ORIGINAL ARTICLE

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A High Amount of Local Adipose Tissue Is Associated With High Cortical Porosity and Low Bone Material Strength in Older Women

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ABSTRACT

Obesity is associated with increased risk of fractures, especially at skeletal sites with a large proportion of cortical bone, such as the humerus and ankle. Obesity increases fracture risk independently of BMD, indicating that increased adipose tissue could have negative effects on bone quality. Microindentation assesses bone material strength index (BMSi) in vivo in humans. The aim of this study was to investigate if different depots of adipose tissue were associated with BMSi and cortical bone microstructure in a population based group of 202 women, 78.2 ± 1.1 (mean ± SD) years old. Bone parameters and subcutaneous (s.c.) fat were measured at the tibia with an XtremeCT device. BMSi was assessed using the OsteoProbe device, and based on at least 11 valid reference point indentations at the mid-tibia. Body composition was measured with dual X-ray absorptiometry. BMSi was inversely correlated to body mass index (BMI) ($r = –0.17$, $p = 0.01$), whole body fat mass ($r = –0.16$,$p = 0.02$), and, in particular, to tibia s.c. fat ($r = –0.33$, $p < 0.001$). Tibia s.c. fat was also correlated to cortical porosity (Ct.Po; $r = 0.19$, $p = 0.01$) and cortical volumetric BMD (Ct.vBMD; $r = –0.23$, $p = 0.001$). Using linear regression analyses, tibia s.c. fat was found to be independent of covariates (age, height, log weight, bisphosphonates or glucocorticoid use, smoking, calcium intake, walking speed, and BMSi operator) and associated with BMSi ($\beta = –0.34$,$p < 0.001$), Ct.Po ($\beta = 0.18$, $p = 0.01$), and Ct.vBMD ($\beta = –0.32$, $p < 0.001$). BMSi was independent of covariates associated with cortical porosity ($\beta = –0.14$, $p = 0.04$) and cortical volumetric BMD ($\beta = 0.21$, $p = 0.02$) at the distal tibia, but these bone parameters could only explain 3.3% and 5.1% of the variation in BMSi, respectively. In conclusion, fat mass was independently and inversely associated with BMSi and Ct.vBMD, but positively associated with Ct.Po, indicating a possible adverse effect of adipose tissue on bone quality and bone microstructure. Local s.c. fat in tibia was most strongly associated with these bone traits, suggesting a local or paracrine, rather than systemic, negative effect of fat on bone. © 2015 The Authors. *Journal of Bone and Mineral Research* published by Wiley Periodicals, Inc. on behalf of American Society for Bone and Mineral Research (ASBMR).

KEY WORDS: CORTICAL POROSITY; BONE MATERIAL STRENGTH; ADIPOSE TISSUE; WOMEN; OSTEOPOROSIS

Introduction

Osteoporosis is a disease characterized by low areal bone mineral density (aBMD) and impaired bone microstructure which leads to lower bone strength and an increased risk of fracture.[1] A high body mass index (BMI) has been shown to reduce the risk of osteoporotic fracture[2] and has been found to be positively correlated to aBMD.[3,4] However, recent studies have revealed that fractures are common in obese women[5] and that obesity increases fracture risk independently of BMD,[6] suggesting that other factors such as bone quality could be negatively affected by excessive adipose tissue.

aBMD has been used as a proxy for bone strength and has, in a large number of studies been shown to predict the risk of fracture.[7,8] However, only about 50% of all postmenopausal women who sustain a fracture have an aBMD below the WHO criteria for osteoporosis.[9] aBMD does not provide any information regarding bone geometry and microstructure, both of which are believed to be of great importance for bone strength.[10] Previously, microstructure could only be estimated by invasive bone biopsies. Today, many parameters of bone microstructure can be obtained at the radius and tibia by high-resolution peripheral quantitative computed tomography (HR-pQCT).[11] Estimates of microstructure by HR-pQCT, such as cortical porosity, has been shown to discriminate between postmenopausal osteopenic women with prior wrist fractures and controls.[12] We recently reported that cortical porosity, independently of aBMD, was associated with prevalent fracture in older men.[13] However, neither aBMD nor HR-pQCT take bone material quality into account, and even though both bone density and

Received in original form July 24, 2015; revised form November 18, 2015; accepted November 20, 2015. Accepted manuscript online December 22, 2015.
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Journal of Bone and Mineral Research, Vol. 31, No. 4, April 2016, pp 749–757
DOI: 10.1002/jbmr.2747

microstructure are important for bone strength, the strength is also dependent on the material properties of the bone.[14]

Reference point indentation (RPI) is a novel in vivo method to assess bone material strength index (BMSi) in humans.[15] It is performed with an OsteoProbe device (Active Life Scientific, Santa Barbara, CA, USA) that creates small microfractures at the anterior surface of the mid-tibia. BMSi has been reported to discriminate patients with osteoporotic fractures from controls.[15] Also, earlier studies have shown lower BMSi in patients with type 2 diabetes[16] and prevalent fragility fractures[17] despite similar BMD as controls, indicating the importance of bone material strength for skeletal strength.

