Influence of Changes in Soft Tissue Composition on Changes in Bone Strength in Peri-Pubertal Girls: The STAR Longitudinal Study

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Supplemental Tables 1-4 are included with the submission

DISCLOSURES

Jennifer W. Bea serves as a consultant to the Women's Health Initiative Study, has received research funding from Disarm Therapeutics, and serves on the Board of Directors for the Global Health and Body Composition Institute, outside the submitted work. Victoria Bland, Robert Blew, Denise Roe, Vinson Lee, Janet Funk, and Scott Going have nothing to disclose.

KEY WORDS

Bone QCT, DXA, Osteoporosis, Bone-Fat Interaction, Fracture Prevention

ABSTRACT

Obesity and osteoporosis remain two major public health concerns. Soft tissue composition and bone are interrelated; however, it is still not well understood how changes in adiposity during adolescence affect bone development. The aim of this study was to assess how changes in DXA-derived total body lean mass (TBLM) and total body fat mass (TBFM) associate with 2-year changes in bone outcomes at the 20% femur, 66% tibia, 66% radius, and 4% tibia, as measured by pQCT, during the years surrounding the onset of menarche in a cohort of 9-12 year-old (baseline) adolescent girls (70% Hispanic). From baseline to 2-year follow-up, girls showed statistically significant increases in all bone outcomes, except radial endosteal circumference. In separate linear regression models, change in TBLM and change in TBFM were both positively associated with 2-year changes in bone outcomes at all measured bone sites, after controlling for relevant covariates. However, when change in TBLM and change in TBFM were included in the same model, change in TBLM was the predominant predictor of bone outcomes, explaining 4-14% of the variance in bone strength outcomes. Change in TBFM remained a positive predictor of tibia polar strength strain index ($SSI_{p)}$ (2% variance explained). A significant interaction between change in TBFM and menarcheal status was identified at the radius for SSI_p and indicated that greater gains in TBFM were beneficial for SSI_p in girls that were pre-menarcheal at baseline but detrimental for girls that were post-menarcheal at baseline. The overall findings suggest that changes in TBLM during the peri-pubertal years have a greater influence on bone outcomes than changes in TBFM. While gains in TBFM might benefit the weight bearing 66% tibia, greater gains in TBFM may be detrimental to bone development at the non-weight bearing 66% radius after the onset of menarche.

INTRODUCTION

Childhood is a critical time for bone development and osteoporosis prevention. Approximately 95% of adult bone mass is accrued by the end of adolescence, with about 40% accrued during the 4 years surrounding peak height velocity (PHV) and the most rapid accrual of bone occurring around the time of menarche.[1,2] Although the majority of adult bone mass is determined by genetics, an estimated 40% can be influenced by environmental and lifestyle factors.[3] It is well established that lean soft tissue mass positively influences bone mineral density (BMD) and structure during bone development; however, the influence of fat mass remains uncertain.

The prevalence of childhood obesity has risen drastically over the past two decades in the United States, increasing from 13.9% of youth 2-19 years old in 2000 to 18.5% in 2016.[4,5] This rate is even higher among Hispanic adolescents, with over 25% of Hispanic youth being classified as obese.[4] Yet, how the increase in body weight and total body adiposity affects developing bones is still poorly understood. While a higher body weight has been considered to be beneficial for bone development due to the increased strain placed on the bone, the higher fracture rate observed in obese adolescents contradicts this idea.[6,7]

Studies assessing the relationship between fat and bone have reported positive[8,9], negative[10,11], and/or null[12,13] results; however, these studies were often limited by cross-sectional design and the use of dual-energy x-ray absorptiometry (DXA) 2-dimensional imaging, which can only obtain measurements of areal bone mineral density (aBMD) and bone mineral content (BMC).[14,15] The advent of peripheral quantitative computed tomography (pQCT) allows for 3-dimensional imaging of the bone to ascertain structural properties of bone and estimates of bone strength to better understand fracture risk. Despite advancements in technology, conflicting

93 results still exist from studies using pQCT, likely due to differences in sex, maturity status,
94 degree of adiposity, and statistical design.[14,15]

95 Considering that soft tissue composition, as well as the rate of bone mineral accrual,
96 changes throughout adolescence, it is logical that cross-sectional analyses may not accurately
97 describe how fat affects bone over time. One of the first longitudinal analyses of the fat-bone
98 relationship that was conducted utilizing pQCT found that the greater bone strength observed in
99 overweight children was due to greater gains in lean mass, while fat mass did not significantly
100 affect bone development.[16] Two more recent longitudinal pQCT studies suggest that excess fat
101 mass may actually be favorable to cortical bone development in weight bearing bones[17] and
102 detrimental to non-weight bearing bones.[18] However, these studies were conducted in cohorts of
103 primarily non-Hispanic white[17,18] or Asian adolescents[16] and may not be indicative of bone
104 development in all adolescents.

105 There is a paucity of data on the relationship between soft tissue composition and bone
106 outcomes in Hispanic adolescents. In a cross-sectional analysis of soft tissue composition and
107 pQCT bone outcomes in Hispanic girls from the Soft Tissue and Bone Development (STAR)
108 study, we recently reported that lean mass was the primary determinant of bone strength at all
109 peripheral bone sites, while fat mass may contribute to increased bone strength in weight bearing
110 bone sites but not at the non-weight bearing site of the radius.[19] Building off this cross-sectional
111 study, the aim of the current longitudinal study was to assess the relationship between changes in
112 total body lean soft tissue mass (TBLM) and total body fat mass (TBFM) with changes in bone
113 strength, density, and structure for weight bearing and non-weight bearing bones in a cohort of
114 primarily Hispanic ethnicity (70% Hispanic) peri-pubertal girls from the STAR study.
115

METHODS

Study Population

The STAR study was a longitudinal cohort study consisting of 9-12 year-old girls recruited from local schools, pediatric clinics, and wellness community events in Tucson, AZ between 2013-2018 (Clinical trials #NCT02654262). Exclusion criteria included: diagnosis of diabetes, taking any medications that may affect body composition, physical disability affecting physical activity, and learning disability that limited ability to complete questionnaires or otherwise made the participant unable to comply with study protocols.

As depicted in **Figure 1**, 358 girls took part in the STAR cross-sectional study. A sub-sample of 297 girls were invited back for the 2-year follow-up visit to ensure a sample size of 150 girls for the longitudinal study, expecting 50% response rate. This longitudinal sample size was based on the primary aim of the STAR study, to detect a significant difference in 2-year pQCT bone strength changes between normal weight girls, obese girls with insulin resistance, and obese girls without insulin resistance. The sample size calculation used two-year longitudinal data from the Jump-In study which showed a mean increase in femur 4% bone strength index (BSI) of 25.25 mg/cm^3 (SD = 15.05 mg/cm^3) in 178 normal weight girls, versus a mean increase of 38.95 mg/cm^3 (SD = 21.97 mg/m^3) in 14 obese girls (unpublished data). Based on these and other results for bone strength measures, 50 girls per group allowed for detection of a statistically significant difference between the normal weight and each of the obese groups with 80% statistical power, accounting for 10% loss to follow-up.

