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Regional Fat Depots and their Relationship to Bone Density and Microarchitecture in Young Oligo-amenorrheic Athletes

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Abstract

Context—Various fat depots have differential effects on bone. Visceral adipose tissue (VAT) is deleterious to bone, whereas subcutaneous adipose tissue (SAT) has positive effects. Also, marrow adipose tissue (MAT), a relatively newly recognized fat depot is inversely associated with bone mineral density (BMD). Bone mass in athletes depends on many factors including gonadal steroids and muscle mass. Exercise increases muscle mass and BMD, whereas, estrogen deficiency decreases BMD. Thus, the beneficial effects of weight-bearing exercise on areal and volumetric BMD (aBMD and vBMD) in regularly menstruating (eumenorrheic) athletes (EA) are attenuated in oligo-amenorrheic athletes (OA). Of note, data regarding VAT, SAT, MAT and

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regional muscle mass in OA compared with EA and non-athletes (C), and their impact on bone are lacking.

Methods—We used (i) MRI to assess VAT and SAT at the L4 vertebra level, and cross-sectional muscle area (CSA) of the mid-thigh, (ii) 1H-MRS to assess MAT at L4, the proximal femoral metaphysis and mid-diaphysis, (iii) DXA to assess spine and hip aBMD, and (iv) HRpQCT to assess vBMD at the distal radius (non-weight-bearing bone) and tibia (weight-bearing bone) in 41 young women (20 OA, 10 EA and 11 C 18-25 years). All athletes engaged in weight-bearing sports for 4 hours/week or ran 20 miles/week.

Main Outcome Measures—VAT, SAT and MAT at L4; CSA of the mid thigh; MAT at the proximal femoral metaphysis and mid-diaphysis; aBMD, vBMD and bone microarchitecture.

Results—Groups had comparable age, menarchal age, BMI, VAT, VAT/SAT and spine BMD Z-scores. EA had higher femoral neck BMD Z-scores than OA and C. Fat mass was lowest in OA. SAT was lowest in OA ($p=0.048$); L4 MAT was higher in OA than EA ($p=0.03$). We found inverse associations of (i) VAT/SAT with spine BMD Z-scores ($r=-0.42$, $p=0.01$), (ii) L4 MAT with spine and hip BMD Z-scores ($r=-0.44$, $p=0.01$; $r=-0.36$, $p=0.02$), and vBMD of the radius and tibia ($r=-0.49$, $p=0.002$; $r=-0.41$, $p=0.01$), and (iii) diaphyseal and metaphyseal MAT with vBMD of the radius ($r=-0.42$, $p=0.01$) and tibia ($r=-0.34$, $p=0.04$). In a multivariate model including VAT/SAT, L4 MAT and thigh CSA, spine and hip BMD Z-scores were predicted inversely by L4 MAT and positively by thigh CSA, and total and cortical radius and total tibial vBMD were predicted inversely by L4 MAT. VAT/SAT did not predict radius or tibia total vBMD in this model, but inversely predicted spine BMD Z-scores. When L4 MAT was replaced with diaphyseal or metaphyseal MAT in the model, diaphyseal and metaphyseal MAT did not predict aBMD Z-scores, but diaphyseal MAT inversely predicted total vBMD of the radius and tibia. These results did not change after adding percent body fat to the model.

Conclusions—VAT/SAT is an inverse predictor of lumbar spine aBMD Z-scores, while L4 MAT is an independent inverse predictor of aBMD Z-scores at the spine and hip and vBMD measures at the distal tibia and radius in athletes and non-athletes. Diaphyseal MAT independently predicts vBMD measures of the distal tibia and radius.

Keywords

visceral fat; marrow fat; athletes; oligo-amenorrhea; BMD; microarchitecture

1. Introduction

Regional fat depots, such as subcutaneous, visceral and marrow fat, have been implicated in the regulation of bone mass at extremes of nutritional status and in older individuals. In adult females, visceral adipose tissue (VAT) has deleterious effects on bone, especially femoral total and cortical bone area, whereas subcutaneous adipose tissue (SAT) has a positive association with bone mass [1]. Similarly, in obese adolescent girls, VAT is negatively associated with whole body BMD [2], whereas SAT is positively associated with tibial total area [3]. Marrow adipose tissue (MAT) has recently been elucidated to have a common progenitor mesenchymal stem cell lineage with osteoblasts. Many osteoporotic states such as old age, diabetes and anorexia nervosa are associated with decreased bone

mineral density (BMD) and increased MAT [4]. Although certain transcription factors and the hormonal milieu have been implicated in the regulation of osteoblast and adipocyte differentiation, much remains unknown. Estrogen deficiency, a known cause of low BMD, increases adipocyte differentiation, and rodent studies have demonstrated a dose-related decrease in MAT following estrogen administration [5]. Furthermore, weight-bearing exercise, known to be beneficial to bone, is now postulated to be a modifier of MAT [6].

Bone mass in athletes depends on many factors including gonadal steroids and muscle mass. Exercise increases muscle mass and BMD, whereas, estrogen deficiency decreases BMD. Thus, the beneficial effects of weight-bearing exercise on areal and volumetric BMD (aBMD and vBMD) in regularly menstruating (eumenorrheic) athletes (EA) are attenuated in oligo-amenorrheic athletes (OA) [7, 8]. Of note, data regarding VAT, SAT, MAT and regional muscle mass in OA compared with EA and non-athletes, and their impact on bone are lacking.

Our objective was to evaluate visceral and subcutaneous adipose tissue and site-specific marrow fat in young OA compared with EA and non-athletes, and to determine associations of these regional fat depots with aBMD using DXA, and vBMD, bone size and structure using high resolution peripheral quantitative computed tomography (HRpQCT).

