ORIGINAL ARTICLE

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Red and White Blood Cell Counts Are Associated With Bone Marrow Adipose Tissue, Bone Mineral Density, and Bone Microarchitecture in Premenopausal Women

Sai Polineni,[1] Megi Resulaj,[1] Alexander T Faje,[1,2] Erinne Meenaghan,[1] Miriam A Bredella,[2,3] Mary Bouxsein,[2,4] Hang Lee,[2,5] Ormond A MacDougald,[6] Anne Klibanski,[1,2] and Pouneh K Fazeli[1,2,7]

[1]Neuroendocrine Unit, Massachusetts General Hospital, Boston, MA, USA
[2]Harvard Medical School, Boston, MA, USA
[3]Department of Radiology, Massachusetts General Hospital, Boston, MA, USA
[4]Center for Advanced Orthopaedic Studies, Beth Israel Deaconess Medical Center, Boston, MA, USA
[5]Biostatistics Center, Massachusetts General Hospital, Boston, MA, USA
[6]Department of Molecular and Integrative Physiology, University of Michigan Medical School, Ann Arbor, MI, USA
[7]Division of Endocrinology and Metabolism, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

ABSTRACT

Bone marrow adipose tissue (BMAT) resides within the bone marrow microenvironment where its function remains poorly understood. BMAT is elevated in anorexia nervosa, a disease model of chronic starvation, despite depletion of other fat depots. In addition to BMAT, the marrow microenvironment also consists of osteoblast and hematopoietic progenitors. BMAT is inversely associated with bone mineral density (BMD) in multiple populations including women with anorexia nervosa, and regulates hematopoiesis in animal models. We hypothesized that BMAT would be associated with circulating populations of hematopoietic cells (red and white blood cells) in humans and performed a post hoc analysis of two studies—a cross-sectional study and a longitudinal study—to investigate this hypothesis. We studied 89 premenopausal women cross-sectionally (median age [interquartile range], 27 [24.5, 31.7] years), including 35 with anorexia nervosa. We investigated associations between red blood cell (RBC) and white blood cell (WBC) counts and BMAT assessed by ^{1}H-magnetic resonance spectroscopy, BMD assessed by DXA, and bone microarchitecture assessed by HR-pQCT. In addition, we analyzed longitudinal data in six premenopausal women with anorexia nervosa treated with transdermal estrogen for 6 months and measured changes in BMAT and blood cell counts during treatment. Cross-sectionally, BMAT was inversely associated with WBC and RBC counts. In contrast, BMD and parameters of bone microarchitecture were positively associated with WBC and RBC. In women with anorexia nervosa treated with transdermal estrogen for 6 months, decreases in BMAT were significantly associated with increases in both RBC and hematocrit (rho = −0.83, $p = 0.04$ for both). In conclusion, we show that BMAT is inversely associated with WBC and RBC in premenopausal women, and there is a potential association between longitudinal changes in BMAT and changes in RBC. These associations warrant further study and may provide further insight into the role and function of this understudied adipose depot. © 2020 American Society for Bone and Mineral Research.

KEY WORDS: ANOREXIA NERVOSA; BONE MICROARCHITECTURE; BONE MINERAL DENSITY; HEMATOPOIESIS; MARROW ADIPOSE TISSUE

Introduction

The bone marrow consists of two types of stem cells: (i) mesenchymal stem cells, which differentiate into osteoblasts or adipocytes, and (ii) hematopoietic stem cells, which differentiate into red blood cells (RBCs), white blood cells (WBCs), or platelets. In murine models, osteoblasts are important regulators of hematopoiesis[1] and induced osteoblast-depletion results in a decrease in erythroid, lymphoid, and myeloid progenitors.[2] In humans, both RBC and WBC populations are associated with bone mineral density (BMD) and anemia has been shown to be an independent predictor of fracture.[3] Further, low hemoglobin levels are associated with low BMD in older adults,[4] and higher rates of bone loss at the hip have been associated with both an increased risk of anemia as well as low lymphocyte counts in men 65 years or older.[5]

Bone marrow adipose tissue (BMAT), another component of the bone marrow microenvironment, is also associated with bone parameters in humans. BMAT is inversely associated with BMD in populations of healthy adults[6,7] and also in a model of

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Address correspondence to: Pouneh K Fazeli, MD, MPH, Division of Endocrinology and Metabolism, University of Pittsburgh School of Medicine, 200 Lothrop Street, BST W1061 Pittsburgh, PA 15213, USA. E-mail: pkfazeli@pitt.edu
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chronic starvation, anorexia nervosa.[8] Women with anorexia nervosa have higher levels of BMAT compared to normal-weight controls despite having significantly lower levels of subcutaneous and visceral adipose tissue depots.[8] Although the function of BMAT is not currently known, the fact that this adipose tissue depot is preserved during chronic starvation, when other fat depots are being used as a source of energy, suggests it may have an important role.