One-hundred and sixty girls participated in the follow-up analysis and comprised the STAR longitudinal cohort. The study protocol was approved by the University of Arizona Human Subjects Protection Committee. Written informed assent was obtained from all

participants and consent was obtained from their parents or legal guardians. Methodology for the cross-sectional study has been previously reported.[19] For the longitudinal study, all measurements were taken at baseline and 2-year follow-up.

Ethnicity and Health History

At study entry, participants' guardians were asked to complete a questionnaire to obtain information on self-identified race and ethnicity. During baseline and 2-year follow-up visits, guardians also completed a health history questionnaire to obtain details about medical diagnoses, medication use, and bone fractures. No participants in the longitudinal study had reported bone fractures in the non-dominant limb, on which pQCT scans were conducted.

Anthropometric Measurements

Anthropometric measurements were assessed using standardized protocols.[20] Body mass was measured to the nearest 0.1 kg using a calibrated scale (Seca, Model 881, Hamburg, Germany). Standing and sitting height were measured at full inhalation to the nearest mm using a stadiometer (Shorr Height Measuring Board, Olney, MD). Centers for Disease Control and Prevention (CDC) BMI-for-age percentiles were used to classify girls as underweight (<5th percentile), normal weight (≥5th and<85th percentiles), overweight (≥85th and<95th percentiles), or obese (≥95th percentile).[21] Femur, tibia, and forearm lengths were measured based on standard anatomical landmarks to the nearest mm using an anthropometric tape.[20]

DXA Soft Tissue Composition

Dual-energy x-ray absorptiometry (DXA) (Prodigy and iDXA models; GE/Lunar Radiation Corp, Madison, WI) was used to measure soft tissue composition, including TBLM, TBFM, and total body percent fat. All scans were performed by a single certified technician using standard subject positioning and data acquisition protocols. DXA machines were calibrated daily according to manufacturer guidelines. Within-subject variation for soft tissue in our laboratory has been previously reported.[22,23]

Maturation

Pubertal status was determined by self-reported questionnaire where the girls indicated their breast and pubic hair development based on pictures depicting the Tanner stages of pubertal maturation.[24] Both age and menarcheal status were used to assess maturity. Self-reported birthdate was used to calculate age at baseline and follow-up. To assess menarcheal status, girls self-reported whether they had experienced their first menstrual period at both measurement time points. If they responded "yes", they were asked to recall the month and year of their first menstrual period.

pQCT Bone Measurements

Changes in bone density, structure, and strength were assessed using pQCT (XCT 3000, Stratec Medizintechnik GmbH, Pforzheim, Germany, Division of Orthometrix; White Plains, NY). Measurements were taken at the 20% femur, 66% tibia, and 66% radius, relative to the respective distal growth plates on the non-dominant limb, to assess diaphyseal (cortical) regions in weight bearing and non-weight bearing bones, and 4% tibia to assess a weight bearing trabeculae-enriched (metaphyseal) region. All imaging scans were analyzed using Stratec XCT

184 software, version 6.0, and operators followed guidelines provided by Bone Diagnostic LLC
185 (Spring Branch, TX). Scans at each bone site were reviewed for movement artifact using a visual
186 inspection rating scale.[25] Coefficients for within-subject variation, as well as instrument, image
187 processing, and analysis protocols for our laboratory have been previously published.[26] The
188 pQCT instrument was calibrated with quality assurance procedures completed daily to maintain
189 measurement precision.
190 Bone density and structural parameters measured at diaphyseal sites included cortical
191 volumetric bone mineral density (vBMD, mg/cm^3), cortical BMC (mg/mm), periosteal
192 circumference (mm), endosteal circumference (mm), and cortical thickness (mm). Polar strength
193 strain index (SSI_p, mm^3), an estimate of cortical bone strength, was calculated using the
194 equation: $SSI_p = \Sigma((d_Z^2 \times A_V) \times (\text{cortical vBMD/ND}))/d_{max}$, where, d_Z is the distance of voxel
195 from the center of gravity, A_V is the area of the voxel (mm^2), vBMD is the cortical bone density
196 (mg/cm^3), ND is the estimated normal physiological bone density (1200 mg/cm^3), and d_{max} is the
197 maximum distance of a voxel from the center of gravity.[27,28] This equation estimates the
198 resistance to bending and torsional loading and has been found to predict up to 80% of the
199 variance in bending failure load in human tibias.[29] Structure and density outcomes of interest at
200 the tibia metaphyseal site were total area (mm^2), total vBMD (mg/cm^3), and trabecular vBMD
201 (mg/cm^3). BSI (mg^2/cm^4) was calculated as total area x total vBMD^2, which estimates up to 85%
202 of variability in the bone's ability to withstand compression at the tibia.[29]
203
204 **Physical Activity and Sedentary Time**
205 Physical activity was measured using GT3X+ tri-axial accelerometers (ActiGraph,
206 Pensacola, FL). Participants received the accelerometer at the imaging visit and were instructed

to wear the accelerometer on their hip for 7 consecutive days during all waking hours, except for showering, or other water activities. Accelerometry data was analyzed in 1-second epochs using ActiLife Software version 6.13.4 (ActiGraph, Pensacola, FL). Non-wear time was classified as ≥ 30 minutes of continuous zeros. Only days with at least 8 hours of wear time were included. Evenson cut points were used to classify moderate physical activity (2296-4011 cpm) and vigorous physical activity (≥ 4012 cpm).[30] Moderate and vigorous activities were combined to estimate average daily time spent in moderate to vigorous physical activity (MVPA).

Statistical Analyses

Descriptive statistics, including means and standard deviations, were calculated for participant demographic, anthropometric, and bone characteristics at baseline and 2-year follow-up. Significant changes in body composition and bone measurements outcomes from baseline to 2-year follow-up were assessed using paired t-tests. Comparisons of Hispanic to non-Hispanic girls and responders to non-responders for 2-year measurements were assessed using independent t-tests. Three girls were missing data pertaining to ethnicity, 2 girls were missing data pertaining to soft tissue composition, and 12 girls were missing accelerometry data at either baseline or follow-up, leaving a sample size of 143 for analysis. Due to missing data for pQCT sites related to movement (n = 1 at radius and n = 3 at femur) and missing repeat data at follow-up (n = 1 at radius, n = 2 at femur, and n = 3 tibia), sample sizes were allowed to vary between the 20% femur (n = 138), 66% tibia (n = 140), 66% radius (n = 141), and 4% tibia (n = 140).