2. Methods

2.1 Subjects

We studied 41 females between the ages of 18-25 years (20 OA, 10 EA and 11 non-athletes). BMI was between the 10th-90th percentiles for subjects per study design. Oligoamenorrhea was defined as the absence of menses for 3 months within a period of oligomenorrhea (cycle length >6 weeks) for 6 months preceding enrollment. Eumenorrheic athletes (EA) had 9 menses (cycle length 21–35 days) in the preceding year. All EA in the study reported normal menstrual cycles since menarche. Per inclusion criteria, athletes were engaged in weight-bearing aerobic sports of the legs (such as track, soccer or field hockey) for 4 hours/week and/or ran 20 miles/week for 6 months preceding the study. For each athlete group, we also calculated the mean years of athletic activity (defined as 4 hours/week of athletic activity or 20 miles/week running or engagement in any team sport for 6 months of the year). None of the participants had a current history of anorexia nervosa. Subjects were recruited through advertisements in medical clinics, the Partners HealthCare system, local colleges and newspapers. Other causes of oligoamenorrhea (premature ovarian failure, hyperprolactinemia, thyroid dysfunction, and hyperandrogenism) and use of oral contraceptives were ruled out. Subjects on medications that affect bone metabolism (including estrogen-progesterone combination pills, glucocorticoids and anti-convulsants) except calcium and vitamin D in the preceding 3 months were excluded. The study was approved by the Institutional Review Board of Partners HealthCare. Written informed consent was obtained from all subjects.

2.2 Study Design

All subjects completed a medical history, physical examination and anthropometric measurements (weight, height and body mass index (BMI)) at a single study visit at the

Clinical Research Center of our institution. BMI was calculated as weight (kilograms)/(height (m)²). Exercise activity was assessed as hours/week of weight-bearing aerobic sports or running over the past year through a detailed history.

2.3 Areal Bone Mineral Density Assessment

DXA (Hologic QDR-Discovery A, Apex software version 13.3; Hologic Inc, Waltham, Massachusetts) was used to assess spine, total hip, femoral neck, and whole body areal BMD (aBMD), as well as body composition. The coefficients of variation for BMD, fat mass, and lean mass for this software are 0.8% to 1.1%, 2.1%, and 1.0%, respectively. The same scanner and software version were used for all participants.

2.4 Bone Microarchitecture Assessment

HRpQCT was used to measure volumetric BMD (vBMD), size parameters and microarchitecture at the distal radius and tibia (XtremeCT; Scanco Medical AG, Bassersdorf, Switzerland) with an isotropic voxel size of 82 μm^3 [9]. Measurements were performed at the non-dominant wrist and leg unless there was an acute fracture at those sites, in which case the non-fracture side was assessed. Outcome variables computed by automated analysis included area (mm^2) and vBMD (mgHA/cm^3) for total, trabecular, and cortical regions; cortical thickness (mm) and porosity (%), trabecular number (mm^{-1}), thickness (mm), and spacing (mm). In this paper, we report area, vBMD and cortical parameters. All HR-pQCT data were acquired on a single instrument by one operator, who performed standard evaluations (periosteal contouring). Short-term reproducibility, computed from repeat scans performed after repositioning on 25 healthy young subjects age 20- 30 years ranged from 0.2-1.7% for density values and from 0.7-8.6% for other variables, consistent with prior reports [9].

2.5 Bone Marrow Fat Assessment

Subjects underwent single voxel proton magnetic resonance spectroscopy (1H-MRS) of bone marrow at the L4 vertebral body, proximal femoral metaphysis and mid-femoral diaphysis to determine lipid content using a 3.0T MR imaging system (Siemens Trio, Siemens Medical Systems, Erlangen, Germany). For lumbar 1H-MRS, a voxel measuring 15×15×15mm (3.4 ml) was placed within the L4 vertebral body. Single-voxel 1H MRS data was acquired using point-resolved spatially localized spectroscopy (PRESS) pulse sequence without water suppression with the following parameters: TE of 30 ms, TR of 3,000 ms, 8 acquisitions, 1024 data points, and receiver bandwidth of 2000 Hz. For femoral 1H-MRS, a voxel measuring 12×12×12mm (1.7 ml) was positioned within the proximal femoral metaphyses in the intertrochanteric region, as well as the mid-diaphysis, and single voxel 1H-MRS using the same non-water suppressed PRESS pulse sequence was performed. Automated procedures for optimization of gradient shimming and transmit and receive gain were used. The coefficient of variation for marrow fat quantification is 5% [10].

The fitting of the 1H-MRS data was performed using LC Model software (version 6.1-4A) (Stephen Provencher, Oakville, ON, Canada). Data were transferred from the scanner to a Linux workstation, and metabolite quantification was performed using an eddy current correction and water scaling. A customized fitting algorithm for bone marrow analysis

provided estimates for all lipid signals combined (0.9, 1.3, and 2.3 ppm). LC Model bone marrow lipid estimates were automatically scaled to unsuppressed water peak (4.7 ppm) and expressed as lipid to water ratio (LWR).

2.6 Subcutaneous and Visceral Fat, and Thigh Muscle Cross Sectional Area Assessment

A single axial MR imaging slice through the abdomen at the level of L4 was obtained (Siemens Trio, 3T, Siemens Medical Systems, Erlangen, Germany) to determine abdominal subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT). A triplane gradient echo localizer pulse sequence with TE of 1.6 ms and TR of 49.0 ms was performed. For the abdomen, an axial T1-weighted image (fast spin-echo pulse sequence, 10 mm slice thickness, 40 cm field of view, TR 300 msec, TE 12 msec, echo train of 4, 512×512 matrix, 1 number of acquisitions) was prescribed through the mid portion of L4. A single slice through the mid thigh was also performed, equidistant to the femoral head and medial femoral condyle. Visceral and subcutaneous fat areas as well as mid-thigh muscle area were determined based on offline analysis of tracings obtained using commercial software (VITRAK; Merge/eFilm, Milwaukee, WI) [10]. Muscle cross sectional area (CSA) is believed to be a good surrogate for muscle force [11]. VAT and SAT are associated with bone density inversely and positively, respectively [1], and we used the ratio of VAT/SAT as an integrated measure of the two fat depots [2].

