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# Regional fat depots and their relationship to bone density and microarchitecture in young oligo-amenorrheic athletes

Vibha Singhal [a,b,1,*], Giovana D.N. Maffazioli [b,1], Natalia Cano Sokoloff [b], Kathryn E. Ackerman [b,c], Hang Lee [d], Nupur Gupta [e], Hannah Clarke [b], Meghan Slattery [b], Miriam A. Bredella [f], Madhusmita Misra [a,b]

[a] Pediatric Endocrine Unit, Massachusetts General Hospital for Children and Harvard Medical School, 175 Cambridge Street, Boston, MA 02114, USA
[b] Neuroendocrine Unit, Massachusetts General Hospital and Harvard Medical School, 55 Fruit Street, Boston, MA 02114, USA
[c] Division of Sports Medicine, Boston Children's Hospital, 319 Longwood Avenue, Boston, MA 02115, USA
[d] Department of Medicine, Massachusetts General Hospital and Harvard Medical School, USA
[e] Department of Adolescent Medicine, Massachusetts General Hospital and Harvard Medical School, 55 Fruit Street, Boston, MA 02114, USA
[f] Department of Radiology, Massachusetts General Hospital and Harvard Medical School, 55 Fruit Street, Boston, MA 02114, USA

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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 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 h/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 ($r \leq -0.42$, $p \leq 0.01$) and tibia ($r \leq -0.34$, $p \leq 0.04$). 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.





* Corresponding author at: 55 Fruit Street, Bulfinch Building (BUL 457B), Boston, MA 02114, USA.
*E-mail addresses:* vsinghal1@partners.org (V. Singhal), gmaffazioli@partners.org (G.D.N. Maffazioli), ncanosokoloff@partners.org (N.C. Sokoloff), keackerman@partners.org (K.E. Ackerman), hlee5@partners.org (H. Lee), ngupta3@mgh.harvard.edu (N. Gupta), clarke.hannahm@gmail.com (H. Clarke), mslattery@partners.org (M. Slattery), mbredella@partners.org (M.A. Bredella), mmisra@partners.org (M. Misra).
[1] Both authors have equal contribution.

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## 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 areas, 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 tissues 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).

## Methods

### Subjects

We studied 41 females between the ages of 18 and 25 years (20 OA, 10 EA and 11 non-athletes). BMI was between the 10th and 90th percentiles for subjects per study design. Oligomenorrhea 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 h/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 h/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 oligomenorrhea (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.

### 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.

### 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.

### 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 HRpQCT 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 to 1.7% for density values and from 0.7 to 8.6% for other variables, consistent with prior reports [9].

### 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 3.0 T MR imaging system (Siemens Trio, Siemens Medical Systems, Erlangen, Germany). For lumbar 1H-MRS, a voxel measuring 15 × 15 × 15 mm (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 3000 ms, 8 acquisitions, 1024 data points, and receiver bandwidth of 2000 Hz. For femoral 1H-MRS, a voxel measuring 12 × 12 × 12 mm (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).



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