Multiple linear regression analyses were used to assess the relationship between soft tissue composition changes and the primary outcomes of interest, change in SSI_p at the 20% femur, 66% tibia, and 66% radius and BSI at the 4% tibia, as well as secondary pQCT bone

density (vBMD) and structure (BMC, periosteal circumference, endosteal circumference, cortical thickness, total area) changes. Three models were developed to assess the independent effects of change in TBLM (model 1), change in TBFM (model 2), and changes in TBLM and TBFM in a single model (model 3). Covariates were selected based on the literature and established relationship with bone outcomes. Covariates included in all models were baseline age, ethnicity, menarcheal status, baseline bone measure (corresponding with outcome of interest), change in limb length, change in MVPA, and DXA machine. Additionally, baseline TBLM was included in models 1 and 3 and baseline TBFM was included in models 2 and 3. Since not all girls had reached menarche at the 2-year follow-up, age at menarche was not a viable covariate; menarche was coded as: 0 = post-menarcheal at baseline, 1 = transitioned from pre-menarcheal to post-menarcheal between baseline and follow-up, 2 = pre-menarcheal at follow-up. Given that the relationship between soft tissue composition and bone outcomes has been shown to vary throughout maturity[15,18], we tested for interactions between change in TBLM and change in TBFM with menarcheal status on bone change.

Outliers were observed at 20% femur (n = 2), 66% tibia (n = 3), and 66% radius (n = 1) for girls with significantly higher or lower bone change variables, yet values were physiologically plausible. A sensitivity analysis was performed on models with statistically significant outliers by re-estimating the models excluding the outliers. However, removal of outliers did not significantly change beta coefficients or overall conclusions, so all participants were included in the models and the summary statistics reported.

All models were checked for the assumptions of linear regression: linearity, normality of residuals, and homoscedasticity. Linearity between outcome variables and each covariate was assessed with scatter plots. Residual versus predictors plots were used to visually assess the

assumptions of linearity and homoscedasticity. Normal distribution of residuals for the model was assessed using a histogram. All analyses were performed in R version 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria) using the stats package lm() function for linear regression. The Bonferonni p-value obtained via the outlierTest() function in the companion to applied regression (car) package was used to identify statistically significant outliers. A p-value of < 0.05 was considered statistically significant.

RESULTS

Demographic, anthropometric, and bone characteristics of girls in the STAR longitudinal study cohort are reported in **Table 1**. Girls were 10.8 ± 1.1 years old at baseline and approximately 70% of girls self-identified as Hispanic ethnicity. The majority of girls remained in the same BMI category from baseline to 2-year follow-up (n = 111); however, 12 girls decreased BMI category (e.g., went from overweight to normal weight) and 20 girls increased BMI category (e.g., went from normal weight to overweight) between time points. On average, girls had a 10% increase in percent body fat with mean percent body fat increasing from 32.3 ± 9.7 at baseline to 35.6 ± 8.2 at follow-up. Of the girls who had reached menarche by 2-year follow-up, the average age at menarche was 11.3 ± 1.0 years old. The majority of the cohort did not meet physical activity guidelines.[31] Only 18% of girls averaged more than 60 min/day MVPA at baseline and this number decreased to 10% of girls at follow-up. All bone outcomes showed a positive percent change, except for radial endosteal circumference.

Girls who identified as Hispanic ethnicity were on average younger (10.7 vs 11.2 yr, p = 0.01), shorter (145.8 vs 149.4 cm, p = 0.047), and had less lean mass (26.7 vs 29.1 kg, p = 0.039) at baseline than girls who identified as non-Hispanic. Additionally, Hispanic girls had

significantly greater gains in fat mass (6.2 vs 4.5 kg, p = 0.02) with higher TBFM at the 2-year follow-up (22.2 vs 18.4 kg, p = 0.04) compared to non-Hispanic girls. No significant differences in limb length, physical activity, nor age at menarche were observed (data not shown). There were also no significant differences in baseline demographic, physical activity, anthropometric, or bone values between girls who participated in the longitudinal study and girls who were invited to participate but did not respond (data not shown).

Results pertaining to changes in TBLM and TBFM from the linear regression analyses are presented in **Table 2**. Full statistical models for model 3 are provided in **Supplemental Tables 1-4**. In their respective models, change in TBLM (model 1) and change in TBFM (model 2) both favorably influenced changes in pQCT-derived bone strength at all bone sites. Additionally, at the cortical sites (20% femur, 66% tibia, 66% radius), change in TBLM and change in TBFM were also consistently, positively associated with change in cortical BMC, but associations with density and other structural outcomes were less consistent. At the trabecular site (4% tibia), both change in TBLM and change in TBFM were positively associated with changes in density.

When change in TBLM and TBFM were included in the same model (model 3), change in TBLM remained a positive predictor of change in strength and structure at the 20% femur and 66% radius, as well as a positive predictor of BMC and cortical thickness at the 66% tibia. At the trabecular site (4% tibia), change in TBLM continued to positively predict change in BSI, total area, and trabecular vBMD. In model 3, change in TBFM was no longer a significant predictor of changes in strength and structure at the 20% femur nor strength and density at the 4% tibia. However, change in TBFM remained a positive predictor of change in 66% tibia SSI_p.

Significant interactions between changes in soft tissue composition and menarcheal status were identified in weight bearing and non-weight bearing cortical sites. At the non-weight bearing 66% radius, there was a significant interaction between change in TBFM and initial menarcheal status on change in SSI_p; in girls who were post-menarcheal at baseline, greater gains in TBFM were negatively associated with change in radius SSI_p while in girls who were pre-menarcheal at baseline (regardless of menarcheal status at follow-up), greater TBFM gains were positively associated with radius SSI_p. At the weight bearing 66% tibia, there was also a significant interaction between change in TBLM and menarcheal status on change in cortical vBMD. This interaction demonstrated that there was a positive relationship between change in TBLM and change in cortical vBMD in girls who remained pre-menarcheal at 2-year follow-up, but a negative relationship between change in TBLM and change in cortical vBMD in girls who were post-menarcheal at follow-up (regardless of baseline menarcheal status). Interactions for the fully adjusted models (model 3) are depicted in **Figure 2**.

Figure 3 demonstrates the variance explained in model 3 for the primary outcomes of interest, SSI_p at the 20% femur, 66% tibia, and 66% radius, and BSI at the 4% tibia. The base model of covariates (baseline age, ethnicity, menarcheal status, baseline bone measure, change in limb length, change in MVPA, DXA machine, baseline TBLM, and baseline TBFM) explained 52% of the variance in strength change at the 66% tibia, ~32% at the 20% femur and 66% radius, and 28% at the 4% tibia. Change in TBLM explained the greatest amount of variance in femur SSI_p change (14%) and change in TBFM explained the greatest amount of variance in 66% radius SSI_p change (3%). Of note, ethnicity was not statistically significant in the majority of models but was positively associated with 20% femur cortical vBMD and negatively associated with 66% tibia cortical BMC and cortical thickness.

DISCUSSION

In this longitudinal analysis, we investigated the effects of changes in TBLM and TBFM on changes in bone strength and structure in a primarily Hispanic cohort of 9-12 year-old girls. Our results showed that change in TBLM and change in TBFM, in separate models, both positively influenced bone development. However, when both body composition measures were included in the same model, change in TBLM maintained a significant, positive influence on changes at all bone sites, particularly at the 20% femur and 66% radius. In the full model for the 66% tibia, change in TBFM was positively associated with SSI_p for the whole cohort. At the 66% radius, change in TBFM was negatively associated with SSI_p for girls who were already post-menarcheal at baseline (20%), but was positively associated with SSI_p for girls who were pre-menarcheal at baseline.

