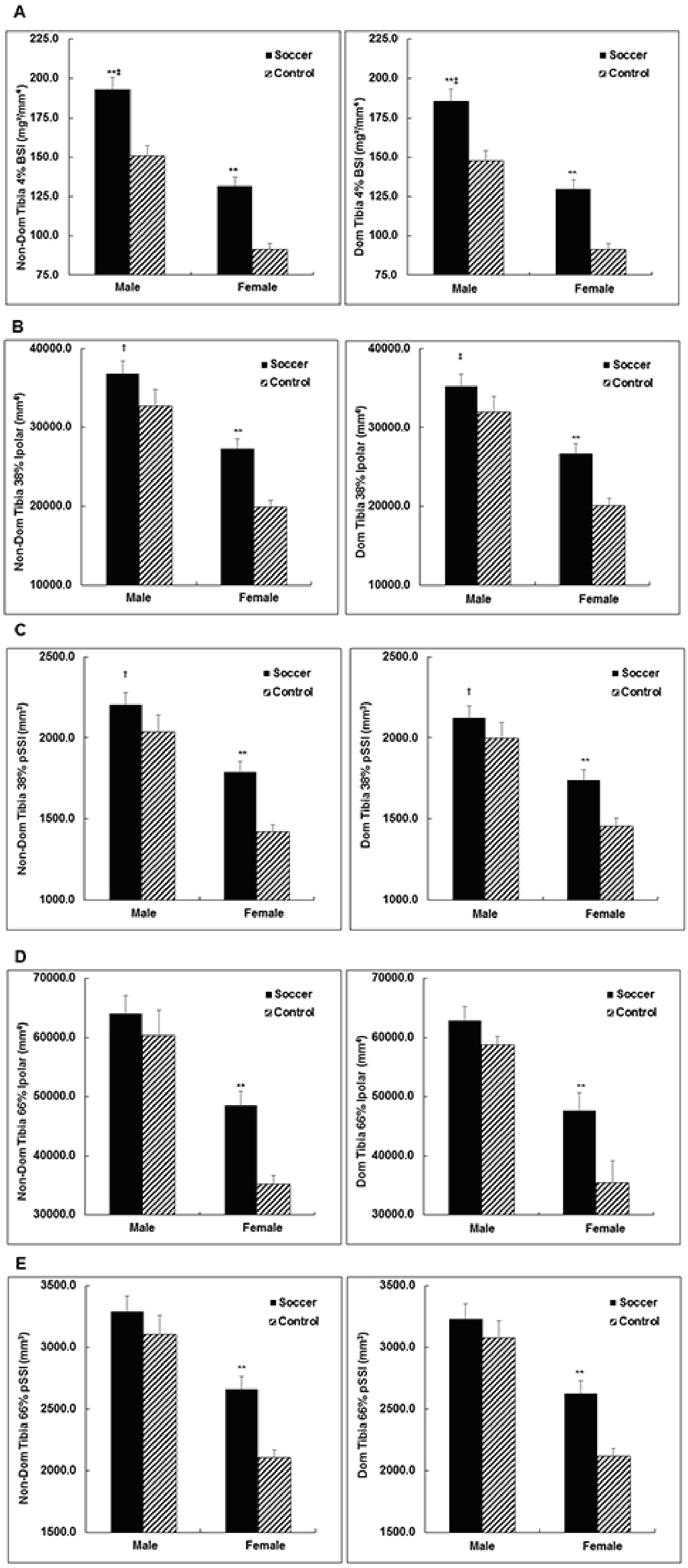

Figure 1. pQCT Strength Indices for 4% (Panel A), 38% (Panels B, C), and 66% (Panel D, E) Tibia Sites (unadjusted means ± SE). $^{*}p \leq 0.05$ significant versus control group, $^{**}p \leq 0.01$ significant versus control group. $^{\dagger}p \leq 0.05$ significant versus female soccer group (adjusted for height, body mass), $^{\ddagger}p \leq 0.01$ significant versus female soccer group (adjusted for height, mass), BSI: Bone Strength Index, Ipolar: Polar Moment of Inertia, pSSI: Strength-Strain Index.

Table 6. Stepwise regression models for pQCT variables in soccer players (n=43).

Dependent Variable	Independent Variables	β	SEE	R^2	p
4% Total vBMD (D)	Sex	0.505	10.21	0.255	0.001
4% Trabecular vBMD (D)	Sex	0.563	8.64	0.317	8.5^{-5}
4% Trabecular BSI (D)	BFLBM	0.946	0.001	0.355	1.5^{-4}
	Sex	-0.579	10.13		
38% Cortical pSSI (ND)	BFLBM	0.689	0.001	0.797	1.3^{-14}
	Sex	0.241	17.92		
66% Cortical vBMD (D)	Sex	-0.374	5.49	0.140	0.014
MCSA (ND)	BFLBM	1.113	0.027	0.608	7.1^{-9}
	Sex	-0.490	517.84		

Predictors used: Sex, BFLBM, FM, Calcium Intake and Current BPAQ. D: Dominant Tibia, ND: Non-dominant Tibia, BFLBM: Bone Free Lean Body Mass, FM: Fat Mass, BPAQ: Bone-Specific Physical Activity Questionnaire, pSSI: Strength-Strain Index, MCSA: Muscle cross-sectional area.

are shown in Supplementary Table 1. Male soccer players had significantly greater total and trabecular BMC and vBMD than female soccer players (all p≤0.003) for the 4% dominant and non-dominant tibia sites. Males also had larger total bone area and Peri C than females for both dominant (all p≤0.042) and non-dominant (all p≤0.020) 38% tibia sites, and greater total and cortical BMC (both p≤0.043) than females for the non-dominant 38% site. No sex differences in bone traits or MCSA were found at the 66% dominant tibia. Males had greater total BMC and cortical area (both p≤0.037) than females for the non-dominant 66% tibia site. Sex, group, and limb comparisons of bone strength variables (BSI, Ipolar, pSSI) for each site are depicted in Figure 1. Male soccer players had greater bone strength indices at the 4% (total BSI, p≤0.0001, Figure 1 Panel A) and 38% (Ipolar and pSSI, p<0.038, Figure 1 Panels B, C) tibia sites than female soccer players. There were no sex differences in Ipolar, or pSSI at the 66% sites (Figure 1 Panel D, E). In female soccer players, the average weekly time spent practicing and competing was positively correlated with 4% BSI, 38% Ipolar and pSSI, and 66% iPolar and pSSI in both limbs (r=0.482-0.619, p=0.031-0.004). However, in male soccer players, a significant negative correlation was found for average weekly time spent practicing and competing and the non-dominant 4% BSI (r=–0.419, p=0.047).