In murine models, granulocyte-colony stimulating factor, which leads to an increase in circulating neutrophils, has been associated with reductions in BMAT[9] and BMAT has also been associated with reduced hematopoiesis.[10] Therefore, to better understand the potential function and determinants of BMAT in humans, we explored the association between BMAT and cells from the hematopoietic lineage, specifically RBC and WBC counts in premenopausal women using both cross-sectional and longitudinal study designs. We hypothesized that BMAT would be negatively associated with cells from the hematopoietic lineage.

Materials and Methods

Subjects

We performed post hoc analyses of two prior studies. The first was a study investigating levels of BMAT in premenopausal women, inclusive of women with anorexia nervosa, normal-weight controls, and obese women.[11–14] The second study was a longitudinal study investigating the effects of low-dose, transdermal estrogen on BMD and BMAT in premenopausal women with anorexia nervosa.[15] Eighty-nine premenopausal women were studied cross-sectionally, inclusive of 35 women with anorexia nervosa (median age [interquartile range], 26.8 [24.6, 31.0] years; range, 20.3 to 45.1 years). In addition, we studied six women with anorexia nervosa (median age [interquartile range], 34.3 [28.5, 45.4] years; range, 25.3 to 45.5 years) longitudinally for 6 months, during which time they were treated with low-dose, transdermal estrogen (Climara Pro; Bayer Pharmaceuticals, Whippany, NJ, USA) a treatment that has been associated with a reduction in BMAT in postmenopausal and premenopausal women.[15,16] This particular transdermal formulation contains 0.045 mg of estradiol/day and 0.015 mg of levonorgestrel/day, a progesterone, which is necessary for the prevention of endometrial hyperplasia in women with an intact uterus. As previously described,[15] this formulation was chosen because it contains a dose of estradiol that we hypothesized would be effective in improving BMD based on data in postmenopausal women.[17] BMAT and/or bone parameters from a subset of subjects were previously reported.[11–15,18] Hematologic data have not been previously reported. Subjects were recruited either through online advertisements or through referrals from local eating disorder providers for both the cross-sectional and longitudinal studies. Subjects with anorexia nervosa met Diagnostic and Statistical Manual of Mental Disorders (DSM-5) criteria for the disorder.[19] Subjects without anorexia nervosa had a BMI > 18.5 kg/m^2 (range, 20.8 to 41.7 kg/m^2), none reported a past or current history of an eating disorder, none were taking medications known to affect bone mass, and all reported having regular menstrual cycles. Participants who had abnormal thyroid function tests or chronic diseases that affect BMD (other than anorexia nervosa) were excluded.

Cross-sectional study

All subjects were evaluated at the Translational and Clinical Research Center at the Massachusetts General Hospital during two study visits: a screening visit and a baseline study visit. All subjects had a complete blood count inclusive of RBC count, WBC count, hemoglobin and hematocrit levels, and a platelet count at the time of their screening visit, performed by a clinical laboratory (LabCorp, Burlington, NC, USA). Subjects then returned for a study visit within 3 months of their screening visit, at which time they were weighed on an electronic scale while wearing a hospital gown, their height was measured on a single stadiometer as the average of three readings, and radiologic imaging, including dual-energy X-ray absorptiometry (DXA), ^{1}H-magnetic resonance spectroscopy (^{1}H-MRS), and high-resolution peripheral quantitative CT (HR-pQCT), was performed (radiologic imaging is described below in the Radiologic Imaging section).

Longitudinal study

All subjects were evaluated at the Translational and Clinical Research Center at the Massachusetts General Hospital for baseline and 6-month study visits. After the baseline visit, subjects were started on low-dose, transdermal estrogen/progesterone and they continued using the patch until their 6-month study visit. At each study visit, all six subjects had a complete blood count, inclusive of RBC count, WBC count, hemoglobin and hematocrit levels, and a platelet count, and ^{1}H-MRS (described in the ^{1}H-MRS section below) performed.

The study complied with the Health Insurance Portability and Accountability Act guidelines and was approved by the Partners Institutional Review Board. All subjects provided written informed consent prior to the initiation of study procedures.

Radiologic imaging

DXA

All subjects underwent DXA to measure areal BMD of the posterior-anterior (PA) lumbar spine (L_1–L_4), lateral spine (L_2–L_4), left total hip, left femoral neck, and total body using a Hologic Discovery A densitometer (Hologic Inc., Bedford, MA, USA). Coefficients of variation (CVs) for the measurement of BMD by DXA have been reported to be less than 2.2%.[20]

High resolution peripheral quantitative computed tomography (HR-pQCT)

HR-pQCT (isotropic voxel size of 82 μm^3) was performed in the nondominant distal radius and tibia (Xtreme CT; Scanco Medical AG, Brüttisellen, Switzerland) as previously described.[21] As previously described,[22,23] linear micro-finite element analysis of HR-pQCT images was subsequently performed in order to estimate the biomechanical properties of the distal radius and the distal tibia under uniaxial compression loading. Outcomes from linear micro-finite element analysis included both compressive stiffness (kN/mm) and failure load (kN). The biomechanical properties estimated from micro-finite element analysis are strongly correlated with those measured through the ex vivo testing of elderly human cadaveric radii.[24]