2.7 Statistical Analysis

We used JMP (version 10; SAS Institute, Inc., Cary, NC) for all analyses and report data as means $\pm$ SD. Data were evaluated for normality of distribution. We used analysis of variance to determine group differences when data were normally distributed, followed by the Dunnett test to control for multiple comparisons (with OA as the reference group). When data were not normally distributed, we used the Kruskal Wallis test to compare differences across groups followed by the Steel Dwass test to control for multiple comparisons. The Fisher's exact test was used to analyze differences between groups for categorical variables. When assessing associations of regional fat measures with clinical characteristics, BMD and microarchitecture parameters for the group as a whole, given the sample size of 41, we assumed normality based on the central limit theorem. Therefore, and also to better estimate the slope, we elected to use Pearson's correlations to determine these associations. Statistical significance was defined as a two-tailed p-value < 0.05 . Data are reported as means $\pm$ standard deviation (SD).

3. Results

3.1 Clinical Characteristics

OA did not differ from EA and non-athletes for age, menarchal age, and BMI (Table 1). Both groups of athletes had similar activity levels, and differed significantly from non-athletes for hours of exercise activity per study design. Also, we found no difference between the mean years of exercise between OA and EA groups (6.3 ± 2.8 vs 5.7 ± 1.8 , $p=0.55$). The median duration of amenorrhea in OA was 5.17 months (interquartile range 3-29.8 months).

3.2 Regional Fat Depots and Muscle Mass

Percentage of body fat and SAT were lower in OA compared with non-athletes (Table 2), whereas VAT and the ratio of VAT/SAT did not differ across groups. OA had higher marrow adipose tissue (MAT; measured as lipid to water ratio) at the L4 vertebra compared with EA. The groups did not differ for MAT at the femoral diaphysis or metaphyses. Although total lean mass did not differ across groups, percent lean mass was higher in the OA group compared to non-athletes. In addition, mid-femur muscle cross sectional area (CSA) was higher in athletes compared with non-athletes.

3.3 Bone Mineral Density, Size Parameters and Bone Microarchitecture

Compared to EA, OA had lower areal BMD Z-scores at the femoral neck, and trended to have lower BMD Z-scores at the total hip. Tibial trabecular density trended lower in OA than EA, whereas cortical area was lower in non-athletes than OA in this cohort, even after adjusting for age and height (Table 1).

3.4 Relationship between Regional Fat Depots, Clinical Characteristics and Body Composition

When evaluating the entire group, there was a trend towards a positive association with age (0.29, $p=0.07$), and a negative association with BMI ($r=-0.38$, $p=0.01$). No associations were observed between L4 MAT and menarchal age, lean mass, fat mass, SAT or VAT. Log metaphyseal MAT was positively associated with lean mass ($r=0.35$, $p=0.02$). VAT/SAT correlated inversely with BMI and percent body fat ($r=-0.41$, $p=0.007$). When assessing only oligoamenorrheic athletes, both log L4 MAT and diaphyseal MAT were positively associated with age ($r=0.54$, $p=0.02$ for both). Log L4 MAT was positively associated with duration of amenorrhea ($r=0.51$, $p=0.02$) and inversely with BMI ($r=-0.47$, $p=0.04$), percent body fat ($r=-0.45$, $p=0.048$) and VAT ($r=-0.47$, $p=0.04$).

3.5 Relationship between Regional Fat Depots, Femoral Muscle Area and Bone Parameters

For areal BMD measures, as shown in Table 3, when evaluating the entire group, inverse associations were observed between VAT/SAT and spine BMD Z-scores. Also, L4 MAT was associated negatively, and thigh muscle CSA positively with lumbar and hip BMD Z-scores. Even after controlling for possible confounders such as age, duration of amenorrhea or lean mass, observed associations persisted of VAT/SAT, L4 MAT and femoral muscle CSA with lumbar spine BMD Z-scores, and of femoral CSA with hip BMD Z-scores.

For HRpQCT parameters (Table 3), L4 MAT was negatively associated with total and cortical vBMD of the radius and tibia. Similarly, diaphyseal and metaphyseal MAT were inversely associated with total vBMD of the radius and tibia. At the radius, L4 MAT, diaphyseal MAT and metaphyseal MAT were inversely associated with cortical thickness, and L4 MAT with cortical area, whereas thigh muscle CSA, diaphyseal and metaphyseal MAT were positively associated with trabecular area. At the tibia, L4 MAT was inversely associated with cortical area and thickness, whereas thigh muscle CSA was positively associated with trabecular vBMD and with total, trabecular and cortical area. Metaphyseal

MAT was inversely associated with tibial trabecular vBMD. VAT/SAT did not predict HRpQCT parameters other than a trend for a negative association with radius cortical area.

L4 MAT remained inversely associated with (i) radius total and cortical vBMD, cortical area and thickness, and tibial total vBMD after controlling for age, duration of amenorrhea, BMI or lean mass, and with (ii) tibial cortical thickness after controlling for age, duration of amenorrhea or lean mass. Inverse associations with tibial cortical vBMD became significant after controlling for age or lean mass.

Diaphyseal MAT remained inversely associated with total vBMD of the radius and tibia and cortical thickness of the radius after controlling for duration of amenorrhea, BMI or lean mass, and with radius total vBMD after controlling for age as well. Positive associations with trabecular area persisted after controlling for duration of amenorrhea or BMI.

Metaphyseal MAT was associated inversely with total vBMD of the radius and tibia, and positively with radius total and trabecular area, after controlling for duration of amenorrhea or BMI. Other significant associations of MAT with bone parameters after controlling for confounders are indicated in Table 3.

After controlling for age, duration of amenorrhea and BMI simultaneously in a single multivariate model, L4 MAT was negatively associated with radius and tibial total and cortical vBMD, cortical thickness, and positively with radius trabecular area. Metaphyseal MAT was inversely associated with total vBMD of the radius and tibia. Metaphyseal and diaphyseal MAT were correlated positively with radius total and trabecular area. Femoral CSA remained positively associated with hip BMD Z-scores and total and trabecular area of the radius and tibia, and tibial cortical area.