Stepwise regression analyses was performed for pQCT dependent variables using sex, BFLBM, FM, calcium intake, and current BPAQ as independent variables. Table 6 shows the significant regression models (all p≤0.014) for 4% total vBMD, 4% trabecular vBMD, 4% trabecular BSI, 38% cortical BSI, 66% cortical vBMD and MCSA that included sex as a significant predictor. In addition to sex, BFLBM was a significant predictor for 4% trabecular BSI, 38% cortical BSI, and MCSA (all p≤0.001). The following is a summary of the findings for regression models not shown. BFLBM was consistently a significant predictor for the dominant 4% tibia site (7/9 variables, β=0.752 to 0.951), in contrast to FM, which was included in the model for 2/9 variables (β=0.225 to 0.230) (model R^2 ranged from 0.525 to 0.719, all p≤0.001 for 9 dependent variables). For the dominant 38% tibia site, both BFLBM (10/12 variables, β=0.433 to 0.886) and FM (8/12 variables, β=0.235 to 0.366) were significant predictors of bone variables (model R^2 ranged from 0.361 to 0.781, all p≤0.004 for all 12 dependent variables). Similarly, both BFLBM (10/12 variables, β=0.408 to 0.864) and FM (9/12 variables, β=0.246 to 0.400) were included in regression models for most of the dominant 66% tibia site variables (model R^2 ranged from 0.167 to 0.726, all p≤0.007 for all 12 dependent variables). In all these regression models, BFLBM was entered first into the model, and had larger regression coefficients than FM. The regression coefficients were positive for both BFLBM and FM indicating their influence on the dependent variables was in the same direction. Current BPAQ was not a significant predictor for any tibia bone variable at any site, and calcium intake was a significant predictor only for 38% cortical vBMD (p=0.013, β=–0.375, R^2 =0.141). The regression models were similar for the non-dominant tibia sites.

Female soccer players had significantly greater values (all p<0.05) than controls for all 4% dominant and non-dominant tibia variables, except trabecular area (Supplementary Table 1). For the 38% and 66% tibia sites, female soccer players had significantly greater total BMC, total area, cortical BMC, Peri C, cortical thickness (all p≤0.05) and bone strength (Ipolar, pSSI) (all p≤0.01), but lower cortical vBMD (both p≤0.01) than controls. Total vBMD was significantly greater (p=0.035) in female soccer players than controls for the 38% dominant tibia. MCSA was significantly greater in female soccer players for both legs (both p=0.001).

Male soccer players had significantly greater total BMC, total vBMD, trabecular vBMD, and BSI for the 4% dominant (all p<0.05) and non-dominant (all p≤0.008) tibia sites than controls (Supplementary Table 1). Few group differences were found for the remaining pQCT variables; male soccer