^{1}H-MRS

The Metabolic Imaging Core of the Nutrition and Obesity Research Center at Harvard performed ^{1}H-MRS as previously described.[8] Briefly, lipid content of the L_4 vertebra, proximal femoral epiphysis, metaphysis, and mid-diaphysis was measured using ^{1}H-MRS (Siemens Trio, 3T; Siemens Medical Systems, Erlangen, Germany). This was performed by placing a voxel measuring $15 \times 15 \times 15$ mm^3 (3.4 mL) within the body of the L_4 vertebra and then using point-resolved spatially localized spectroscopy (PRESS) pulse sequence without water suppression (parameters: echo time [TE] of 30 ms, repetition time [TR] of 3000 ms, eight acquisitions, 1024 data points, and receiver bandwidth of 2000 Hz), single-voxel ^{1}H-MRS data were obtained. Similarly, in the femur, single-voxel ^{1}H-MRS, using the same non-water suppressed PRESS pulse sequence, was performed in the femur after a $12 \times 12 \times 12$ mm^3 (1.7 mL) voxel was placed in the proximal femoral epiphysis and repeated after voxel placement in the mid-diaphysis and the intertrochanteric region of the femoral metaphysis. Automated procedures were used to optimize gradient shimming and transmit and receive gain. We have reported that the CV for marrow fat quantification, obtained by scanning five subjects two times each, is 3%.[25]

^{1}H-MRS data were fitted using LCModel software (version 6.1-4A; Stephen Provencher, Oakville, ON, Canada). Metabolite quantification was obtained using eddy current correction and water scaling after data were transferred to a Linux workstation. A fitting algorithm customized for analysis of bone marrow was used to provide estimates for all of the lipid signals combined (0.9, 1.3, and 2.3 ppm). The lipid estimates for bone marrow using LCModel software were automatically scaled to an unsuppressed water peak (4.7 ppm) and results were expressed as a lipid to water ratio.

Statistical analysis

JMP Pro 13.0 (SAS Institute, Inc., Cary, NC, USA) software was used to perform statistical analyses. The Student's *t* test was used to compare means and standard deviations (SDs) unless data were not normally distributed, in which case the Wilcoxon test was used to compare median (interquartile range). Univariate associations were assessed using Pearson correlation coefficients (R), or if data were non-normally distributed, Spearman coefficients (rho). Spearman coefficients were calculated in the analysis of the longitudinal data, given the small sample size ($n = 6$). A p value <0.05 was used to indicate significance.

Results

Clinical characteristics

Clinical characteristics of the study subjects, including BMD and bone microarchitecture data, are listed in Table 1. Subjects were all premenopausal. Subjects in the cross-sectional study were a median of 27 years of age (median age [interquartile range], 27 [24.5, 31.7] years; range, 20.0 to 45.7 years). Subjects in the longitudinal study had anorexia nervosa for a median of 13 years (range, 3 to 17 years) and had amenorrhea for a median of 135 months (range, 34 to 204 months). BMAT was inversely associated with BMD, parameters of bone microarchitecture, and bone strength (Table 2).

Association between blood count parameters and BMAT

BMAT at the vertebra and femur was inversely associated with WBC and/or RBC counts. In particular, BMAT at the L_4 vertebra was significantly inversely associated with WBC count (rho = −0.37, $p = 0.0003$) (Fig. 1*A* and *B*). BMAT at the femoral diaphysis and femoral metaphysis were both significantly and inversely associated with WBC and RBC counts (Table 3). There was a trend toward an inverse association between BMAT at the femoral diaphysis and both hemoglobin and hematocrit levels (rho = −0.20, $p = 0.057$ for both). There were no significant associations between BMAT and platelet count.

When we divided the group based on diagnosis/BMI: anorexia nervosa ($n = 35$), normal-weight ($n = 28$), and overweight/obese ($n = 26$), there were no significant associations between BMAT and any hematologic parameters in the women with anorexia nervosa or normal-weight women. In the women who were overweight/obese, in contrast to what was observed in the group as a whole, there was a significant positive association between L_4 vertebral BMAT and RBC count (rho = 0.43, $p = 0.03$) and between BMAT at the femoral metaphysis and WBC count (rho = 0.46, $p = 0.02$). There was also a positive association between BMAT at the femoral metaphysis and platelet count in this group ($R = 0.48$, $p = 0.01$).

Association between blood counts and bone parameters

BMD

There were significant and positive associations between WBC and RBC counts and BMD at the spine and hip (Table 4). BMD at the PA spine was significantly associated with RBC count (rho = 0.32, $p = 0.002$) and WBC count (rho = 0.45, $p < 0.0001$). There were no significant associations between hemoglobin or hematocrit and BMD. Platelet count was positively associated with both total hip BMD ($R = 0.21$, $p = 0.04$) and femoral neck BMD ($R = 0.21$, $p = 0.04$).