The effects of adipose tissue on the cortical and trabecular bone compartments have not been fully elucidated. Obese women have been reported to have reduced cortical but increased trabecular volumetric BMD.[18] Fractures at bone sites with a large proportion of cortical bone, such as the upper arm and ankle,[2,19] are associated with obesity. We therefore hypothesized that adipose tissue could have adverse effects on cortical bone quality and microstructure. The aim of this study was to investigate if different depots of adipose tissue were associated with BMSi and cortical bone microstructure, near the indentation site, in a population based group of 202 women (78.2 ± 1.1 years [mean ± SD]).

Subjects and Methods

Study subjects

The present study is a cross-sectional population-based study with participants from the greater Gothenburg area. Letters where sent to a randomly selected group of women (75 to 80 years old), identified in the Swedish national population registry. These women were thereafter contacted by telephone. A total of 496 women were asked to participate in the study. From this group, a subsample of women ($n = 202$), who agreed to BMSi testing and had valid BMSi and HR-pQCT measurements, were used for further analyses. There were no differences between included subjects and those who did not participate ($n = 294$) regarding age (78.2 ± 1.1 versus 78.4 ± 1.1 years, $p = 0.19$), height (161.2 ± 57.6 versus 161.4 ± 59.3 cm, $p = 0.74$), and weight (69.2 ± 12.6 versus 70.3 ± 13.2 kg, $p = 0.35$).

Height and body weight were measured with standardized equipment and two consecutive measurements were made. If the two measurements differed by ≥5 mm, a third measure was performed and a calculated average of the two most similar estimates was used. Tibia length was measured from the medial malleolus to the medial condyle of the tibia. A walk test was performed to assess current physical function. All subjects were instructed to walk a distance of 10 m in a self-chosen pace. This test was performed twice and the average time was used to calculate a walking speed (m/s). A standardized questionnaire was used to collect information regarding the participant's intake of calcium, medical history, use of medications, and smoking. A validated questionnaire about daily intake of dairy-containing products (eg, milk, hard and soft cheese) was used to assess intake of food containing calcium.[20] Total calcium intake was calculated by summarizing food-derived calcium intake with possible supplementation. Prevalent fractures from the age of 50 years and onward were collected using a questionnaire. Information about trauma type was not available. Subjects with fractures located at the lower leg, ankle, or foot were compared against controls without any fracture. All fractures were self-reported. All study participants signed an informed consent before entering the study, which was approved by the ethical review board at the University of Gothenburg.

Assessment of bone microarchitecture

Volumetric BMD (vBMD) and bone microstructure were investigated in the tibia with a high-resolution three-dimensional peripheral quantitative computed tomography (HR-pQCT) (XtremeCT; Scanco Medical AG, Brüttisellen, Switzerland). Images were taken in accordance with an earlier described protocol.[21] The tibia, ipsilateral to the nondominant arm, was measured with the standard program (ultradistal site) recommended by the manufacturer and also with an extra acquisition more proximally at 14% of the bone length, in order to obtain a more optimal measure of cortical bone. In case of previous fracture of the ipsilateral tibia, the contralateral side was measured. The operator placed a reference line at the distal articular plateau. From that reference line, the first image was captured at a distance of 22.5 mm when using the standard settings. For the more proximal acquisition, the first image was captured at 14% of the measured bone length. At each measuring site, a total of 110 cross-sectional images were captured with an isotropic resolution of 82 μm resulting in a 3D representation of the tibia. The total scan time was approximately 3 minutes with an effective dose of about 3 μSv per section.

Obtained images where processed according to Laib and colleagues,[22] resulting in the following parameters: trabecular bone volume fraction (BV/TV, %), trabecular number (Tb.N, mm^{-1}), and trabecular thickness (Tb.Th, μm). Cortical parameters were obtained from the distal measurement (14% of the bone length) and trabecular parameters from the ultradistal measurements. The coefficient of variation (CV) ranged from 0.80% to 2.6% for the trabecular parameters obtained at the ultradistal bone site. Image quality was assured by using of a grading scale recommended by the manufacturer (Scanco Medical AG), where grades 1 to 3 were regarded as acceptable quality and grades 4 to 5 as unacceptable quality. All of the 202 women enrolled in this study had acceptable measurements at the tibia.