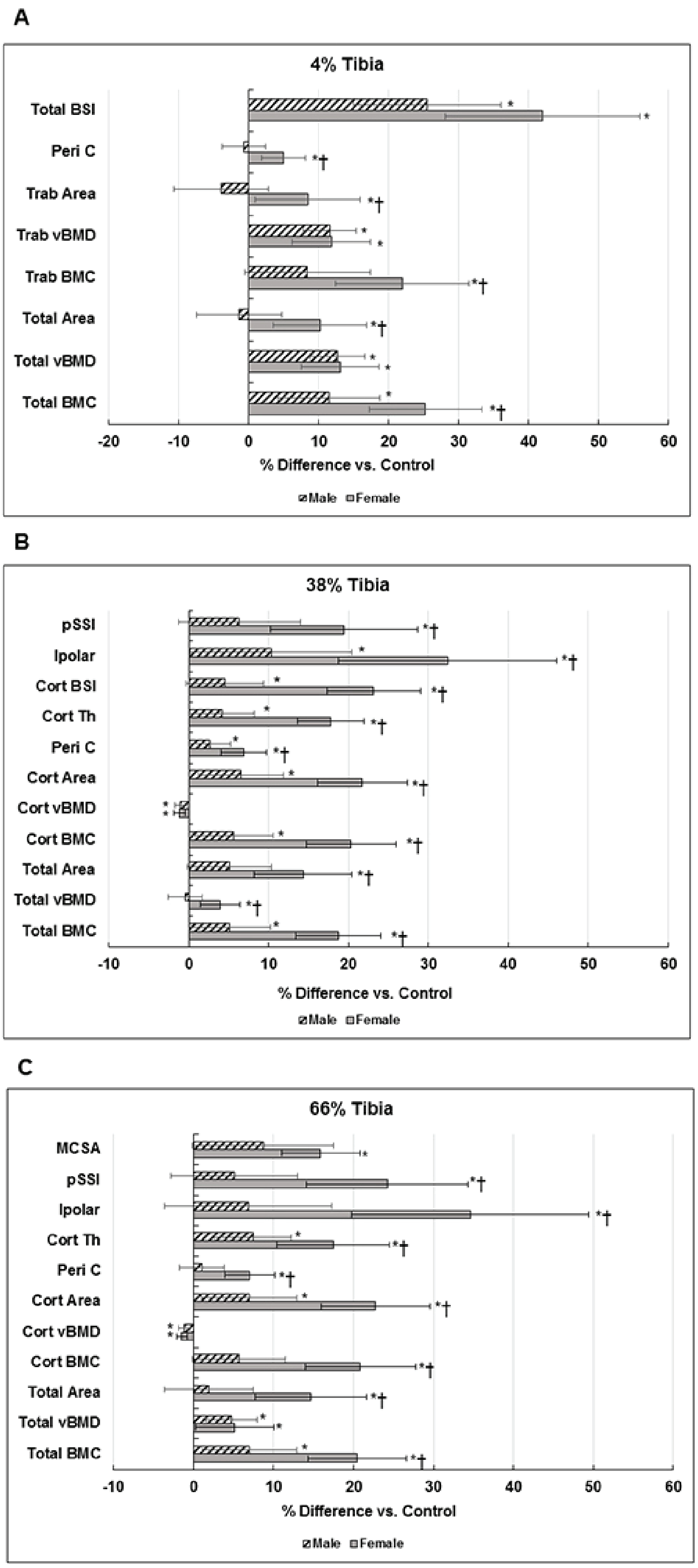

Figure 2. Percent Differences between Soccer and Control Groups for 4% (Panel A), 38% (Panel B), and 66% (Panel C) Dominant Tibia Bone Characteristics (unadjusted means ± 95% CI). 0 represents the control group mean; *p≤0.05 significantly different from 0; †p≤0.05 significantly different between males and females, vBMD: Volumetric Bone Mineral Density, BMC: Bone Mineral Content, Trab: Trabecular, Peri C: Periosteal Circumference, Cort: Cortical, Th: Thickness, BSI: Bone Strength Index, Ipolar: Polar Moment of Inertia, pSSI: Strength-Strain Index, MSCA: Muscle Cross-sectional Area.

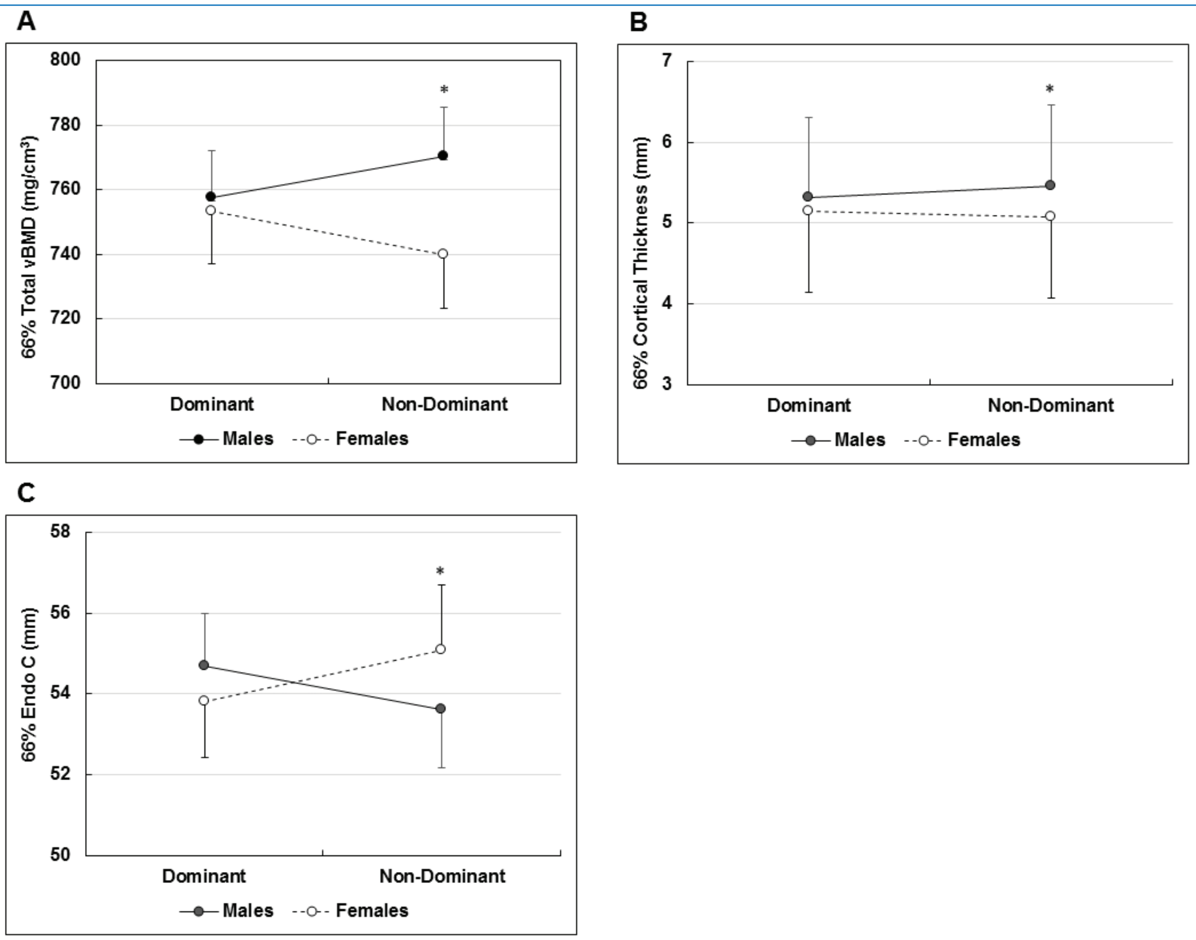

Figure 3. Sex × Side Interaction Effects for Total vBMD (Panel A), Cortical Thickness (Panel B), and Endocortical Thickness (Endo C) (Panel C) for 66% Tibia (means ± SE adjusted for height and body mass) *p≤0.05 significant sex × side interaction effects.

players had lower cortical vBMD (p=0.016) and greater cortical area (p=0.032) for the 38% non-dominant tibia, and greater total vBMD and cortical thickness (all p≤0.05) for the 66% site of both tibiae than controls. There were no significant group differences for MCSA in males.