Bone microarchitecture and estimated bone strength

Parameters of bone microarchitecture and estimated bone strength (stiffness and failure load) at the radius and tibia were significantly associated with WBC and RBC counts (Table 4). WBC count was positively associated with estimates of failure load at the radius (rho = 0.40, $p < 0.0001$) and tibia (rho = 0.41, $p < 0.0001$) (Fig. 2*A-D*). RBC count was also positively associated with estimates of failure load at both the radius (rho = 0.26, $p = 0.01$) and tibia (rho = 0.24, $p < 0.03$). There were no significant associations between microarchitecture parameters and hemoglobin or hematocrit levels. Platelet count was only associated with trabecular bone volume fraction at the radius (rho = 0.24, $p < 0.03$) and trabecular thickness at the radius (rho = 0.22, $p < 0.04$).

Association between changes in blood counts and changes in BMAT

Treatment with transdermal estrogen is associated with changes in BMAT in both postmenopausal women and premenopausal women.[15,16] In an exploratory analysis, we measured complete blood counts at baseline and after 6 months of treatment with transdermal estrogen in six amenorrheic women with anorexia nervosa. Changes in BMAT at the femoral diaphysis were significantly inversely associated with changes in red blood cell count over the 6-month study (Fig. 3*A* and *B*) and with changes in

Table 1. Clinical Characteristics of Participants in Cross-Sectional Study and Baseline Characteristics of Subjects With Anorexia Nervosa in Longitudinal Study

Characteristic	Cross-sectional study participants (n = 89)	Longitudinal study participants (n = 6)
Age (years)	27.0 (24.5, 31.7); range, 20–45.7	35.7 ± 3.5 (range, 25.3–45.5)
BMI (kg/m^2)	22.1 (17.8, 30.8)	17.1 ± 1.4
BMD		
Posterior-anterior spine BMD (g/cm^2)	0.955 (0.854, 1.078)	0.808 ± 0.111
Lateral spine BMD (g/cm^2)	0.743 ± 0.149	0.628± 0.109
Total hip BMD (g/cm^2)	0.919 ± 0.170	0.739± 0.093
Femoral neck BMD (g/cm^2)	0.799 ± 0.159	0.647± 0.087
Total body BMD (g/cm^2)	1.020 ± 0.097	0.982 ± 0.052
Bone marrow adipose tissue		
L_4 vertebra (lipid/water)	0.54 (0.40, 0.87)	1.18 ± 0.42
Femoral epiphysis (lipid/water)	7.25 ± 2.90	8.17 ± 2.17
Femoral diaphysis (lipid/water)	4.84 (2.61, 7.09)	7.58 ± 1.71
Femoral metaphysis (lipid/water)	3.18 (2.04, 4.89)	5.77 ± 1.96
Microarchitecture parameters at radius		
Trabecular BV/TV (%)	0.128 (0.109, 0.151)	0.108 ± 0.024
Trabecular number (1/mm)	1.91 (1.71, 2.12)	1.67 ± 0.24
Trabecular thickness (mm)	0.066 (0.060, 0.074)	0.061 (0.057, 0.072)
Trabecular separation (mm)	0.452 (0.407, 0.520)	0.546 ± 0.093
Cortical thickness (mm)	0.77 ± 0.18	0.61 ± 0.08
Estimates of bone strength at radius		
Stiffness (kN/mm)	71.1 ± 15.8	60.0 ± 2.6
Failure load (kN)	3.6 ± 0.8	2.9 ± 0.2
Microarchitecture parameters at tibia		
Trabecular BV/TV (%)	0.142 ± 0.038	0.115 ± 0.028
Trabecular number (1/mm)	1.87 ± 0.42	1.70 (1.49, 1.81)
Trabecular thickness (mm)	0.076 ± 0.013	0.071 ± 0.008
Trabecular separation (mm)	0.458 (0.398, 0.540)	0.510 (0.483, 0.638)
Cortical thickness (mm)	1.17 (0.98, 1.33)	0.97 ± 0.22
Estimates of bone strength at tibia		
Stiffness (kN/mm)	200.4 ± 44.9	165.6 ± 23.9
Failure load (kN)	10.1 ± 2.2	8.2 ± 1.3
Hematologic parameters		
White blood cell count (×10E3/μL)	5.6 (4.7, 7.1)	4.4 ± 1.0
Red blood cell count (×10E6/μL)	4.30 ± 0.34	4.21 ± 0.42
Hemoglobin (g/dL)	12.9 ± 1.0	13.1 ± 1.0
Hematocrit (%)	38.3 ± 2.7	38.9 ± 3.2
Platelet count (×10E3/μL)	254 ± 60	247 ± 63

Values are mean ± SD, or median (interquartile range) when data were not normally distributed. BMD = bone mineral density.

hematocrit level (Fig. 3*C* and *D*). There were no significant associations between change in WBC count and change in BMAT.

Discussion

In this exploratory analysis, we have shown that BMAT, a component of the bone marrow microenvironment, is inversely associated with RBC and WBC counts in a population of premenopausal women. Although the function of BMAT is not known, it is inversely associated with bone parameters and therefore may have a role in mineral metabolism. We now show that there may be an association between BMAT and hematopoietic cells in humans, suggesting a possible role for BMAT in hematopoiesis.