When evaluating **only OA**, inverse associations were observed of (i) VAT/SAT with spine BMD Z-scores ($r = -0.68$, $p = 0.001$) and cortical area of the radius ($r = -0.51$, $p = 0.03$), (ii) L4 MAT with spine BMD Z-scores, radius cortical area ($r = -0.46$, $p = 0.046$), and tibial total vBMD, and cortical area and thickness ($r = -0.48$, $p = 0.03$), (iii) diaphyseal-MAT with radius total vBMD ($r = -0.50$, $p = 0.04$) and (iv) metaphyseal MAT with tibial total vBMD ($r = -0.51$, $p = 0.02$). Femoral CSA was associated positively with hip BMD Z-scores, and total and trabecular area of the radius and tibia ($r = 0.55$, $p = 0.01$).

3.6 Independent Associations of Regional Fat Depots and Femoral Muscle Cross Sectional Area with Bone Parameters

We next assessed whether the various fat depots and muscle mass predicted bone parameters independent of each other. Table 4 shows the various multivariate models for aBMD and vBMD measures. Data for area and thickness are not shown. In a multivariate model that included VAT/SAT, log L4 MAT and thigh muscle CSA for the group as a whole, spine BMD Z-scores were predicted inversely by VAT/SAT and log L4 MAT and positively by thigh muscle CSA, and hip BMD Z-scores were predicted inversely by log L4 MAT and positively by thigh muscle CSA. Total vBMD at the radius and tibia and cortical vBMD at the radius were predicted inversely by L4 MAT ($p < 0.05$). Radius cortical vBMD was predicted inversely by L4 MAT and thigh muscle CSA ($p < 0.05$). Thigh muscle CSA was a positive determinant of tibial trabecular vBMD and total, cortical and trabecular area

($p < 0.05$). Associations of L4 MAT with HRpQCT parameters persisted after controlling for percent body fat (not shown). VAT/SAT did not predict HRpQCT parameters in this model. When evaluating diaphyseal or metaphyseal MAT (instead of L4 MAT) in the model with VAT/SAT and thigh CSA, diaphyseal or metaphyseal MAT did not predict lumbar and hip aBMD Z-scores, but diaphyseal MAT inversely predicted radius and tibial total vBMD. Both MAT measures inversely predicted radius cortical thickness, and positively predicted radius and tibial total and trabecular cross sectional area ($p < 0.05$). Trends were observed for inverse associations with tibia cortical thickness. Metaphyseal MAT inversely predicted tibial trabecular vBMD in this model. Most associations held even after controlling for percent body fat.

4. Discussion

This is the first study to evaluate the relative impact of various fat depots (visceral, subcutaneous and marrow fat) and muscle mass on bone parameters in young athletes with exercise-induced oligo-amenorrhea in relation to eumenorrheic athletes and non-athletes. OA had higher femoral muscle CSA and lower SAT than non-athletes and higher L4 MAT than EA. We report positive associations of thigh muscle CSA, and inverse independent associations of VAT/SAT and L4 MAT with spine BMD. We also report inversely associations of L4, diaphyseal and metaphyseal MAT with multiple cortical structural parameters at the radius and tibia. Marrow adipose tissue at L4 is a stronger predictor of axial and appendicular structural parameters than marrow fat at femoral sites.

In this cohort, we found lower femoral neck BMD Z-scores and a trend for lower hip BMD Z-scores (site of primarily cortical bone) in OA compared with EA, although HRpQCT indicated lower trabecular vBMD at the distal radius in OA vs. EA, with no differences across groups for cortical parameters. While it is true that the femoral neck is a site of primarily cortical bone, it does also include trabecular bone. A report by Bohr and Schaadt quantified the femoral neck as containing 57% cortical bone and 43% trabecular bone [12]. In fact, studies in postmenopausal women report reduced bone trabecular volume in the femoral neck in osteoporosis in addition to decreases in cortical thickness [13] and that trabecular changes at the femoral neck contribute to changes in bone strength estimates independent of cortical changes [14]. Thus, changes in trabecular vBMD would be expected to impact areal BMD at the femoral neck, and may contribute to lower aBMD at this site despite a lack of differences across groups for cortical parameters. An important consideration is that HRpQCT measures were available at the distal tibia (and not the hip) and while both the tibia and the hip are sites of weight bearing and primarily cortical bone, changes at the distal tibia may not translate perfectly to changes at the hip.

Visceral and Subcutaneous Adipose Tissue and Bone

Lovejoy *et al.* have shown that menopausal (thus hypoestrogenic) women have higher VAT than premenopausal women [15]. The hypoestrogenic state in OA may thus contribute to lesser decreases in VAT (compared to SAT). Previous studies in other populations have reported positive associations of SAT with bone [3], whereas VAT and the ratio of VAT/SAT have inverse associations with bone [1, 2, 16], suggesting that the distribution of SAT vs. VAT is an important determinant of bone status. Specifically, Deere *et al.* [3]

showed that SAT is positively associated with periosteal circumference in young males and other investigators have reported inverse associations of VAT with bone in both healthy [3] and obese female adolescents [8]. Consistent with these reports, our study demonstrated an inverse association between VAT/SAT and lumbar BMD Z-scores in the group as a whole and within OA. As previously described by Gilsanz *et al.* in normal weight females, VAT is negatively and SAT is positively related to femoral CSA. Similarly, we found a trend for a negative correlation between VAT/SAT and cortical area in our subjects. The deleterious effect of VAT on bone may be a consequence of inflammatory cytokines and adipokines produced by visceral fat [8]. The presence of lower SAT in OA may also contribute to adverse bone changes seen in OA. Of note, VAT/SAT was an inverse predictor of lumbar spine BMD Z-scores, but not of other bone parameters in a multivariate model that included MAT and thigh CSA.