Figure 2 depicts the percent differences (±95% CI) in bone characteristics between soccer players and controls for the 4% (Panel A), 38% (Panel B), and 66% (Panel C) dominant tibia sites. There were significant sex differences in the magnitude of the percent difference variables. Generally, female soccer players exhibited greater percent differences (all p≤0.05) from controls for BMC, area, and bone strength variables at each tibia site than male soccer players. All bone variable percent differences for female soccer players vs. controls were significant (p≤0.05), with values ranging from 5% to 41% greater than the control group mean. Male soccer players had fewer significant percent differences from control means; total BMC for all sites (all p≤0.05), cortical thickness for the 38% and 66% sites (both p≤0.05), and bone strength (4%, 66%) were greater than controls, with percent differences ranging from 4.2% to 25.5%. Cortical vBMD (38%, 66%) was 1 to 1.5% lower (all p≤0.05) than the control mean for both male and female soccer players.

Limb asymmetries

Sex × side differences in proximal femur aBMD, HSA, and pQCT variables were determined within soccer players by two-way repeated measures ANCOVA adjusted for height and body mass. No significant sex × side interaction effects were observed for femur aBMD or HSA variables (Tables 2 and 3, respectively). Similarly, there were no significant sex × side interactions for 4% and 38% tibia pQCT variables (Supplementary Table 1). However, significant sex × side interactions were found for total vBMD (p=0.029) (Figure 3 Panel A), cortical thickness (p=0.025) (Figure 3 Panel B), and Endo C (p=0.024) (Figure 3 Panel C) at the 66% tibia site. Female soccer players had greater total vBMD and cortical thickness, and lower Endo C for the dominant (kicking) leg compared to the non-dominant support leg. The side difference was opposite for male soccer players with the non-dominant leg showing greater total vBMD and cortical

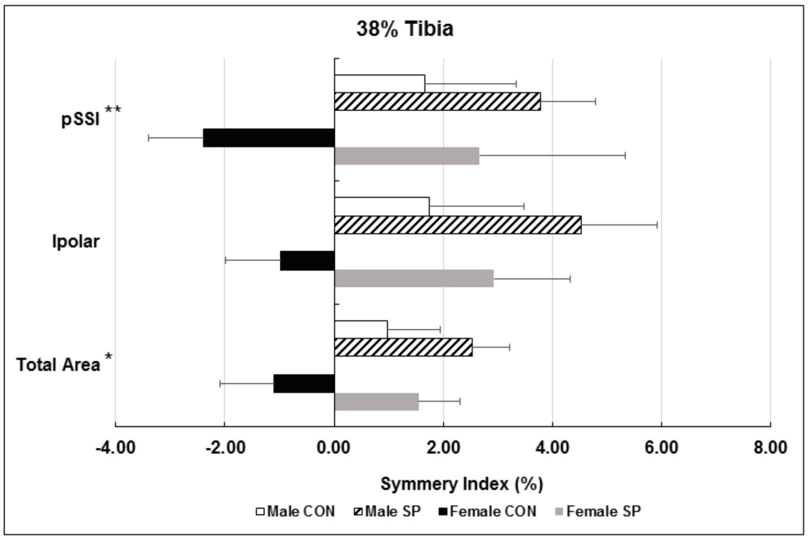

Figure 4. Symmetry Index in Soccer Players and Controls for Selected 38% Tibia Variables (unadjusted means ± SE) *p≤0.05 significant group effect **p≤0.01 significant group effect, Ipolar: Polar Moment of Inertia, pSSI: Strength-Strain Index.

thickness, and lower Endo C than the dominant leg.

Correlations between MCSA and tibia bone variables were performed within each limb and within sex for soccer players. In female soccer players, moderate positive correlations between MCSA and 66% tibia total area (r=0.68, p=0.001) and pSSI (r=0.641, p=0.002) were found for the dominant limb, whereas lower correlations between these variables (total area r=0.52, p=0.019; pSSI r=0.49, p=0.028) were found for the non-dominant tibia. However, male soccer players had high positive correlations between MCSA and total bone area and pSSI for the non-dominant tibia (r=0.809, p<0.001, r=0.803, p<0.001, respectively), and moderate positive correlations between theses variables (total area r=0.66, p=0.001, pSSI r=0.67, p=0.001) for the dominant tibia.

The height and body mass adjusted sex × group SI analyses showed no significant sex or sex × group interaction effects for any SI variables. Significant group effects were found for 38% total area SI (p=0.017) and pSSI SI (p=0.007), with soccer players having greater limb asymmetries favoring the non-dominant (support) leg for these variables (2.1% and 3.8%, respectively) than controls (Figure 4).

Stepwise regression analyses within soccer players for SI variables using sex, BFLBM, FM, calcium intake, and current BPAQ as independent variables resulted in few significant predictors. Calcium intake was a significant predictor of 4% total vBMD SI (p=0.017, β=0.257, R^2=0.066), and BFLBM significantly predicted 38% total area SI (p=0.029, β=0.235, R^2=0.055) and 38% pSSI SI (p=0.015, β=0.260, R^2=0.068).

Discussion

To our knowledge, this is the first study to directly compare skeletal characteristics of male and female soccer players relative to age and body mass matched controls. There are several novel aspects to this study that add to the literature. Although male soccer players had greater total body and hip aBMD, and larger bone size, strength, and vBMD for the tibia sites compared to female soccer players, these sex differences were diminished when the influence of BFLBM and FM was accounted for in regression analysis. We used limb asymmetry as a model for examining sex differences in bone adaptations to loading. There was no sex difference in the magnitude of the limb differences; however, male and female soccer players showed different asymmetry patterns for total vBMD, cortical thickness, and endosteal circumference at the 66% tibia site with female soccer players exhibiting greater bone adaptations in the dominant tibia, while male soccer players had greater adaptations in the non-dominant tibia. Another unique finding was that female soccer players showed greater percent differences (5% to 41%) in tibia bone characteristics relative to their controls than male soccer players. The SI analyses for the 38% tibia total bone area and strength favored the non-dominant (support) leg, confirming previous findings in other groups of athletes[23,26,31].