Changes in TBLM and Bone Changes

Consistent with the literature[14], we observed a positive relationship between change in TBLM and bone changes in our sample. This relationship was the strongest at the 20% femur and 4% tibia, explaining approximately 14% of the variance in SSI_p change and 12% of the variance in BSI change, respectively, after adjusting for relevant covariates. At the femur, change in TBLM was also significantly (positively) associated with all structural parameters and although somewhat counterintuitive, greater gains in TBLM were negatively associated with change in cortical vBMD. Given that density is a function of mass divided by volume, this potentially indicates an increase in bone size (periosteal circumference, cortical thickness) with a more modest increase in mineral content, resulting in an overall stronger bone despite the lower

cortical vBMD. Another likely explanation is that this negative relationship is a result of intracortical remodeling in girls that had greater gains in lean mass.[32] At the 4% tibia, change in TBLM was also positively associated with total area and trabecular density.

The mechanostat theory states that bone adaptation is driven by mechanical load, with muscle mass being the largest load, particularly in lower body bones.[33] Therefore, it is not surprising that the strongest relationships were seen at the 20% femur and 4% tibia. Although a stronger relationship between lean mass and 66% tibia bone outcomes was also expected, much of the change in 66% tibia bone outcomes was explained by change in tibia length (e.g., 20% of variance in SSI_p and 33% of variance in periosteal circumference), which negated the effect of lean mass. The strength of the relationship between change in TBLM with 20% femur and 4% tibia bone outcomes, relative to other bone sites, is likely explained by muscle size and insertion points. While only a surrogate measure for muscle force, DXA TBLM has been shown to be significantly correlated with muscle force.[34] The muscles surrounding the femur (e.g., quadriceps muscles) are much larger than the muscles surrounding the tibia and radius. Additionally, there are muscle insertion points near the 20% femur and 4% tibia bone site. Thus, it would be expected that the muscular forces placed on the bone at the femur and distal tibia, and subsequent bone adaption in strength and size, would be larger at these sites. Given that muscle forces place a greater mechanical strain on bone than overall body mass[33], it is plausible that change in TBLM may mask the influence of change in TBFM at the femur and 4% tibia.

Changes in TBFM and Bone Changes

There is no consensus on the relationship between fat and bone in adolescents. Prior studies that used pQCT and a longitudinal research design showed positive[17], negative[18], and

null[16] relationships between fat and bone in Caucasian and/or Asian populations. These studies assessed the relationship in weight bearing bones[16,17] and non-weight bearing bones[18], but there is a paucity of studies assessing pQCT outcomes for both weight bearing and non-weight bearing bones in a single cohort. Our findings from the STAR study cohort adds to the literature by showing that the relationship between fat and bone is bone site specific and may be at least partially influenced by weight bearing status.

At the weight bearing bone sites, greater gains in TBFM were associated with greater increases in 66% tibia SSI_p and 20% femur cortical vBMD, but not bone strength at the 20% femur nor 4% tibia. In a previous study of preadolescent girls from our laboratory (Jump-In), a similar positive relationship of TBFM with tibia SSI_p and femur cortical vBMD, but null relationship between TBFM and 20% femur BSI were identified[17]; however, unlike Jump-In, we did not identify a positive association between TBFM and 4% tibia BSI. This difference may relate to our use of change in TBFM in this longitudinal study (versus baseline TBFM in Jump-In). Additionally, the Jump-In cohort was primarily comprised of non-Hispanic Caucasian girls (77%), unlike the STAR study which was a predominantly Hispanic cohort (70% Hispanic). Regardless, our current findings for 66% tibia SSI_p deviate from the findings of Wetzsteon et al.[16], which demonstrated a lack of correlation between change in mid-tibial SSI_p and change in fat mass after controlling for lean mass in the Action Schools! British Columbia (AS! BC) study. Differences in findings could be a result of differences in statistical design, length of follow-up (AS! BC: 16 months, STAR: 24 months), sex (AS! BC: males and females, STAR: females only), and/or race/ethnicity (AS! BC: primarily Asian cohort; STAR: primarily Hispanic cohort).

At the non-weight bearing site, the 66% radius, a statistically significant interaction between change in TBFM and menarcheal status on radius SSI_p was identified. Greater gains in

TBFM were negatively associated with change in bone strength in girls who were post-menarcheal at baseline, but favorably influenced bone strength in girls who were pre-menarcheal at baseline. We are not the first to identify that the onset of puberty and menarche, likely due to hormonal changes, may attenuate or reverse the positive relationship observed between fat and bone in earlier adolescence.[15] In a study by Wey et al. that assessed how changes in TBFM influenced 20% radius bone outcomes in Hutterite children[18], a significant interaction between TBFM and age on radius SSI_p was identified for females. This interaction showed that TBFM positively influenced radius SSI_p at younger ages but was detrimental to SSI_p at older ages. While menarche did not modify the relationship between TBFM and SSI_p in this study, Wey et al. observed a positive influence of menarche on the relationship between TBFM and cortical area, BMC, and vBMD at the radius[18], whereas we observed a negative association between menarche and change in TBFM and SSI_p in the STAR study. Differences in findings between the current study and prior study[18] may be at least partially explained by the greater baseline TBFM observed in our sample (15.3 ± 9.3 kg) compared to that of the 10-12 year-old girls in the sample from Wey et al. (10.5 ± 5.5 kg). It is well accepted that overweight and obesity is associated with early menarche[35], and despite similar ages between the studies at baseline (Bland et al.: 10.9 ± 1.1 years, Wey et al.: 11.0 ± 0.6 years), the present study had a higher percentage of post-menarcheal girls (20%) at the baseline sample than that from Wey et al. (5%).

Though we have focused on the natural trajectory of weight gain with growth, it is important to note that a supervised weight loss trial in adolescents did not significantly influence bone outcomes at the radius or tibia in obese adolescent girls.[36] Although there are notable limitations in comparing the two studies (e.g., differences in age, race/ethnicity, follow-up time, bone sites measured, study design), this indicates there may be a capacity for the skeleton to