Bone Marrow Adiposity and Areal Bone Mineral Density

Athletic activity is a known powerful inducer of osteogenesis [17] and has recently been shown to impact mesenchymal stem cell differentiation, such that there is increased differentiation along the osteoblast vs. the adipocyte pathway. David *et al.* have demonstrated in a mouse model that mechanical loading decreases PPAR- γ expression, thereby decreasing adipogenesis [17]. Furthermore, marrow adiposity has been shown to decrease with exercise [2]. However, despite having similar exercise activity as EA, OA have lower BMD associated with higher marrow adiposity at the lumbar spine (weight-bearing site in runners and mostly trabecular bone). This supports the reciprocal relationship between adipogenesis and osteoblastogenesis reported in other osteoporotic states such as post-menopausal age, diabetes, and obesity [18]. This also suggests that the beneficial effect of exercise at the spine is counteracted by other factors that differ across the groups (such as the state of amenorrhea) leading to increased MAT. Interestingly, at the femur, an appendicular weight-bearing site, marrow adiposity appears to be preserved in OA despite lower bone density measures than in EA. In our study, although femoral diaphyseal and metaphyseal marrow fat was somewhat higher in OA compared with EA, this did not reach statistical significance.

Bone Marrow Adiposity and Bone Microarchitecture

Most studies have shown a negative association between MAT and trabecular bone [16, 19]. Our study supports findings by Wren *et al.* who reported negative associations of MAT and cortical cross-sectional area [20], although we did find positive associations of trabecular area with MAT, and inverse associations of metaphyseal MAT with tibial trabecular vBMD. There were stronger associations of lumbar MAT with microarchitectural parameters at the distal radius than at the distal tibia, and this may reflect (i) the non-weight-bearing effects at the radius given the inclusion criteria for this study (weight-bearing activities of the legs), with minimal benefits of exercise at this site, and (ii) the fact that spine BMD typically correlates strongly with radius BMD [21]. In contrast, although femoral diaphyseal and metaphyseal marrow fat did not differ across groups, MAT at these sites was inversely associated with total vBMD of the radius and tibia, and cortical thickness of the radius, sites of predominantly cortical bone (one weight-bearing and the other non-weight-bearing).

Hypoestrogenemia is a likely contributor to higher MAT at the spine in OA compared with EA, and higher marrow adiposity has previously been reported in other states of estrogen deficiency, such as postmenopausal osteoporosis and anorexia nervosa [22, 23]. Estrogen has bone antiresorptive effects and also stimulates mesenchymal stem cell differentiation towards osteoblastogenesis and away from adipogenesis [24, 25]. Estrogen deficiency is associated with decreased expression of SIRT1 which leads to increased PPAR- γ levels and decreased Runx2 expression in mesenchymal stem cells [26]. The increase in marrow adiposity in OA was positively associated with the duration of amenorrhea in these athletes, consistent with the reported impact of hypoestrogenemia (and its duration) on marrow adiposity. However, negative associations of L4 MAT with radius total and cortical vBMD, cortical area and cortical thickness; and tibial vBMD, cortical area and cortical thickness, persisted after controlling for duration of amenorrhea, suggesting that other paracrine/endocrine factors (independent of estrogen deficiency) may also contribute to these effects.

Another possible hormonal determinant of higher marrow fat in OA is lower leptin. Leptin stimulates osteogenesis and following leptin administration, BMD increases while MAT decreases [27]. We have previously reported lower leptin levels in OA compared with EA [28]. MAT appears to be regulated differently from other white adipose tissue depots, as suggested by studies in women with anorexia nervosa, who have lower fat mass (both subcutaneous and visceral) but higher MAT [4]. Of note, leptin levels correlate strongly with fat mass and particularly subcutaneous fat [28].

Bone Marrow Adiposity, Visceral and Subcutaneous Adipose Tissue, and Bone

Bredella et al. reported an inverse association between MAT and SAT in women with anorexia nervosa [10], and another study reported a positive correlation between MAT and VAT in obese premenopausal women [16]. In contrast to these studies, we found no association between MAT and SAT or VAT in our study of athletes and non-athletes, when all groups were taken together. A possible explanation for this difference is that our cohort included only normal-weight women, and thus our sample may not have had the variability necessary to demonstrate such associations. However, the oligo-amenorrheic athletes in our study did demonstrate inverse associations of L4 MAT with BMI, percent body fat and VAT.

In a multivariate model that included VAT/SAT, log L4 MAT and thigh CSA, areal and volumetric BMD were predicted inversely by L4 MAT and positively by thigh mid-femur muscle CSA. This suggests that bone marrow fat and muscle mass have independent effects on BMD at the spine, with MAT being deleterious to bone and mid-femur muscle CSA having positive effects. Similarly, for the whole group, diaphyseal and log metaphyseal MAT independently predicted vBMD measures. MAT measures at the metaphysis and diaphysis were positively associated with trabecular area and inversely with cortical thickness, suggestive of an increase in trabecular area at the cost of cortical thickness, as has been reported in other hypogonadal conditions consequent to increased endosteal bone resorption [29]. However, in our study, OA did not differ from the other groups for cortical thickness or trabecular area. Interestingly, L4 MAT was a stronger predictor of most bone parameters than was MAT at femoral diaphyseal and metaphyseal sites. This may reflect

more metabolically active bone at the spine than that at the hip [30, 31]. VAT/SAT did not predict HRpQCT bone parameters in this model, but remained a significant negative predictor of lumbar spine BMD Z-scores.

Of note, thigh muscle CSA is reported to be an excellent surrogate for muscle force [11, 32] and reflects whole body muscle strength [33]. Mid-femur muscle CSA was higher in athletes than non-athletes in our study. Positive effects of muscle CSA were evident at weight bearing sites, such as the lumbar spine and hip, using DXA measures, and at the weight-bearing tibia using HRpQCT for bone size parameters, namely total, cortical and trabecular bone area, and also trabecular volumetric BMD. However, positive associations were not evident of CSA with tibial cortical vBMD, and we speculate that this may be because of a delay in mineralization of the outwardly expanding cortex [34]. In fact, in the multivariate model that included VAT/SAT, MAT and thigh muscle CSA, muscle CSA was a positive predictor of tibial trabecular, but not cortical, vBMD. Weight bearing activity likely first leads to an increase in tibial cross-sectional area, followed by mineralization of the expanding tibial trabecular and cortical compartments. However, because the denser expanding cortex may take longer to mineralize than the spongy trabecular compartment, the expected positive association of muscle CSA with cortical vBMD may not be evident (as in our sample). In addition, while some impact of weight bearing activity is likely evident at the non-weight bearing radius consequent to circulating humoral factors, it is possible that mineralization of the distal radius lags behind that of the distal tibia. Preferential mineralization at distal tibia (compared to the distal radius) would be important in preserving bone strength at a site subject to continued weight bearing activities. As a consequence, however, mineralization may be delayed at the non-weight bearing distal radius. This may result in the observed negative association between thigh muscle CSA and radius cortical vBMD. DXA integrates information from cortical and trabecular compartments as well as size parameters, and positive associations of mid-femur muscle CSA with DXA measures of areal BMD at weight bearing sites (spine and femoral neck) likely reflect the sum total and positive effects of muscle CSA on bone.