Sex differences in bone characteristics are well-established, with men having larger, denser, stronger bones than women[32-33]. There also are sex differences in bone microarchitecture as assessed by HR-pQCT. Men, 16-32 years, had greater tibia cross-sectional size, trabecular number and thickness, cortical porosity, and ultimate failure load than women in the same age range[34]. There are several factors that could account for the sex differences in bone characteristics. In males, bone geometry changes during growth are achieved by increasing periosteal dimensions and cortical thickness, whereas females increase cortical thickness through endosteal bone formation without periosteal expansion, thus decreasing endosteal circumference[8]. These bone growth patterns are partly explained by the effects of sex steroids during puberty as estrogens in females limit periosteal apposition and androgens and estrogens in males stimulate periosteal apposition and cortical bone growth[8]. Statistically correcting for body size factors (e.g., body mass, height, BFLBM) generally reduces the magnitude of the sex differences in femur aBMD[32] and tibia BMC[33], however bone geometry differences between males and females remain. Our findings confirm that sex differences in soccer players persisted in aBMD and bone geometry variables after adjusting for body size. However, BFLBM was a stronger predictor than sex for most of the hip and tibia bone variables based on regression analysis. FM also was a significant predictor for many 38% and 66% tibia variables in the models. Regression coefficients for BFLBM and FM indicated both had positive effects on bone outcomes.

An interesting finding was the sex difference in the comparison of soccer players to their controls, as female soccer players had greater percent differences in pQCT variables than male soccer players. Generally, soccer participation characteristics were not different between males and females; however, female soccer players reported more months weight lifting than males. Another possible explanation for this finding is that female soccer players reported being more active than their controls in childhood and adolescence, while there was no difference in past BPAQ scores between the male groups. Since mechanical loading performed before and during puberty can have profound effects on skeletal density and geometry later in life[35] the higher loading levels of female soccer players may have amplified the differences from their controls.

As athletes perform sport related movements such as jumping, running, cutting, or striking a ball, uneven loading of the limbs may occur, resulting in skeletal asymmetries. Males and female soccer players showed different asymmetry patterns for 66% tibia bone density, cortical thickness, and endosteal circumference variables. In male soccer players, the greater bone adaptation to loading occurred in the non-dominant support leg as indicated by greater total vBMD and cortical thickness in conjunction with endocortical constriction compared to the dominant leg. However, in female soccer players, this adaptation was found for the dominant kicking leg. Muscle size may be a contributing factor to these bone adaptations as female soccer players had greater MCSA in the dominant leg and stronger correlations between bone area and strength and MCSA for the dominant leg, while male soccer players had these findings for the non-dominant leg. Previously reported data in young tennis players suggested there may be a sex difference in bone sensitivity to loading, as males exhibited greater limb differences in dominant vs. non-dominant radii, ulnae, and humeri bone traits than females[18]. However, our SI analyses indicated there were no sex differences in magnitude of the asymmetries, and sex was not a significant predictor of SI variables, supporting male and female bones did not differ in sensitivity to loading. Our findings agree with Rantalainen et al. who reported that associations between loading history (BPAQ) and bone characteristics of the non-dominant tibia were not sex-specific[17]. The strongest evidence for sex differences in bone responses to loading comes from animal models, where male rodents exhibited greater improvements in BMC, bone area, and fracture load to mechanical stimuli than female rodents[15,16]. Randomized control trials sufficiently powered for sex and training effects are needed to address this question in humans.

Generally, we found few sport-specific asymmetries for pQCT variables. However, similar to previous studies[23,26], soccer players exhibited greater symmetry indices for 38% total area and pSSI than controls, indicative of greater bone size and strength for the non-dominant (support) leg. Anliker et al.[23] reported a larger side difference in 38% pSSI (4.21%) than ours (2.1-3.8%), but they tested young male soccer players (12-18 years) who may have been a playing at a higher level than our male soccer players. They also tested the 14% tibia site, which showed the largest pSSI side difference (5.64%) favoring the non-dominant leg, suggesting that this site may be important to assess in future asymmetry studies.

As expected, both male and female soccer players had greater total body, lumbar spine, and hip aBMD, greater BFLBM, and lower fat mass compared to their respective controls, supporting previous reports of positive soccer participation effects in children[20,36], young adults[22,25,37], and elderly[21]. Minett et al.[37] found that NCAA Division I female soccer players had greater pre-season total hip and femoral neck aBMD than controls after adjusting for height, lean and fat mass. Our male and female soccer players had DXA-derived hip bone strength variables (strength index, section modulus, CSMI) that were 17-25% greater than their controls, suggesting the odd impact loading from soccer is associated with greater resistance to bending and torsion forces. El Hage et al.[19] also reported that female soccer players had greater resistance to bending, torsion, and compressive forces as indicated by greater section modulus and lower buckling ratio than controls.

Our pQCT data corroborate previous studies documenting that participation in exercise or athletics results in favorable skeletal adaptations of the lower limbs compared to sedentary or non-athletic controls[7,38,39]. We found that female soccer players consistently had greater skeletal geometry measures (BMC, area, cortical thickness, Peri C) than controls at all tibiae sites, but male soccer players overall showed fewer significant pQCT variables versus controls. Similarly, female soccer players had greater bone strength indices than controls at most sites for both limbs, while male soccer players only had greater 4% BSI than controls. Cortical vBMD was significantly lower in female soccer players than controls for 38% and 66% dominant and non-dominant tibia, but it was lower in male soccer players only for 38% non-dominant site. There are mixed findings for cortical vBMD in the literature as athletes have had lower[7,40], higher[26] and similar[5,37] values compared to controls. Cortical vBMD indicates the degree of calcification of the matrix and it is related to bone strength; however, Cointry et al.[41] reported that moment of inertia variables had stronger influences on bone strength indices than cortical vBMD, suggesting that the impact of mechanical loading protocols are driven to a greater extent by bone architecture rather than material properties. Another explanation for the discrepant findings is the location of the tibia site assessed (e.g., 20%, 50%, 66%) in those studies, since cortical vBMD differs along the axial length of the tibia[33].