Although BMAT, a component of the bone marrow microenvironment, has been associated with low bone density,[6–8] its function has not been fully described. Paradoxically, in models of chronic starvation, such as anorexia nervosa, although subcutaneous and visceral adipose tissue stores are reduced, levels of BMAT are elevated.[8] Why lipid would be stored in the marrow while other adipose tissue depots are actively being utilized as an energy source during periods of caloric deprivation is not known, but understanding this paradox may allow us to better understand the function of this fat depot.

Given the inverse association between marrow adiposity and bone density, BMAT has been hypothesized to be an important determinant of the low BMD and increased fracture risk observed in women with anorexia nervosa.[26] BMAT is associated with decreased bone integrity[27] and BMAT is inversely associated with finite element analysis–derived estimates of bone strength both in adolescents with anorexia nervosa[28] and, as our data here show, also in premenopausal adult women, but whether BMAT is a causative factor of the increased fracture risk observed in anorexia nervosa remains to be determined.

In addition to marrow adipocytes, the bone marrow microenvironment also consists of osteoblast and hematopoietic

Table 2. Univariate Associations Between BMAT at the Spine and Hip and Bone Parameters in Cross-Sectional Study Participants ($n = 89$)

Parameter	BMAT at L_4 vertebra (lipid/water)	BMAT at femoral epiphysis (lipid/water)	BMAT at femoral diaphysis (lipid/water)	BMAT at femoral metaphysis (lipid/water)
BMD				
Posterior–anterior spine BMD (g/cm^2)	rho = −0.49; $p < 0.0001$	NS	rho = −0.41; $p < 0.0001$	rho = −0.60; $p < 0.0001$
Lateral spine BMD (g/cm^2)	rho = −0.57; $p < 0.0001$	NS	rho = −0.31; $p < 0.004$	rho = −0.62; $p < 0.0001$
Total hip BMD (g/cm^2)	rho = −0.55; $p < 0.0001$	NS	rho = −0.40; $p = 0.0001$	rho = −0.63; $p < 0.0001$
Femoral neck BMD (g/cm^2)	rho = −0.54; $p < 0.0001$	NS	rho = −0.40; $p < 0.0001$	rho = −0.63; $p < 0.0001$
Total body BMD (g/cm^2)	rho = −0.51; $p < 0.0001$	NS	rho = −0.34; $p = 0.001$	rho = −0.52; $p < 0.0001$
Microarchitecture parameters at radius				
Trabecular BV/TV (%)	rho = −0.42; $p < 0.0001$	rho = −0.27; $p = 0.01$	rho = −0.31; $p < 0.004$	rho = −0.50; $p < 0.0001$
Trabecular number (1/mm)	rho = −0.30; $p = 0.004$	NS	rho = −0.23; $p = 0.03$	rho = −0.43; $p < 0.0001$
Trabecular thickness (mm)	rho = −0.32; $p = 0.002$	rho = −0.23; $p = 0.03$	rho = −0.22; $p = .04$	rho = −0.30; $p < 0.005$
Trabecular separation (mm)	rho = 0.34; $p = 0.001$	NS	rho = 0.27; $p = 0.01$	rho = 0.47; $p < 0.0001$
Cortical thickness (mm)	rho = −0.45; $p < 0.0001$	NS	rho = −0.35; $p = 0.0009$	rho = −0.40; $p = 0.0001$
Estimates of bone strength at radius				
Stiffness (kN/mm)	rho = −0.40; $p < 0.0001$	NS	NS	rho = −0.42; $p < 0.0001$
Failure load (kN)	rho = −0.41; $p < 0.0001$	NS	NS	rho = −0.44; $p < 0.0001$
Microarchitecture parameters at tibia				
Trabecular BV/TV (%)	rho = −0.33; $p < 0.002$	NS	NS	rho = −0.37; $p = 0.0003$
Trabecular number (1/mm)	rho = −0.38; $p = 0.0003$	NS	rho = −0.33; $p < 0.002$	rho = −0.49; $p < 0.0001$
Trabecular thickness (mm)	NS	NS	NS	NS
Trabecular separation (mm)	rho = 0.37; $p = 0.0003$	NS	rho = 0.31; $p < 0.003$	rho = 0.49; $p < 0.0001$
Cortical thickness (mm)	rho = −0.37; $p = 0.0004$	NS	rho = −0.33; $p < 0.002$	rho = −0.42; $p < 0.0001$
Estimates of bone strength at tibia				
Stiffness (kN/mm)	rho = −0.35; $p = 0.0008$	NS	NS	rho = −0.39; $p = 0.0002$
Failure load (kN)	rho = −0.35; $p = 0.0008$	NS	NS	rho = −0.39; $p = 0.0001$

BMD = bone mineral density; BV/TV = bone volume/total volume; NS = not significant.