The study is limited by sample size and inability to control for all potential confounders that may affect bone. Also, we cannot generalize our findings to all athletes as all our participants were athletes engaged in weight-bearing sports of the lower extremities alone. Furthermore, given the cross-sectional nature of the study, we cannot establish causality. Larger, prospective studies are necessary to evaluate changes in body fat composition in relation to bone in female athletes with oligo-amenorrhea, and the impact of estrogen replacement on these regional fat depots.

To conclude, we demonstrate, for the first time, increased spine marrow fat in oligo-amenorrheic athletes (which inversely predicts BMD and cortical thickness at weight bearing and non-weight bearing sites), and inverse associations of femoral marrow fat with volumetric BMD at weight bearing and non-weight bearing sites. Understanding effects of exercise and amenorrhea on marrow fat may result in a better understanding of the pathogenesis of bone fragility in female athletes, and eventually lead to novel therapies to treat bone fragility in this population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Oligoamenorrheic athletes have higher marrow adipose tissue (MAT) at the L4 vertebra and lower subcutaneous fat than eumenorrheic athletes.
- Spine BMD is associated negatively with visceral/subcutaneous fat ratio and L4 MAT and positively with thigh muscle cross-sectional area.
- L4, diaphyseal and metaphyseal MAT are inversely associated with volumetric BMD at radius and tibia.

Table 1
Clinical and Bone Characteristics of Oligo-amenorrheic Athletes (OA), Eumenorrheic Athletes (EA) and Non-athletes (NA)

Characteristics	OA (n=20)	EA (n=10)	NA (n=11)	P value
Age (years)	21.2±2.2	21.1±2.3	21.7±1.9	0.76
Bone Age (years)	18±0	18±0	17.9±0.32	0.23
Age at menarche (years)	13.3±1.8	12.0±1.6	12.6±0.82	0.10
Duration of Amenorrhea (months)**	5.17 (3-29.75)			
Height (cm)	162.7±6.7	162.7±5.3	164.0±9.0	0.87
Weight (kg)	56.37±7.58	60.32±6.80	60.96±11.50	0.29
BMI (kg/m ²)	21.2±2.1	22.8±2.3	22.5±2.4	0.13
Activity (hours/week)	9.7±5.5	8.9±3.6	1.2±2.4	0.003^b
Areal Bone Mineral Density (BMD)				
Lumbar spine BMD Z-scores	-0.58±0.94	-0.28±0.69	-0.43±1.09	0.71
Femoral neck BMD Z-scores	-0.28±0.90	0.65±0.71	-0.17±0.94	0.03^a
Total Hip BMD Z-scores	0.13±0.89	0.82±0.78	0.06±0.79	0.07
Microarchitecture CT radius				
Total volumetric bone mineral density(vBMD) (mgHA/cm ³)	312.4±48.0	325.2±58.1	319.1±53.7	0.82
Cortical vBMD (mgHA/cm ³)	846.1±40.9	854.0±40.7	871.4±38.0	0.28
Trabecular vBMD (mgHA/cm ³)	164.2±34.2	170.3±50.6	158.1±29.4	0.78
Total area (mm ²)	261.0±52.0	263.1±27.6	267.2±61.1	0.95
Cortical area (mm ²)	50.8±87	53.9±11.4	55.2±11.2	0.50
Trabecular area (mm ²)	210.4±56.1	202.4±33.2	209.1±57.0	0.93
Cortical Thickness (mm)	0.76±0.15	0.81±0.19	0.82±0.17	0.51
Microarchitecture CT tibia				
Total vBMD (mgHA/cm ³)	346.4±44.7	341.0±68.1	305.0±45.9	0.12
Cortical vBMD (mgHA/cm ³)	885.6±36.0	874.7±36.4	900.0±35.8	0.32
Trabecular vBMD (mgHA/cm ³)	201.5±28.3	215.0±48.1	177.4±24.6	0.05^a
Total area (mm ²)	646.4±127.4	703.1±66.5	664.9±163.1	0.55
Cortical area (mm ²)	131.1±18.9	133.3±27.3	111.9±27.0	0.04^{*b}
Trabecular area (mm ²)	515.6±132.9	571.1±82.4	550.6±147.7	0.52
Cortical Thickness (mm)	1.34±0.23	1.30±0.31	1.13±0.22	0.09

^a p<0.05 OA vs EA;

^b p<0.05 OA vs NA. Data presented as means ± SDs. Significant p values are bolded.

OA: oligomenorrheic athletes; EA eumenorrheic athletes; NA non-athletes ANOVA used for 3 group comparisons followed by Dunnett's testing when ANOVA was significant with OA as the comparison group.

* Non-parametric data. Kruskal Wallis was used for the 3 groups comparison followed by Steel Dwass test to control for multiple comparisons.