Cortical evaluation

All images were further processed by the manufacturer's customized version of the Image Processing Language (IPL v5.08b; Scanco Medical AG) in accordance with a described method.[23] The cortical evaluation software automatically placed a contour around the bone at the periosteal surface on all 110 cross-sectional images obtained at each measuring site, to delineate the bone from extraosseous soft tissue. Another automatically-placed contour on the endosteal side of the cortical bone was used to separate cortical from trabecular bone. Obtained contours from both segmentation processes were carefully inspected and manually corrected if necessary. If the automated algorithms, for example, included trabecular bone or soft tissue within either of the two regions of interest (ROIs), the operator corrected the mistake. After all contours had been inspected, cortical porosity was defined within the two contours and artifacts such as surface roughness and transcortical foramen or erosions were excluded. Finally, the segmented cortical bone was combined with the cortical porosity images, resulting in a more refined cortical compartment. With this method the following parameters were obtained: cortical pore volume (Ct.Po.V, mm^3), cortical bone

volume (Ct.BV, mm^3), cortical volumetric bone mineral density (Ct.BMD, mg/cm^3), cortical thickness (Ct.Th, mm), cortical area (Ct.Ar, mm^2), and total area (Tt.Ar, mm^2). This segmentation process makes it possible to calculate cortical porosity (Ct.Po, %) by the following formula[23,24]: Ct.Po (%) = Ct.Po.V/(Ct.Po.V + Ct.BV). Out of the 202 individuals with approved tibia measurements, two cortical evaluation analyses at the distal tibia were excluded due to unresolvable segmentation errors. The following CVs were obtained for the cortical parameters at the distal tibia measurements: cortical porosity (4.1%), cortical vBMD (0.30%), cortical thickness (1.2%), cortical area (0.71%), and total area (0.12%).

Assessment of bone material strength index by microindentation

BMSi was measured by reference point indentation (RPI) using the OsteoProbe device (Active Life Scientific, Santa Barbara, CA, USA). Under local anesthesia the subject's HR-pQCT–measured leg was measured and the indentation was performed at the middle of the tibia length. The handheld OsteoProbe was inserted through the skin and periosteum at the anterior face of the mid-tibia. With the device held perpendicular against the bone surface, a preload force up to 10 N was applied to establish the probe. When established, a trigger mechanism released an impact force of 30 N (indentation measurement), for less than a millisecond, pushing the probe into the cortical bone introducing a small microfracture, 375 μm across.[15,25] The distance the probe moves from the position where it was established to the position right after the impact force has been actuated, is called the indentation distance increase (IDI). At least 11 valid reference point indentations were made on each patient. Five additional measurements were made on a polymethylmethacrylate plastic calibration phantom to calibrate the measurements. BMSi was then calculated by the ratio of an harmonic mean IDI obtained from the polymethylmethacrylate-material relative to the IDI obtained from the impact into the bone, multiplied by 100.[16] A low BMSi-value indicates that the probe created a larger cavity reflecting lower bone material strength.

Four different operators performed the assessment of BMSi. Possible differences between operators were investigated by an interobserver CV. This CV was 5.2% and was obtained from measurements on 30 subjects where two different operators performed indentations within 2 cm on the same leg. The intraobserver CV was 3.2% and calculated from duplicate measurements performed on another set of 30 study subjects. The two measurements took place within 2 cm and the measuring probe was exchanged between measurements. All operators participated in the assessment of both CVs. BMSi differed between operators (OP) (OP1 [$n = 36$] 75.7 ± 7.8; OP2 [$n = 39$] 75.3 ± 8.3; OP3 [$n = 57$] 79.4 ± 6.9; OP4 [$n = 70$] 72.5 ± 6.3; $p < 0.001$) analyzed by ANOVA. All 202 included women had complete microindentation measurements.

Ex vivo experiments were performed to evaluate the potential effect of soft tissue on microindentation measurements. Microindentation, by standard OsteoProbe routine, was therefore performed on eight donated porcine tibias. Two consecutive measurements were made: the first indentation was performed at an intact tibial bone (through the soft tissue), followed by a second indentation where the soft tissue was removed and thus performed directly on the bone surface. The same operator performed all indentations. A comparison by a paired-sample *t* test showed no significant difference in BMSi between the measurements directly on bone and through soft tissue (64.1 ± 8.6 versus 66.6 ± 9.7; $p = 0.27$, respectively).