Our findings are contingent upon a number of limitations. First, female soccer players were recruited from NCAA Division I teams while male soccer players came primarily from Division II teams since there are no Division I male soccer teams in the region. This makes a direct comparison based on competition level not possible. Another consideration is that while soccer training characteristics were similar for males and females, they were not engaging in exactly the same training programs. We did not control for hormonal contraceptive use in female participants, but the prevalence of use was similar for female soccer players and controls. Lastly, these data are cross-sectional in nature, therefore, we are not able to make causal inferences on the effects of soccer participation on bone characteristics in young adults.

In conclusion, our findings do not support a sex difference in responsiveness to mechanical loading. As expected, we found sex differences in bone characteristics with male soccer players having larger, denser, and stronger bones than female soccer players. However, sex was a significant predictor for fewer bone outcomes (lumbar spine, 4% tibia variables), than BFLBM, which was the strongest predictor of hip and the majority of pQCT variables. This finding suggests body composition differences were more important than sex differences at these bone sites. Female soccer players had greater percent differences from controls for tibia bone characteristics than male soccer players, but there were no sex differences in limb asymmetries. Our findings overall support the beneficial effects of soccer participation on bone health in both males and females.

Acknowledgements

We also thank Igor Manfro and Kendall Elliott for their assistance with the data collection.

Compliance with Ethical Standards
Human and animal rights and informed consent

All procedures performed in this study were in accordance with the Institutional Review Board at the University of Oklahoma Health Sciences Center and the 1964 Declaration of Helsinki and its later amendments. Informed consent was obtained from all individual participants included in the study.

References

1. Rizzoli R, Bianchi ML, Garabedian M, McKay HA, Moreno LA. Maximizing bone mineral mass gain during growth for the prevention of fractures in the adolescents and the elderly. Bone 2010;46(2):294-305.
2. Bonjour JP, Chevalley T, Rizzoli R, Ferrari S. Gene-environment interactions in the skeletal response to nutrition and exercise during growth. In: Daly R, Petit M, editors. Optimizing Bone Mass and Strength. The Role of Physical Activity and Nutrition during Growth. Basel, Karger: Med Sport Sci; 2007. p. 64-80.
3. Turner CH. Mechanical loading responses in genetically engineered mice. J Bone Miner Res 2004; 19(S1):S493-S493.
4. Turner CH. Three rules for bone adaptation to mechanical stimuli. Bone 1998;23(5):399-407.
5. Weidauer L, Minett M, Negus C, Binkley T, Vukovich M, Wey H, Specker B. Odd-impact loading results in increased cortical area and moments of inertia in collegiate athletes. Eur J Appl Physiol 2014;114(7):1429-1438.
6. Nikander R, Kannus P, Rantalainen T, Uusi-Rasi K, Heinonen A, Sievänen H. Cross-sectional geometry of weight-bearing tibia in female athletes subjected to different exercise loadings. Osteoporos Int 2010; 21(10):1687-1694.
7. Nikander, Sievänen H, Uusi-Rasi K, Heinonen A, Kannus P. Loading modalities and bone structures at nonweight-bearing upper extremity and weight-bearing lower extremity: a pQCT study of adult female athletes. Bone 2006;39(4):886-894.
8. Nieves JW. Sex-differences in skeletal growth and aging. Curr Osteoporos Rep 2017;15(4):70-75.
9. Jarvinen TL, Kannus P, Sievanen H. Estrogen and bone-a reproductive and locomotive perspective. J Bone Miner Res 2003;18(11):1921-1931.
10. Kriemler S, Zahner L, Puder JJ, Braun-Fahrlander C, Schindler C, Farpour-Lambert NJ, Kranzlin M, Rizzoli R. Weight-bearing bones are more sensitive to physical exercise in boys than in girls during pre- and early puberty: a cross-sectional study. Osteoporos Int 2008;19(12):1749-1758.
11. Ireland A, Rittweger J, Degens H. The influence of muscular action on bone strength via exercise. Clin Rev Bone Miner Metab 2014;12(2):93-102.
12. Saxon LK, Galea G, Meakin L, Price J, Lanyon LE. Estrogen receptors α and β have different gender-dependent effects on the adaptive responses to load bearing in cancellous and cortical bone. Endocrinol 2012;153(5):2254-2266.
13. Callewaert F, Sinnesael M, Gielen E, Boonen S, Vanderschueren D. Skeletal sexual dimorphism: relative contribution of sex steroids, GH-IGF-I, and mechanical loading. J Endocrinol 2010;207(2):127-134.
14. Almeida M, Laurent MR, Dubois V, Claessens F, O'Brien CA, Bouillion R, Vanderschueren D, Manolagas S. Estrogens and androgens in skeletal physiology and pathophysiology. Physiol Rev 2017;97(1):135-187.
15. Jarvinen TLN, Kannus P, Pajamaki I, Vuohelainen T, Tuukkanen J, Jarvinen M, Sievanen H. Estrogen deposits extra mineral into bones of female rats in puberty, but simultaneously seems to suppress the responsiveness of female skeleton to mechanical loading. Bone 2003;32(6):642-651.
16. Wallace JM, Rajachar RM, Allen MR, Bloomfield SA,

Robey PG, Young MF, Kohn DH. Exercise-induced changes in the cortical bone of growing mice are bone- and gender-specific. Bone 2007;40(4):1120-1127.