** Data presented as median + interquartile range

Table 2
Regional Fat Distribution Parameters in Oligo-amenorrheic Athletes (OA),
Eumenorrheic Athletes (EA) and Non-athletes (NA)

Characteristics	OA (n=20)	EA (n=10)	NA (n=11)	P value
Percent body fat (%)	23.7±5.3	27.1±4.1	33.2±5.6	<0.001^b
Total lean mass (kg)	44.88±12.47	42.48±3.51	39.16±7.65	0.31
Percentage lean mass (%)	72.9±5.3	69.4±4.1	63.5±5.2	<0.001^b
Visceral adipose tissue (VAT) (cm ²)	33.4±15.1	34.6±7.1	46.1±24.5	0.13
Subcutaneous adipose tissue (SAT) (cm ²)	110.0±61.5	147.0±70.1	178.4±91.0	0.048^b
VAT/SAT	0.33±0.10	0.27±0.11	0.27±0.09	0.19
Mid-femur muscle cross sectional area (cm ²)	1.37±0.21	1.41±0.19	1.18±0.25	0.03^b
L4 marrow adipose tissue (MAT) (Lipid/Water ratio)	0.89±0.53	0.48±0.22	0.62±0.28	0.03^a
Femoral metaphyseal MAT (Lipid/Water ratio)	4.72±3.33	2.32±1.03	3.68±1.89	0.18 [*]
Femoral diaphyseal MAT (Lipid/Water ratio)	6.13±3.60	4.04±2.13	6.34±2.58	0.16

^a p<0.05 OA vs EA;

^b p<0.05 OA vs NA. Data presented as means ± SDs. Significant p values are bolded.

^{*} Non-parametric values were evaluated by Kruskal Wallis Test, followed by the Steel Dwass test with OA as the comparison group.

ANOVA used for 3 group comparisons followed by Dunnett's testing when ANOVA was significant with OA as the comparison group.

Table 3
Associations of VAT/SAT, MAT and thigh muscle cross-sectional area (CSA) with bone parameters for all subjects

All Subjects (n=41)	VAT/SAT		Log L4 MAT		Log Metaphyseal MAT		Diaphyseal MAT		Thighmuscle CSA	
	r	p	r	p	r	p	r	p	r	p
BMD Z-scores										
Lumbar spine	-0.42	0.01 ^{a,b,d}	-0.44	0.01 ^{a,b,d}	-0.13	0.42	-0.18	0.27	0.40	0.01 ^{a,b,d}
Total Hip	-0.18	0.26	-0.36	0.02 ^{a,d}	-0.11	0.49	-0.09	0.60	0.53	0.0003 ^{a,b,c,d,e}
HRpQCT radius										
Total vBMD (mgHA/cm ³)	-0.02	0.92	-0.49	0.002 ^{a,b,c,d,e}	-0.42	0.01 ^{a,b,c,e}	-0.44	0.01 ^{a,b,c,d}	-0.12	0.49
Cortical vBMD (mgHA/cm ³)	-0.20	0.22	-0.42	0.01 ^{a,b,c,d,e}	-0.30	0.07	-0.26	0.12	-0.39	0.02 ^{a,b,c,d,e}
Trabecular vBMD (mgHA/cm ³)	0.06	0.71	-0.15	0.37	-0.18	0.28	-0.17	0.34	0.24	0.14 ^d
Total area (mm ²)*	-0.22	0.18	0.10	0.57	0.34	0.04 ^{b,c,e}	0.28	0.10 ^{c,e}	0.59	0.0001 ^{a,b,c,d,e}
Cortical area (mm ²)	-0.30	0.07	-0.52	0.001 ^{a,b,c,d,e}	-0.16	0.34	-0.24	0.15	0.12	0.47
Trabecular area (mm ²)*	-0.10	0.56	0.25	0.13 ^{c,e}	0.41	0.01 ^{a,b,c,e}	0.33	0.049 ^{b,c,e}	0.54	0.0004 ^{a,b,c,d,e}
Cortical thickness (mm)	-0.15	0.36	-0.57	0.0002 ^{a,b,c,d,e}	-0.34	0.04 ^c	-0.40	0.02 ^{b,c,d}	-0.15	0.37
HRpQCT tibia										
Total vBMD (mgHA/cm ³)	0.08	0.62	-0.41	0.01 ^{a,b,c,d,e}	-0.37	0.02 ^{a,b,c,d,e}	-0.34	0.04 ^{b,d}	0.17	0.30
Cortical vBMD (mgHA/cm ³)	-0.14	0.41	-0.31	0.06 ^{a,d,e}	-0.12	0.48	-0.08	0.63	-0.09	0.58
Trabecular vBMD (mgHA/cm ³)	0.04	0.80	-0.24	0.15	-0.33	0.04	-0.19	0.25	0.39	0.01 ^{a,b,d}
Total area (mm ²)*	-0.14	0.40	0.18	0.28	0.29	0.08	0.28	0.10 ^c	0.53	0.001 ^{a,b,c,d,e}
Cortical area (mm ²)	-0.09	0.56	-0.32	0.04 ^{b,d}	-0.10	0.56	-0.14	0.41	0.55	0.0003 ^{a,b,c,d,e}
Trabecular area (mm ²)	-0.08	0.64	0.23	0.16	0.30	0.07	0.30	0.07 ^c	0.43	0.01 ^{a,b,c,d,e}
Cortical thickness (mm)	-0.001	1.00	-0.39	0.01 ^{a,b,d,e}	-0.23	0.17	-0.27	0.10	0.26	0.11

^a p< 0.05 after controlling for age

^b p<0.05 after controlling for duration of amenorrhea

^c P<0.05 after controlling for BMI

^d p<0.05 after controlling for lean mass

p<0.05 after controlling for age, duration of amenorrhea and BMI

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Table 4
Stepwise Regressions of VAT/SAT, MAT (L4 MAT, metaphyseal MAT or diaphyseal MAT) and thigh muscle cross-sectional area (CSA) with bone parameters for all subjects