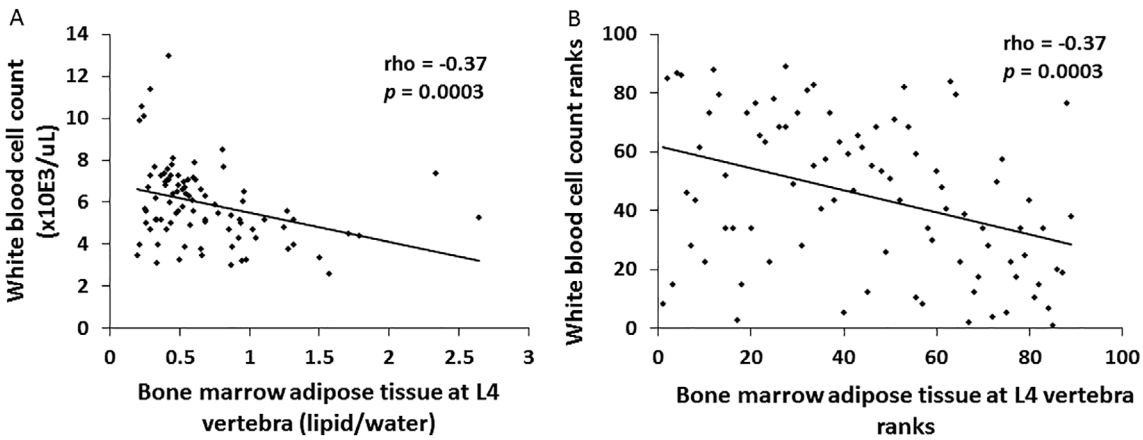

Fig. 1. There was a significant inverse association between white blood cell count and bone marrow adipose tissue at the L_4 vertebra (rho = −0.37, $p = 0.0003$) (*A*). When we performed a sensitivity analysis and excluded the two subjects with L_4 BMAT ≥2 lipid/water and the five subjects with WBC count ≥9 × 10E3/μL, the association remained significant (rho = −0.33, $p = 0.002$). Data are also plotted as ranks (*B*).

progenitors. A number of studies in both animal models and humans have investigated the association between hematopoiesis and bone formation. In murine models, osteoblastic cells have been shown to be potential important regulators of hematopoiesis.[1,2] In addition, in human studies, bone loss at the hip has been associated with an increased risk of anemia[5] and hemoglobin levels have been positively associated with BMD measured by pQCT[4] and ultrasound.[29] Anemia has also been associated with increased fracture risk.[3] Our data demonstrate that the association between RBC and WBC counts and bone parameters is also significant and positive in premenopausal women; both RBC and WBC counts were significantly associated with BMD as well as parameters of bone microarchitecture and estimates of bone strength.

Table 3. Univariate Associations Between BMAT at the Spine and Hip and Hematopoietic Parameters

	White blood cell count (×10E3/μL)	Red blood cell count (×10E6/μL)	Hemoglobin (g/dL)	Hematocrit (%)	Platelet count (×10E3/μL)
BMAT at L_4 vertebra (lipid/water)	rho = −0.37 p = 0.0003	NS	NS	NS	NS
BMAT at femoral epiphysis (lipid/water)	NS	NS	NS	NS	NS
BMAT at femoral diaphysis (lipid/water)	rho = −0.24 p < 0.03	rho = −0.31 p < 0.004	rho = −0.20 p = 0.057	rho = −0.20 p = 0.057	NS
BMAT at femoral metaphysis (lipid/water)	rho = −0.36 p = 0.0005	rho = −0.32 p = 0.002	NS	NS	NS

NS = not significant.

Table 4. Univariate Associations Between BMD, Bone Microarchitecture, Estimated Bone Strength, and Hematopoietic Parameters

Parameter	White blood cell count (×10E3/μL)	Red blood cell count (×10E6/μL)
BMD		
Posterior–anterior spine BMD (g/cm^2)	rho = 0.45; p < 0.0001	rho = 0.32; p = 0.002
Lateral spine BMD (g/cm^2)	rho = 0.52; p < 0.0001	R = 0.29; p = 0.006
Total Hip BMD (g/cm^2)	rho = 0.49; p < 0.0001	R = 0.30; p = 0.004
Femoral neck BMD (g/cm^2)	rho = 0.50; p < 0.0001	R = 0.32; p = 0.002
Total body BMD (g/cm^2)	rho = 0.34; p = 0.001	NS
Radial microarchitecture parameters		
Trabecular BV/TV (%)	rho = 0.33; p = 0.002	rho = 0.25; p < 0.02
Trabecular number (1/mm)	rho = 0.26; p = 0.01	rho = 0.29; p < 0.006
Trabecular thickness (mm)	rho = 0.25; p < 0.02	NS
Trabecular separation (mm)	rho = −0.29; p < 0.01	rho = −0.29; p < 0.007
Cortical thickness (mm)	rho = 0.24; p < 0.03	NS
Estimates of bone strength at radius		
Stiffness (kN/mm)	rho = 0.39; p = 0.0002	R = 0.25; p = 0.02
Failure load (kN)	rho = 0.40; p < 0.0001	R = 0.26; p = 0.01
Microarchitecture parameters at tibia		
Trabecular BV/TV (%)	rho = 0.31; p = 0.003	R = 0.28; p = 0.007
Trabecular number (1/mm)	rho = 0.38; p = 0.0003	R = 0.26; p < 0.02
Trabecular thickness (mm)	NS	NS
Trabecular separation (mm)	rho = −0.37; p = 0.0004	rho = −0.29; p < 0.007
Cortical thickness (mm)	rho = 0.24; p = 0.02	NS
Estimates of bone strength at tibia		
Stiffness (kN/mm)	rho = 0.40; p = 0.0001	R = 0.24; p < 0.03
Failure load (kN)	rho = 0.41; p < 0.0001	R = 0.24; p < 0.03