413 respond to increasing adiposity during growth and development. Therefore, degree of adiposity
414 should be considered when assessing the relationship between fat and bone.
415 Our finding that greater gains in TBFM may be beneficial for cortical bone strength at the
416 tibia, yet potentially detrimental at the radius after the onset of menarche, is significant given the
417 high rate of forearm fractures in adolescents, with obese adolescents having more forearm
418 fractures than their normal weight counterparts.[6] From a mechanical standpoint, the tibial shaft
419 may strengthen and adapt to the strain and bending of bearing the greater body mass associated
420 with overweight and obesity, yet the non-weight bearing radius does not undergo the same
421 physiological strain. Therefore, overweight and obese adolescents may be particularly
422 susceptible to forearm fractures due to the higher force placed on the bone during a fall and
423 without a compensatory strengthening of the bone with regular loading. While we did not
424 measure the distal forearm in this study, the most common site of forearm fractures, our findings
425 at the 66% radius may help to provide insight into the relationship between obesity and fractures.
426 Furthermore, when comparing the relationship of TBFM on weight bearing versus non-
427 weight bearing bones, it is noteworthy that an interaction between TBFM and maturity (age,
428 menarcheal status) has only been identified at the radius. It is possible that this influence of
429 maturity and menarcheal status is more apparent in the bone outcomes at the radius due to the
430 non-weight bearing status of the bone, whereas the negative relationship between TBFM and
431 bone outcomes hypothesized in post-menarcheal and/or older girls may be offset by the positive
432 strain that increased fat mass (and associated increase in lean mass) places on weight bearing
433 bone. To our knowledge, we are the first to assess this interaction in the weight bearing bones, so
434 further studies are needed to validate this finding.
435

Cross-Sectional vs. Longitudinal

Much of the previous literature assessing the association between fat and bone is based on cross-sectional analyses due to the paucity of longitudinal data. Yet, findings from cross-sectional studies likely do not accurately reflect the influence of excess adiposity on bone over time, especially considering the importance of hormonal changes during puberty in females. There is little data assessing agreement between cross-sectional and longitudinal studies. In the study by Wey et al., cross-sectional analyses showed that higher fat mass was associated with smaller bone size, but longitudinal analyses showed that increased fat mass was associated with decreased cortical area but not significant differences in bone size.[18]

In a previously published analysis of cross-sectional data from the STAR study, Hetherington-Rauth et al. found that fat mass was positively associated with bone strength and size at the 66% tibia, bone size at the 20% femur and 4% tibia, and cortical thickness and BMC at the radius.[19] The present longitudinal analysis showed a positive relationship between change in TBFM and 66% tibia bone strength, after adjusting for TBLM, but change in TBFM did not significantly influence bone outcomes at the 20% femur nor 4% tibia after adjusting for TBLM. Additionally, our longitudinal analysis identified a significant relationship between change in TBFM on radius SSI_p (dependent on menarcheal status) that was not observed in the cross-sectional analysis. Consistent with the results of Hetherington-Rauth et al., we found that TBLM was a more reliable and stronger predictor of pQCT outcomes at all bone sites, and that there was potential for TBFM to negatively influence bone outcomes at the radius.

It should be noted that comparison of the cross-sectional and longitudinal analyses from the STAR study has inherent limitations due to differences in the statistical outcomes (i.e., baseline values used in cross-sectional analysis vs. change values used in longitudinal analysis).

Additionally, the cross-sectional study used maturity offset as an estimate of maturation while the present analysis used menarcheal status. Although this difference limited comparison, the ability to capture menarcheal transition in the longitudinal analysis was a strength of this study. The months surrounding menarche are a period of rapid bone accrual in females[37] and capturing this with a hard marker of maturity, rather than a proxy or estimate (e.g., maturity offset), was valuable considering that almost 50% of the STAR cohort transitioned from premenarcheal to post menarcheal during the 2-year follow-up.

Strengths and Limitations

Our study had many strengths, including the 2-year longitudinal study design that allowed us to observe changes in weight bearing and non-weight bearing bone development during the years surrounding the onset of menarche. Another strength was our use of pQCT, which allows for assessment of bone structural parameters and estimation of bone strength that cannot be ascertained from DXA imaging. Finally, our sample was primarily comprised of Hispanic girls, an understudied population with an increased risk for obesity.

This study also had some limitations. First, we utilized total body measures of soft tissue composition. TBLM was used as a surrogate measure for muscle strength and although the two measures are highly correlated, TBLM does not directly measure muscle strength. TBFM was used as a generalizable measure of adiposity but does not account for the physiological differences in adipose tissue depots, particularly visceral adipose tissue and intra-muscular fat. Finally, it is not possible to measure the exact same bone cross-section over time; however, as is standard practice, we used anatomical landmarks to measure the same site relative to the growth plate over time.

Conclusions and Future Directions

This study fills several gaps in knowledge regarding the relationship between adiposity and bone in adolescents by assessing the influence of changes in soft tissue composition on changes in bone strength in both weight bearing and non-weight bearing bones during the years surrounding the onset of menarche in a cohort of primarily Hispanic girls. In separate models, both change in TBLM and change in TBFM were positively associated with changes in bone outcomes. After controlling for both fat and lean mass in the same model, change in TBLM was the strongest soft tissue predictor of bone changes. Greater gains in TBFM may be favorable for change in tibia cortical bone strength, but detrimental for radius cortical bone strength after the onset of menarche. Further research utilizing longitudinal data is needed to better understand how adipose tissue distribution, metabolic biomarkers, and hormonal changes during puberty may impact the relationship between fat and bone, particularly in previously under-represented populations.

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Approving final version of manuscript: VB, JB, RB, DR, VL, JF, SG. VB takes responsibility for the integrity of the data analysis.