	Estimate	Std error	t-ratio	p-value	R ²	Estimate	Std error	t-ratio	p-value	R ²
DXA Lumbar Spine						DXA Hip				
L BMD Z-score				0.0002	0.41	Hip BMD Z-score			0.0004	0.38
VAT/SAT	-2.87	1.18	-2.42	0.02		VAT/SAT	1.16	-0.52	0.61	
Thigh muscle CSA	0.0001	5.14	2.53	0.02		Thigh muscle CSA	4.97	3.82	0.001	
<u>Log L4 MAT</u>	-1.15	0.44	-2.62	0.01		<u>Log L4 MAT</u>	0.43	-2.21	0.03	
L BMD Z-score				0.01	0.26	Hip BMD Z-score			0.01	0.29
VAT/SAT	-2.93	1.32	-2.22	0.03		VAT/SAT	1.20	-0.21	0.84	
Thigh muscle CSA	0.0001	5.69	2.44	0.02		Thigh muscle CSA	5.12	3.77	0.001	
<u>Metaphyseal MAT</u>	-0.02	0.05	-0.48	0.63		<u>Metaphyseal MAT</u>	0.04	-0.80	0.43	
L BMD Z-score				0.004	0.32	Hip BMD Z-score			0.004	0.31
VAT/SAT	-3.24	1.35	-2.40	0.02		VAT/SAT	1.31	-0.58	0.57	
Thigh muscle CSA	0.0002	5.62	2.59	0.01		Thigh muscle CSA	5.37	3.78	0.001	
<u>Diaphyseal MAT</u>	-0.05	0.04	-0.87	0.39		<u>Diaphyseal MAT</u>	0.04	-0.44	0.66	
HRpQCT RADIUS (total, cortical and trabecular)						HRpQCT TIBIA (total, cortical and trabecular)				
Total vBMD (mgHA/cm³)				0.01	0.28	Total vBMD (mgHA/cm³)			0.03	0.22
VAT/SAT	53.30	74.33	0.72	0.48		VAT/SAT	101.11	1.26	0.22	
Thigh muscle CSA	-0.003	0.003	-1.04	0.30		Thigh muscle CSA	0.003	0.003	1.01	0.32
<u>Log L4 MAT</u>	-97.72	27.53	-3.55	0.001		<u>Log L4 MAT</u>	-84.91	29.73	-2.86	0.01
Total vBMD (mgHA/cm³)				0.26	0.11	Total vBMD (mgHA/cm³)			0.19	0.13
VAT/SAT	65.73	83.24	0.79	0.45		VAT/SAT	122.54	1.44	0.16	
Thigh muscle CSA	-0.002	0.004	-0.56	0.58		Thigh muscle CSA	0.005	0.004	1.29	0.21
<u>Metaphyseal MAT</u>	-5.24	3.05	-1.72	0.10		<u>Metaphyseal MAT</u>	-5.34	3.11	-1.72	0.10
Total vBMD (mgHA/cm³)				0.03	0.25	Total vBMD (mgHA/cm³)			0.04	0.22
VAT/SAT	95.63	77.72	1.23	0.23		VAT/SAT	135.35	1.62	0.12	
Thigh muscle CSA	-0.002	0.003	-0.57	0.57		Thigh muscle CSA	0.005	0.003	1.44	0.16
<u>Diaphyseal MAT</u>	-7.67	2.49	-3.08	0.004		<u>Diaphyseal MAT</u>	-6.60	2.65	-2.49	0.02
Cortical vBMD (mgHA/cm³)				0.001	0.38	Cortical vBMD (mgHA/cm³)			0.22	0.12

Estimate	Std error	t-ratio	p-value	R ²	Estimate	Std error	t-ratio	p-value	R ²
VAT/SAT	54.80	-1.02	0.32		VAT/SAT	58.55	-0.56	0.58	
Thigh muscle CSA	0.008	-3.30	0.002		Thigh muscle CSA	0.003	-0.82	0.42	
<u>Log L4 MAT</u>	20.30	-3.15	0.003		<u>Log L4 MAT</u>	21.68	-1.85	0.07	
Cortical vBMD (mgHA/cm³)			0.03	0.23	Cortical vBMD (mgHA/cm³)			0.73	0.04
VAT/SAT	64.08	-1.16	0.26		VAT/SAT	63.83	-0.97	0.34	
Thigh muscle CSA	0.003	-2.45	0.02		Thigh muscle CSA	0.003	-0.54	0.60	
<u>Metaphyseal MAT</u>	2.34	-1.03	0.31		<u>Metaphyseal MAT</u>	2.33	-0.14	0.89	
Cortical vBMD (mgHA/cm³)			0.03	0.24	Cortical vBMD (mgHA/cm³)			0.91	0.02
VAT/SAT	60.78	-0.65	0.52		VAT/SAT	61.89	-0.27	0.79	
Thigh muscle CSA	0.003	-2.68	0.01		Thigh muscle CSA	0.003	-0.51	0.62	
<u>Diaphyseal MAT</u>	1.95	-1.56	0.13		<u>Diaphyseal MAT</u>	1.96	-0.41	0.68	
Trabecular vBMD (mgHA/cm³)			0.36	0.09	Trabecular vBMD (mgHA/cm³)			0.04	0.21
VAT/SAT	60.49	0.75	0.46		VAT/SAT	53.32	0.92	0.36	
Thigh muscle CSA	0.003	1.47	0.15		Thigh muscle CSA	0.002	2.55	0.02	
<u>Log L4 MAT</u>	22.41	-0.90	0.37		<u>Log L4 MAT</u>	19.74	-1.50	0.14	
Trabecular vBMD (mgHA/cm³)			0.29	0.11	Trabecular vBMD (mgHA/cm³)			0.01	0.30
VAT/SAT	58.71	1.36	0.18		VAT/SAT	46.78	2.01	0.05	
Thigh muscle CSA	0.003	1.52	0.14		Thigh muscle CSA	0.002	3.13	0.004	
<u>Metaphyseal MAT</u>	2.15	-0.99	0.33		<u>Metaphyseal MAT</u>	1.71	-2.39	0.02	
Trabecular vBMD (mgHA/cm³)			0.26	0.12	Trabecular vBMD (mgHA/cm³)			0.04	0.22
VAT/SAT	63.35	1.05	0.30		VAT/SAT	56.74	1.07	0.29	
Thigh muscle CSA	0.003	1.60	0.12		Thigh muscle CSA	0.002	2.69	0.01	
<u>Diaphyseal MAT</u>	2.03	-1.18	0.25		<u>Diaphyseal MAT</u>	1.79	-1.44	0.16	