BMD = bone mineral density; BV/TV = bone volume/total volume; NS = not significant.

In contrast, less is known about the association between marrow adipocytes and hematopoiesis. In a murine model, skeletal regions with greater BMAT have been associated with fewer hematopoietic stem cells and in mice who are irradiated and receive a bone-marrow transplant, inhibition of adipogenesis after irradiation is associated with improved bone-marrow engraftment and increased WBC counts.[10] More recently, reductions in BMAT in a bariatric surgery mouse model have been associated with increased granulocyte-colony stimulating factor which, in turn, leads to increased circulating neutrophils.[9] Our data now show an inverse association between BMAT and both RBC and WBC counts in humans. Whether BMAT is a negative regulator of hematopoiesis[10] or whether it may be utilized to fuel myelopoiesis[9] in humans remains unknown, and future studies will be necessary to further explore this association. Given the known association between BMAT and decreased bone integrity,[27] whether this association between BMAT and hematopoietic parameters is a potential mediator in the association between anemia and increased fracture risk, which has been shown in some populations to be independent of BMD,[30] also warrants further study.

Interestingly, when we divided the group by BMI, we found that the association between BMAT and WBC and RBC counts in overweight/obese women was opposite to that observed in the group as a whole. In overweight/obese women, the relationship between BMAT and WBC/RBC counts was positive. These data are consistent with prior studies suggesting potential differences in BMAT in women with anorexia nervosa as compared to those who are normal-weight or overweight/obese. For example, in overweight/obese women, BMAT is positively associated with visceral adipose tissue,[31] whereas this association is not observed in lower-weight women.[14] These data may provide further support for the concept that BMAT may have a different function in states of nutrient insufficiency as compared to nutrient sufficiency.

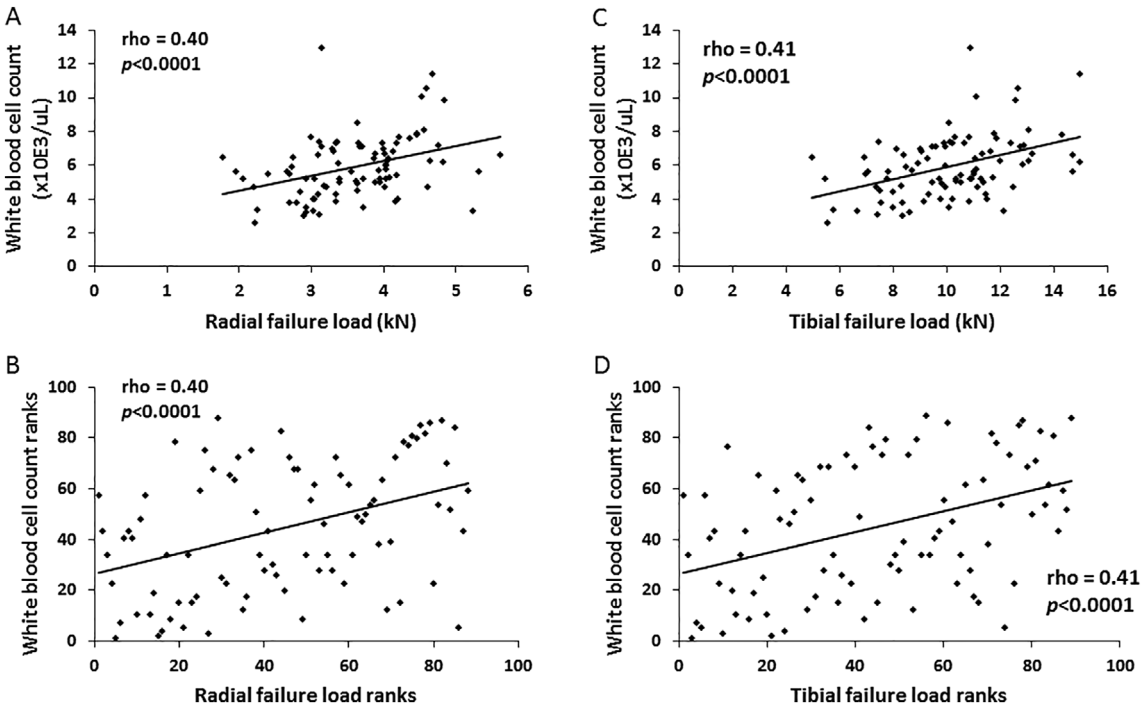

Fig. 2. There was a significant positive association between white blood cell count and failure load, an estimate of bone strength, at the radius (rho = 0.40, $p < 0.0001$) (*A*, with ranks plotted in *B*), and at the tibia (rho = 0.41, $p < 0.0001$) (*C*, with ranks plotted in *D*).