17. Rantalainen T, Weeks BK, Nogueira RC, Beck BR. Effects of bone-specific physical activity, gender and maturity on tibial cross-sectional bone material distribution: a cross-sectional pQCT comparison of children and young adults aged 5-29 years. Bone 2015;72(March):101-108.
18. Ireland A, Maden-Wilkinson T, McPhee J, Cooke K, Narici M, Degens H, Rittweger J. Upper limb muscle-bone asymmetries and bone adaptation in elite youth tennis players. Med Sci Sports Exerc 2013;45(9):1749-1758.
19. El Hage R.Geometric indices of hip bone strength in young female football players. J Musculoskelet Neuronal Interact 2013;13(2):206-212.
20. Zouch M, Zribi A, Alexandre C, Chaari H, Frere D, Tabka Z, Vico L. Soccer increases bone mass in prepubescent boys during growth: a 3-yr longitudinal study. J Clin Densitom 2015;18(2):179-186.
21. Helge EW, Andersen TR, Schmidt JF, Jorgensen NR, Hornstrup T, Krustrup P, Bangsbo J. Recreational football improves bone mineral density and bone turnover marker profile in elderly men. Scand J Med Sci Sports 2014;24 (Suppl 1):98-104.
22. Jackman SR, Scott S, Randers MB, Ørntoft C, Blackwell J, Zar A, Krustrup P. Musculoskeletal health profile for elite female footballers versus untrained young women before and after 16 weeks of football training. J Sports Sci 2013;31(13):1468-1474.
23. Anliker E, Sonderegger A, Toigo M. Side-to-side differences in the lower leg muscle-bone unit in male soccer players. Med Sci Sports Exerc 2013; 45(8):1545-1552.
24. Fousekis K, Tsepis E, Vagenas G. Lower limb strength in professional soccer players: profile, asymmetry, and training age. J Sports Sci Med 2010;9(3):364.
25. Calbet J, Dorado C, Diaz-Herrera P, Rodriguez-Rodriguez L. High femoral bone mineral content and density in male football (soccer) players. Med Sci Sports Exerc 2001;33(10):1682-1687.
26. Sone T, Imai Y, Joo YI, Onodera S, Tomomitsu T, Fukunaga M. Side-to-side differences in cortical bone mineral density of tibiae in young male athletes. Bone 2006;38(5):708-713.
27. Weeks BK, Beck BR. The BPAQ: a bone-specific physical activity assessment instrument. Osteoporos Int 2008;19(11):1567-1577.
28. Musgrave KO, Giambalvo L, Leclerc HL, Cook RA, Rosen CJ. Validation of a quantitative food frequency questionnaire for rapid assessment of dietary calcium intake. J Am Diet Assoc 1989;89(10):1484-1488.
29. Ohnaru K, Sone T, Tanaka K, Akagi K, Ju YI, Choi HJ, Tomomitsu T, Fukunaga M. Hip structural analysis: a comparison of DXA with CT in postmenopausal Japanese women. Springerplus 2013;2(1):331.
30. Blew RM, Lee VR, Farr JN, Schiferl DJ, Going SB. Standardizing evaluation of pQCT image quality in the presence of subject movement: qualitative versus quantitative assessment. Calcif Tissue Int 2014; 94(2):202-211.
31. Hart NH, Nimphius S, Weber J, Spiteri T, Rantalainen T, Dobbin M, Newton R. Musculoskeletal asymmetry in football athletes: a product of limb function over time. Med Sci Sports Exerc 2016;48(7):1379-1387.
32. Looker AC, Beck TJ, Orwoll ES. Does body size account for gender differences in femur bone density and geometry? J Bone Miner Res 2001;16(7):1291-1299.
33. Sherk VD, Bemben DA, Bemben MG, Anderson MA. Age and sex differences in tibia morphology in healthy adult Caucasians. Bone 2012;50(6):1324-1331.
34. Walsh JS, Paggiosi MA, Eastell R. Cortical consolidation of the radius and tibia in young men and women. J Clin Endocrinol Metab 2017;97(9):3342-3348.
35. Gunter KB, Almstedt HC, Janz KF. Physical activity in childhood may be the key to optimizing lifespan skeletal health. Exerc Sport Sci Rev 2012;40(1):13-21.
36. Lozano-Berges G, Matute-Llorente Á, González-Agüero A, Gómez-Bruton A, Gómez-Cabello A, Vicente-Rodriguez G, Casajús JA. Soccer helps build strong bones during growth: a systematic review and meta-analysis. Eur J Pediatr 2017;177(3)295-310.
37. Minett MM, Binkley TB, Weidauer LA, Specker BL. Changes in body composition and bone of female collegiate soccer players through the competitive season and off-season. J Musculoskelet Neuronal Interact 2017;17(1):386-398.
38. Wilks DC, Winwood K, Gilliver S, Kwiet A, Chatfield M, Michaelis I, Sun L, Ferretti JL, Sargeant AJ, Felsenberg D. Bone mass and geometry of the tibia and the radius of master sprinters, middle and long distance runners, race-walkers and sedentary control participants: a pQCT study. Bone 2009;45(1):91-97.
39. Schipilow J, Macdonald H, Liphardt A, Kan M, Boyd S. Bone micro-architecture, estimated bone strength, and the muscle-bone interaction in elite athletes: an HR-pQCT study. Bone 2013;56(2):281-289.
40. Rantalainen T, Nikander R, Daly R, Heinonen A, Sievänen H. Exercise loading and cortical bone distribution at the tibial shaft. Bone 2011;48(4):786-791.
41. Cointry G, Ferretti JL, Reina P, Nocciolono L, Rittweger J, Capozza R. The pQCT “Bone Strength Indices” (BSIs, SSI). Relative mechanical impact and diagnostic value of the indicators of bone tissue and design quality employed in their calculation in healthy men and pre-and post-menopausal women. J Musculoskelet Neuronal Interact 2014;14(1):29-40.

Supplementary Table 1. pQCT tibiae bone variables (unadjusted means ± SD).