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FIGURES

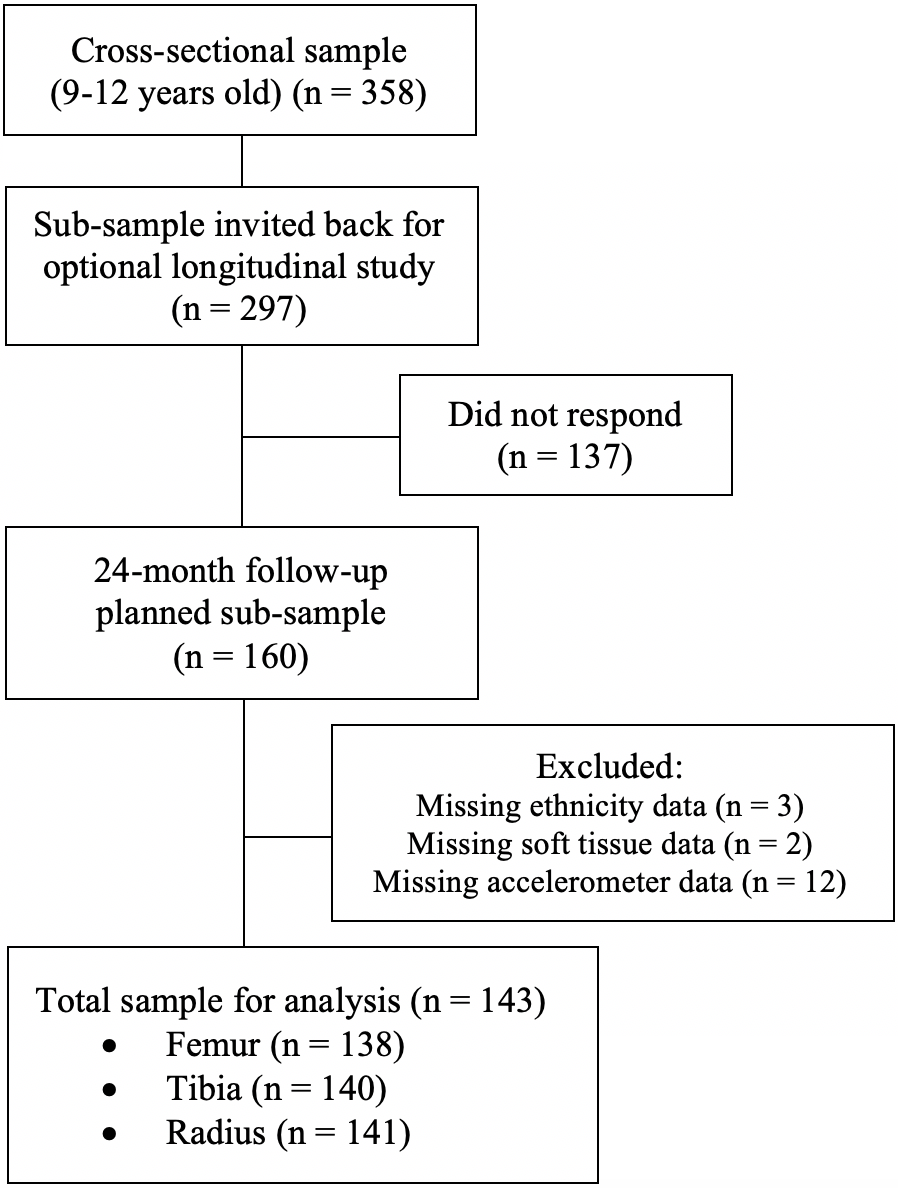
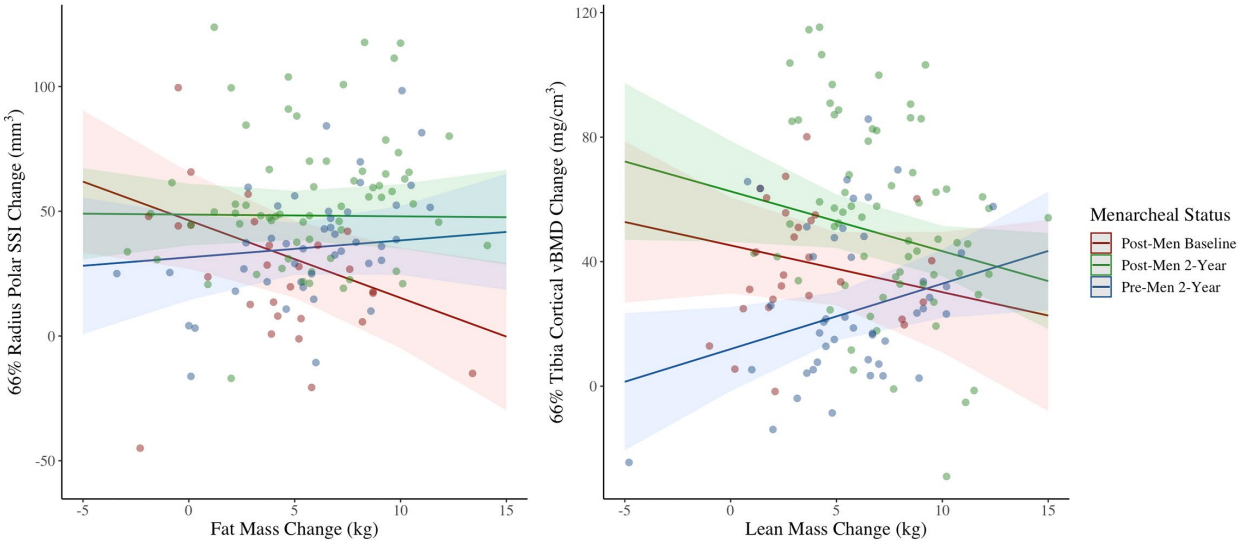


Figure 1: Flow diagram depicting stepwise process of sample size determination



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624 **Figure 2:** Interaction plots depicting the influence of menarcheal status on the relationship

625 between 2-year changes in soft tissue and bone outcomes with 95% confidence intervals

626

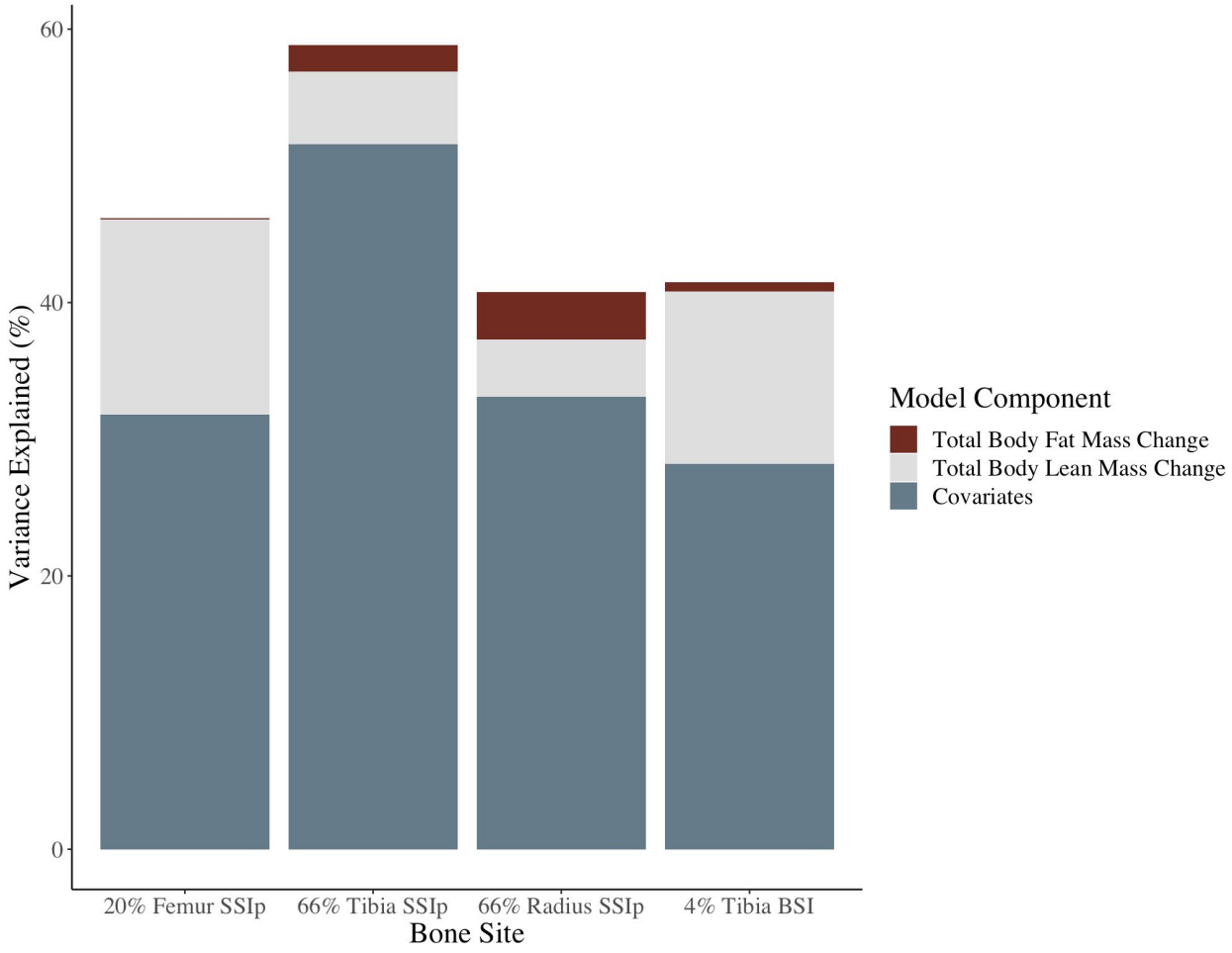

Figure 3: Variance explained by the covariates and predictor variables of interest for polar strength strain index (SSI_p) at the femur, tibia, and radius and bone strength index (BSI) at the tibia