Assessment of body composition

Lean and fat mass were measured with the whole body software and further divided into more specific subregions (eg, trunk, leg, and arm) as described[26] with a Hologic Discovery A (S/N 86491) device (Hologic, Waltham, MA, USA). Fat and lean mass, of the leg (as a subregion) was categorized and used on the same leg, as measured with the HR-pQCT device. All measurements were performed with the same DXA device. The CVs for these body composition measurements ranged from 0.063% to 2.4%.

Assessment of subcutaneous fat

Subcutaneous (s.c.) fat was measured at the distal tibial measurement (14% of the tibia length) with the HR-pQCT device. Two lines were drawn in connection to and on both sides of the tibia and fibula. These lines created an angle that was divided into four compartments. With these compartments, three evenly distributed measurement points (M1, M2, and M3) were established in between the two main lines (Fig. 1*A*). Measures of the depth of subcutaneous fat at theses measurement points were made directly on the HR-pQCT images, from the skin surface to the periosteum of the tibia. In total, nine measurements in millimeters were obtained in each subject in M1, M2, and M3 at image numbers 1, 55, and 110, and an average of these was used. Interobserver and intraobserver CVs for this method were 2.5% and 1.1%, respectively. CVs were obtained from measurements on 30 out of the 202 participating women.

In order to determine if this method captures s.c. fat, a correlation study was performed between the HR-pQCT and a peripheral quantitative computed tomography (pQCT) (XCT-2000; Stratec Medizintechnik, Pforzheim, Germany). Measurements, at 14% of tibial bone length, were made with both the pQCT and HR-pQCT in 30 women with similar age (76.5 ± 0.98 years) and from the same region as the original 202 elderly women. pQCT-obtained images were further processed with BoneJ, which is a software plug-in for the open source program ImageJ (NIH, Bethesda, MD, USA). This software enables segmentation analyses of the soft tissue.[27] The procedure has been explained elsewhere[28]; in short BoneJ, soft tissue analyses applies a 7×7 median filter to minimize noise. The analyzed images are binarized in accordance to the given threshold values: muscle density (41 to 139 mg/cm^3) illustrated as crimson area, intermuscular adipose tissue (–40 to 40 mg/cm^3) illustrated as green area, and subcutaneous adipose tissue (–40 to 40 mg/cm^3) illustrated as purple area, excluding the three outermost pixels as skin. The single processed image, obtained from the pQCT device, was analyzed with the same angle-method as for the HR-pQCT. With an established angle, three measurement locations (M1, M2, and M3) were obtained and an average was calculated of the measured fat (purple area) at these locations (Fig. 1*B*). A correlation was calculated between measured s.c. fat on the HR-pQCT and pQCT images ($r = 0.95$; $p < 0.001$). To receive a regression equation, a linear regression was performed with fat measurements from the pQCT as dependent variable and HR-pQCT fat measurements as independent variable. From this linear regression, a regression coefficient (0.95) and constant (–2.31) were given for fat measurements. Subcutaneous fat measurements with the HR-pQCT device were all adjusted according to the regression equation.