	Males		Females	
	Soccer (n=23)	**Control (n=23)**	**Soccer (n=20)**	**Control (n=20)**
4% Dominant				
Total vBMD (g/cm^3)	385.2±30.6 **††	341.7±36.4	346.9±36.4 **	306.6±37.5
Total BMC (g)	479.5±72.4 *††	429.9±64.5	372.1±50.9 **	296.9±31.5
Total Area (mm^2)	1247.5±177.2	1264.8±196.3	1077.3±139.8 *	977.4±119.6
Trab vBMD (g/cm^3)	328.0±25.6 **††	293.8±32.8	290.3±31.1 **	259.6±33.2
Trab BMC (g)	342.4±63.8 ††	316.6±56.3	252.2±42.2 **	206.7±28.1
Trab Area (mm^2)	1042.4±169.0	1081.5±186.1	871.3±125.3	803.2±109.9
Peri C (mm)	124.9±9.0	125.7±9.8	116.1±7.4 *	110.6±7.0
4% Non-Dominant				
Total vBMD (g/cm^3)	388.2±32.2 **††	344.2±34.6	348.2±36.4 **	305.8±36.0
Total BMC (g)	494.2±72.7 **††	436.2±67.8	375.3±49.2 **	297.8±33.1
Total Area (mm^2)	1275.0±166.5	1273.3±206.7	1082.1±133.0 *	982.0±121.7
Trab vBMD (g/cm^3)	331.4±25.1 **††	294.3±30.9	289.3±31.4 **	258.5±31.4
Trab BMC (g)	352.5±58.8 ††	319.0±57.6	251.8±40.5 **	207.0±28.7
Trab Area (mm^2)	1063.5±156.8	1087.2±192.7	872.6±121.3	807.0±111.5
Peri C (mm)	126.3±8.4	126.1±10.2	116.4±7.0 *	110.9±7.0
38% Dominant				
Total vBMD (g/cm^3)	937.5±46.4	941.2±62.8	960.0±48.8 *	923.8±55.4
Total BMC (g)	427.9±48.3	407.1±50.2	380.1±36.5 **	320.2±32.4
Total Area (mm^2)	457.3±52.6 †	435.1±69.4	397.1±45.3 **	347.4±37.3
Cort vBMD (g/cm^3)	1158.7±20.2	1169.9±19.3	1171.7±18.0 **	1185.6±13.8
Cort BMC (g)	412.8±45.6	391.0±47.3	367.6±36.8 **	305.5±31.3
Cort Area (mm^2)	356.5±41.2	334.58±43.3	313.7±31.0 *	257.7±27.1
Cort Thickness (mm)	6.4±0.6	6.2±0.6	6.1±0.5 **	5.2±0.5
Peri C (mm)	75.7±4.5 †	73.7±5.7	70.5±4.0 **	66.0±3.6
38% Non-Dominant				
Total vBMD (g/cm^3)	936.9±50.5	937.1±62.5	959.4±52.7	926.6±53.3
Total BMC (g)	438.9±51.8 †	409.8±54.9	385.4±36.5 **	317.4±28.6
Total Area (mm^2)	468.8±51.9 ††	439.7±72.3	403.1±43.9 **	343.4±35.5
Cort vBMD (g/cm^3)	1156.3±20.9 *	1170.6±17.5	1173.1±20.0 **	1189.9±15.5
Cort BMC (g)	423.5±49.3 †	396.4±51.9	372.2±34.0 **	303.9±27.4
Cort Area (mm^2)	366.5±44.0 *†	337.2±46.0	317.3±27.9 **	255.5±23.6
Cort Thickness (mm)	6.5±0.7	6.2±0.6	6.1±0.5 **	5.2±0.4
Peri C (mm)	76.6±4.3 ††	74.1±5.9	71.1±3.8 **	65.6±3.4
66% Dominant				
Total vBMD (g/cm^3)	754.1±53.3 *	719.7±52.8	757.1±75.0	719.9±54.2
Total BMC (g)	485.6±62.5	453.9±64.1	422.8±46.1 **	351.5±32.
Total Area (mm^2)	645.7±81.8	633.4±98.4	562.1±72.8 **	490.0±51.0
Cort vBMD (g/cm^3)	1125.5±19.1	1139.3±18.6	1139.7±16.6 **	1156.8±12.8
Cort BMC (g)	442.1±55.8	418.2±57.6	389.9±47.9 **	322.3±31.0
Cort Area (mm^2)	393.0±50.5	367.5±53.7	342.0±40.6 **	278.6±26.8
Cort Thickness (mm)	5.4±0.6 *	5.0±0.5	5.1±0.7 *	4.3±0.4
Peri C (mm)	89.9±5.8	89.0±6.7	83.9±5.3 *	78.4±4.1
Muscle CSA (mm^2)	8485.2±1592.2	7803.7±1300.4	7277.7±657.9 **	6278.2±999.9
66% Non-Dominant				
Total vBMD (g/cm^3)	760.2±73.8 *	716.6±58.1	751.1±63.7	717.4±56.3
Total BMC (g)	490.6±62.6 †	454.6±69.3	423.9±40.8 **	347.9±31.4
Total Area (mm^2)	650.5±92.7	637.9±108.9	567.7±72.6 **	486.7±48.9
Cort vBMD (g/cm^3)	1124.1±22.1	1135.1±16.4	1138.9±17.8 **	1157.2±12.9
Cort BMC (g)	448.6±56.3	418.1±63.7	391.1±42.7 **	319.3±30.4
Cort Area (mm^2)	399.4±51.9 †	368.6±57.8	343.2±35.6 **	275.9±26.7
Cort Thickness (mm)	5.5±0.6 **	5.0±0.6	5.0±0.5 **	4.3±0.4
Peri C (mm)	90.2±6.9	89.2±7.4	84.3±5.2 **	78.1±3.9
Muscle CSA (mm^2)	8312.1±1938.1	7835.1±1375.4	7055.2±598.9 **	6139.6±1012.6

*p≤0.05, **p≤0.01 significant vs. respective control group; †p≤0.05, ††p≤0.01 significant vs. female soccer group (adjusted for height, and body mass); vBMD: Volumetric Bone Mineral Density, BMC: Bone Mineral Content, Trab: Trabecular, Peri C: Periosteal Circumference, Cort: Cortical, CSA: Cross Sectional Area.