Table 1: Characteristics of girls in the STAR Longitudinal Study (n = 143)

	Baseline	2-Years	Change	% Change
Age (years)	10.8 ± 1.1	12.9 ± 1.1	2.1 ± 0.1	---
Ethnicity (n (%) Hispanic)	100 (70%)	100 (70%)	---	---
Race (n (%))				
Caucasian	95 (66%)	95 (66%)	---	---
African American	5 (3%)	5 (3%)	---	---
American Indian/Alaska Native	4 (3%)	4 (3%)	---	---
Asian	3 (2%)	3 (2%)	---	---
Multiple Races	14 (10%)	14 (10%)	---	---
Not Indicated	22 (15%)	22 (15%)	---	---
Tanner breast stage (n: 1/2/3/4/5)	15/59/54/13/2	2/19/54/55/13	---	---
Tanner pubic hair (n: 1/2/3/4/5)	80/46/12/5/0	14/56/37/30/6	---	---
Post-menarche (n (%))	29 (20%)	98 (69%)	---	---
Height (cm)	146.9 ± 9.4	157.5 ± 7.8	10.6 ± 4.4	6.8
Weight (kg)	45.1 ± 14.5	57.0 ± 16.1	11.9 ± 6.5	21.1
BMI (kg/m^2)	20.6 ± 4.9	22.8 ± 5.4	2.2 ± 2.1	9.3
Weight category (n (%))				
Underweight	4 (3%)	4 (3%)	---	---
Normal weight	83 (58%)	75 (52%)	---	---
Overweight	22 (15%)	31 (22%)	---	---
Obese	34 (24%)	33 (23%)	---	---
Total body lean soft tissue mass (kg)	27.4 ± 5.9	33.4 ± 6.5	5.9 ± 3.3	17.6
Total body fat mass (kg)	15.3 ± 9.3	21.0 ± 10.3	5.7 ± 4.0	28.5
MVPA (min/day)	45.6 ± 16.6	38.2 ± 16.7	-7.4 ± 19.0	-33.6
Femur length (cm)	34.3 ± 2.8	37.8 ± 8.2	3.6 ± 7.7	8.3
Tibia length (cm)	34.7 ± 2.6	37.0 ± 2.2	2.3 ± 1.3	6.3
Forearm length (cm)	23.2 ± 1.8	24.8 ±1.5	1.6 ± 0.9	6.7
Femur 20% SSI_P (mm^3)	1648.1 ± 450.0	2179.6 ± 489.2	531.3 ± 243.6	24.6
Femur 20% cortical BMC (mg/mm)	199.2 ± 36.7	243.8 ± 37.2	44.5 ± 20.4	18.3
Femur 20% cortical vBMD (mg/cm^3)	1056.4 ± 29.6	1082.2 ± 36.1	25..6 ± 27.7	2.3
Femur 20% periosteal circumference (mm)	80.7 ± 8.8	88.7 ± 8.3	7.9 ± 3.7	9.0
Femur 20% endosteal circumference (mm)	64.4 ± 9.1	70.9 ± 8.9	6.5 ± 3.6	9.2
Femur 20% cortical thickness (mm)	2.6 ± 0.4	2.8 ± 0.4	0.2 ± 0.2	8.0
Tibia 66% SSI_P (mm^3)	1498.5 ± 399.8	1862.4 ± 418.9	363.9 ± 174.0	19.8
Tibia 66% cortical BMC (mg/mm)	210.4 ± 43.2	253.8 ± 42.0	43.8 ± 20.2	17.4
Tibia 66% cortical vBMD (mg/cm^3)	1033.6 ± 40.7	1075.9 ± 40.1	42.3 ± 29.4	3.9
Tibia 66% periosteal circumference (mm)	75.2 ± 7.1	79.9 ± 6.8	4.7 ± 3.0	5.9
Tibia 66% endosteal circumference (mm)	55.7 ± 7.3	58.2 ± 7.6	2.6 ± 3.5	4.3
Tibia 66% cortical thickness (mm)	3.1 ± 0.5	3.4 ± 0.5	0.3 ± 0.3	9.7
Radius 66% SSI_P (mm^3)	173.2 ± 49.7	216.2 ± 61.4	43.3 ± 29.2	19.4
Radius 66% cortical BMC (mg/mm)	55.8 ± 14.2	70.2 ± 15.3	14.5 ± 8.4	20.2
Radius 66% cortical vBMD (mg/cm^3)	1047.7 ± 48.8	1091.7 ± 50.9	43.9 ± 38.3	4.0
Radius 66% periosteal circumference (mm)	33.2 ± 3.5	35.2 ± 3.4	2.0 ± 2.2	5.6
Radius 66% endosteal circumference (mm)	20.8 ± 3.9	20.8 ± 3.7	-0.1 ± 2.2	-0.7
Radius 66% cortical thickness (mm)	2.0 ± 0.4	2.3 ± 0.4	0.3 ± 0.2	14.3
Tibia 4% BSI (mg^2/cm^4)	57.9 ± 16.2	74.2 ± 21.0	16.6 ± 9.7	21.4
Tibia 4% total area (mm^2)	626.6 ± 119.3	709.8 ± 116.6	84.2 ± 93.4	11.4
Tibia 4% total vBMD (mg/cm^3)	304.0 ± 40.0	323.2 ± 49.1	19.6 ± 27.0	5.5
Tibia 4% trabecular vBMD (mg/cm^3)	228.1 ± 28.0	236.2 ± 31.5	8.2 ± 17.7	3.1

Notes: Values presented as mean (SD) unless otherwise indicated. All change values significantly different ($p<0.05$) between baseline and 2-year follow-up, except radius endosteal circumference. Available data in bone outcomes at the femur 20% (n = 138), tibia 66% and 4% (n = 140), and radius (n = 141).