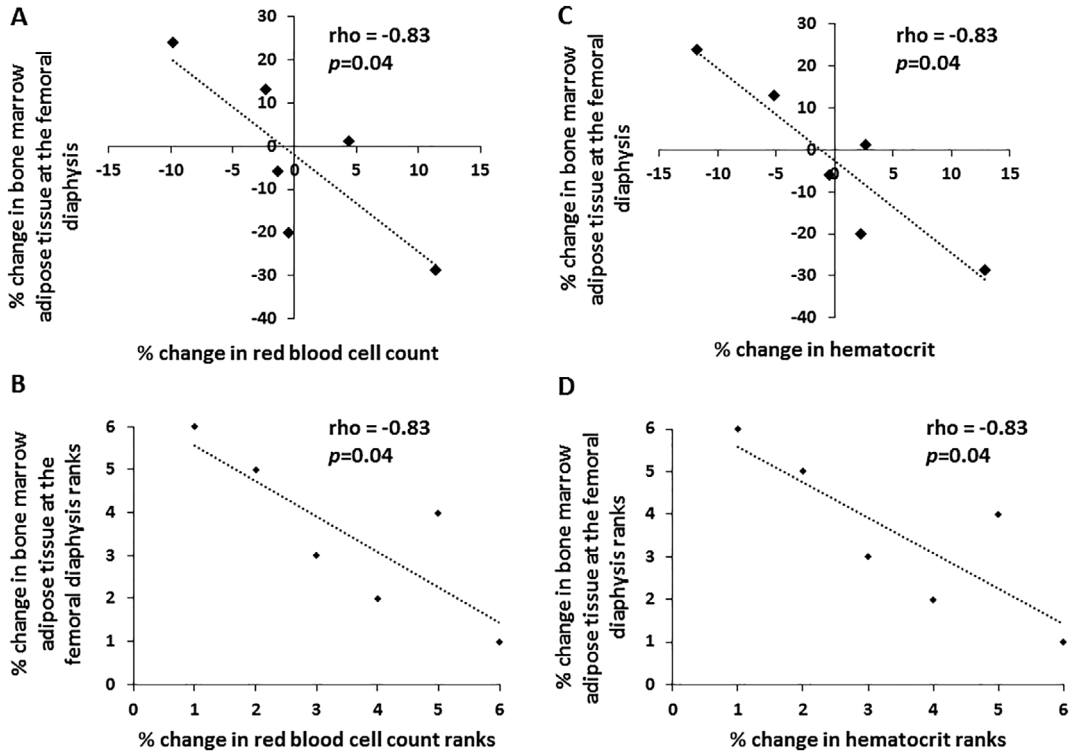

Fig. 3. There was a significant inverse association between % change in bone marrow adipose tissue at the femoral diaphysis and % change in red blood cell count (rho = −0.83, $p = 0.04$) (*A*, with ranks plotted in *B*) and % change in hematocrit (rho = −0.83, $p = 0.04$) (*C*, with ranks plotted in *D*) after 6 months of transdermal estrogen treatment in six women with anorexia nervosa.

Although a major limitation of our study is its cross-sectional nature, which does not allow us to make any inferences about causality, the longitudinal component of our study shows a strong association between changes in BMAT and changes in RBC counts over a short-time interval. Importantly, this is a small, exploratory study and therefore meant to be hypothesis-generating, given the very small sample size, but these data suggest that BMAT may potentially be a determinant of RBC count. Estrogen has been shown to increase hematopoietic stem cell division and therefore it will be important to determine in future studies whether this association between changes in BMAT and changes in RBC count is independent of estrogen.[32] An additional limitation is that we did not have data on specific WBC populations (lymphocytes and neutrophil counts) and prior human studies have demonstrated differential findings with respect to the association between BMD and specific WBC populations.[5] Furthermore, the univariate associations we observed in this study are prone to confounding, including potential confounding by inflammatory cytokines. Inflammatory cytokines, specifically interleukin-6 (IL-6), have been shown to be associated with low hemoglobin levels in frail, elderly adults[33] and IL-6 is also a mediator of osteoclastogenesis,[34] suggesting a possible link between anemia and decreased BMD. Although our population included premenopausal women, increased IL-6 levels have been reported both in women with anorexia nervosa and obese individuals[35,36] and therefore future studies will be necessary to further explore the possible association between inflammatory cytokines, BMD, and blood counts in these populations.

In conclusion, in this exploratory study, we show an association between BMAT and RBC and WBC counts. Further studies will therefore be necessary to better understand the complex relationship between adipocyte, osteoblast, and hematopoietic precursors in the marrow microenvironment in order to gain insight into the function of BMAT.

Disclosures

All authors state that they have no conflicts of interest.

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