Abbreviations: *BMI* body mass index, *MVPA* moderate to vigorous physical activity, SSI_p polar strength strain index, *BMC* bone mineral content, *vBMD* volumetric bone mineral density, *BSI* bone strength index

Table 2: Standardized beta coefficients for changes in total body soft tissue composition from linear regression models for pQCT outcomes of bone strength (SSI_p, BSI), density (vBMD), and structure (BMC, PC, EC, Ct Th, Tt Area) at the femur (n = 138), tibia (n = 140), and radius (n = 141) for girls in the STAR longitudinal study

	Model 1		Model 2		Model 3			
	β Lean Δ	**p**	**β Fat Δ**	**p**	**β Lean Δ**	**p**	**β Fat Δ**	**p**
20% Femur								
Δ SSI_p	**113.03 (75.65, 150.35)**	**<0.0001**	**65.59 (29.93, 101.26)**	**0.0004**	**105.30 (56.58, 154.01)**	**<0.0001**	11.80 (-30.68, 54.28)	0.59
Δ Ct vBMD	**-7.32 (-11.21, -3.43)**	**0.0003**	0.60 (-2.99, 4.18)	0.74	**-11.17 (-16.09, -6.25)**	**<0.0001**	**6.02 (1.88, 10.16)**	**0.005**
Δ Ct BMC	**9.32 (6.06, 12.58)**	**<0.0001**	**6.51 (3.42, 9.59)**	**<0.0001**	**8.52 (4.29, 12.76)**	**0.0001**	2.09 (-1.53, 5.70)	0.26
Δ PC	**1.59 (1.11, 2.07)**	**<0.0001**	**0.83 (0.37, 1.30)**	**0.0006**	**1.60 (0.99, 2.22)**	**<0.0001**	0.00 (-0.52, 0.53)	0.99
Δ EC	**1.11 (0.58, 1.65)**	**<0.0001**	**0.59 (0.10, 1.09)**	**0.02**	**1.06 (0.36, 1.79)**	**0.003**	0.06 (-0.53, 0.64)	0.85
Δ Ct Th	**0.07 (0.03, 1.10)**	**0.0006**	0.03 (-0.00, 0.07)	0.05	**0.08 (0.03, 0.13)**	**0.002**	-0.01 (-0.05, 0.03)	0.69
66% Tibia								
Δ SSI_p	**43.62 (19.51, 67.72)**	**0.0005**	**45.26 (24.42, 66.09)**	**<0.0001**	26.67 (-3.71, 57.05)	0.09	**32.75 (6.67, 58.82)**	**0.02**
Δ Ct vBMD	Lean*Men1: -1.13 (-10.53, 8.28) **Lean*Men2: 12.62 (2.83, 22.42)**	0.81 **0.01**	0.21 (-2.95, 3.37)	0.90	Lean*Men1: -1.36 (-10.75, 8.02) **Lean*Men2: 11.76 (1.95, 21.56)**	0.77 **0.02**	0.64 (-3.20, 4.48)	0.74
Δ Ct BMC	**8.36 (5.28, 11.44)**	**<0.0001**	**5.36 (2.52, 8.20)**	**0.0003**	**7.62 (3.51, 11.72)**	**0.0004**	1.44 (-2.01, 4.89)	0.41
Δ PC	0.35 (-0.02, 0.71)	0.06	0.33 (0.00, 0.65)	0.05	0.25 (-0.23, 0.73)	0.31	0.22 (-0.19, 0.63)	0.29
Δ EC	-0.44 (-0.94, 0.06)	0.09	-0.07 (-0.53, 0.39)	0.76	-0.49 (-1.15, 0.18)	0.15	0.18 (-0.39, 0.74)	0.54
Δ Ct Th	**0.12 (0.08, 0.16)**	**<0.0001**	**0.06 (0.02, 0.10)**	**0.008**	**0.12 (0.06, 0.17)**	**0.0001**	0.00 (-0.05, 0.05)	0.96
66% Radius								
Δ SSI_p	**7.40 (1.88, 12.92)**	**0.01**	**FatΔ*Men1: 11.08 (0.16, 22.00)** **FatΔ*Men2: 16.65 (4.25,29.06)**	**0.048** **0.01**	**10.27 (2.82, 17.71)**	**0.008**	**Fat*Men1: 12.18 (1.44, 22.91)** **Fat*Men2: 15.18 (3.03, 27.33)**	**0.03** **0.02**
Δ Ct vBMD	-1.53 (-8.04, 4.99)	0.65	2.96 (-2.09, 8.01)	0.25	-2.26 (-10.76, 6.23)	0.60	3.67 (-2.74, 6.23)	0.26
Δ Ct BMC	**2.55 (1.01, 4.09)**	**0.001**	**1.66 (0.39, 2.93)**	**0.01**	**3.24 (1.23, 5.26)**	**0.002**	-0.02 (-1.53, 1.50)	0.98
Δ PC	**0.51 (0.10, 0.92)**	**0.02**	0.08 (-0.25, 0.41)	0.64	**0.82 (0.28, 1.36)**	**0.004**	-0.31 (-0.71, 0.08)	0.12
Δ EC	0.09 (-0.37, 0.56)	0.68	-0.23 (-0.58, 0.13)	0.21	0.35 (-0.25, 0.96)	0.25	-0.41 (-0.86, 0.04)	0.08
Δ Ct Th	**0.06 (0.02, 0.10)**	**0.007**	**0.05 (0.02, 0.08)**	**0.003**	**0.06 (0.00, 0.11)**	**0.04**	0.02 (-0.02, 0.06)	0.31
4% Tibia								
Δ BSI	**3.98 (2.43, 5.53)**	**<0.0001**	**2.92 (1.49, 4.34)**	**<0.0001**	**3.53 (1.48, 5.58)**	**0.001**	1.05 (-0.67, 2.77)	0.23
Δ Tt Area	**25.08 (11.41, 38.76)**	**0.0005**	5.61 (-6.91, 18.13)	0.38	**32.87 (14.88, 50.86)**	**0.0005**	-11.38 (-26.33, 3.57)	0.14
Δ Tt vBMD	**4.19 (0.04, 8.33)**	**0.049**	**5.48 (1.89, 9.07)**	**0.003**	1.71 (-0.12, 9.02)	0.54	4.45 (-0.12, 0.92)	0.06
Δ Tb vBMD	**5.88 (2.69, 9.07)**	**0.0004**	**3.72 (0.87, 6.56)**	**0.01**	**4.65 (0.38, 8.91)**	**0.03**	1.30 (-2.28, 4.87)	0.48

Notes: Model 1 includes Δ lean mass only, Model 2 includes Δ fat mass only, and model 3 includes both Δ lean and Δ fat mass. All models controlled for ethnicity, baseline age, menarcheal status, baseline bone, change in limb length, change in MVPA, and DXA machine. Models 1 and 3 additionally controlled for baseline lean mass. Models 2 and 3 additionally controlled for baseline fat mass. Menarcheal status coded as Men0 = post-menarche at baseline (reference group), Men1 = post-menarche at follow-up only, Men2 = pre-menarche at follow-up.

Abbreviations: *BMC* bone mineral content, *BSI* bone strength index, *Ct* cortical, *EC* endosteal circumference, *PC* periosteal circumference, SSI_p polar strength strain index, *Tb* trabecular, *Th* thickness, *Tt* total, *vBMD* volumetric bone mineral density