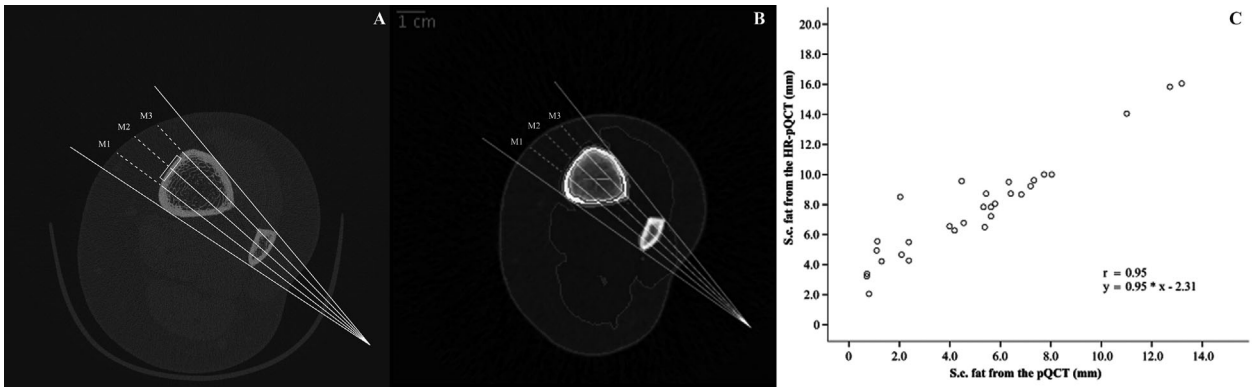

Fig. 1. An illustration of how subcutaneous fat was measured at the distal tibia with both HR-pQCT (*A*) and pQCT (*B*) at 14% of the bone length. Two lines were drawn that touched both tibia and fibula on each side. These two lines coincided at one place creating an angle. This angle was divided into four equal sectors and resulted in three measurement sites (M1, M2, and M3). The dashed lines were measured with a ruler in between the skin surface and the periosteum of the tibia for the HR-pQCT. The same procedure was performed on HR-pQCT image numbers 1, 55, and 110, and an average distance of tissue between the skin surface and the periosteum was calculated from these nine measurements in total. For the pQCT, the same method resulted in three measurement sites (M1, M2, and M3). An average of the three dashed lines covering the purple area (s.c. fat) was measured with ImageJ measuring tool. The correlation between measured s.c. fat with the two devices (*C*) is presented together with the corresponding regression equation. The applied box in *A* illustrates the indentation site (performed at 50% of the bone length).

Statistical analyses

Bivariate correlations were used to analyze the association between bone geometry, microstructure, body composition, and BMSi. Multivariate linear regressions, with a stepwise function, were used to analyze associations with adjustments for covariates. Because BMSi differed between operators, adjustments for this factor were made by dummy variables in the stepwise multivariate linear regressions. The beta and *p* values of the independent variables that were excluded (not significantly associated with the dependent variables) in the stepwise models are presented in terms of their nonsignificant contribution, when forced into the final model. Quartiles of tibia s.c. fat were used to investigate whether associations between s.c. fat, BMSi, and cortical porosity were nonlinear. Differences between quartiles were investigated with an ANOVA followed by a Bonferroni post hoc test or with χ^2 (for fracture proportions). Multivariate logistic regression was used to calculate an odds ratio (OR) for the predictive value of tibial s.c. fat on lower extremity fractures. Log-transformed values of weight, tibia s.c fat, and body composition variables were used in all performed parametric tests. Obtained p values <0.05 were regarded as statistically significant and all statistical analyses were performed with SPSS, version 22 (IBM Corp., Armonk, NY, USA).

Results

BMSi, parameters of body composition, and bone microstructure were measured in 202 older women (78.2 ± 1.1 years). Group characteristics are presented in Table 1.

Associations between body composition, BMSi, and bone parameters

BMSi was negatively correlated to BMI ($r = -0.17$, $p = 0.01$) (Table 2). Negative correlations were apparent for BMSi and whole body fat mass ($r = -0.16$, $p = 0.02$), but not for whole body lean mass ($r = -0.11$, $p = 0.13$; Table 2). Both total DXA-derived fat mass and lean mass of the BMSi-measured whole leg correlated with BMSi (Table 2). Large adipose tissue depots (android and gynoid fat) were not correlated to BMSi, but a clear inverse correlation between tibia s.c. fat and BMSi was found ($r = -0.33$, $p < 0.001$). Cortical porosity ($r = 0.19$, $p = 0.01$) and cortical vBMD ($r = -0.23$, $p = 0.001$) of the distal tibia were both correlated to tibia s.c. fat but not to any other body composition parameter (Table 2). Cortical cross-sectional area was most strongly correlated with BMI ($r = 0.34$, $p < 0.001$) and with all other body composition parameters but tibia s.c. fat. Positive correlations between trabecular bone volume fraction and all lean and fat measures, except for tibia s.c. fat, were found (Table 2). Tibia s.c. fat was positively correlated to trabecular number ($r = 0.37$, $p < 0.001$), but inversely correlated to trabecular thickness ($r = -0.27$, $p < 0.001$).

In order to investigate the independent association between lean, fat, and BMSi, a stepwise linear regression model with age, height, weight, per oral glucocorticoids, bisphosphonates, calcium intake, walking speed, smoking, and operator as covariates was used. In these analyses total body fat mass ($\beta = -0.16$, $p = 0.02$) but not total body lean mass (both parameters entered simultaneously) was independently associated with BMSi. Using the same covariates, only total fat mass, but not total lean mass, of the BMSi measured leg was associated with BMSi ($\beta = -0.19$, $p = 0.006$). The fat parameters total body fat, leg fat, and tibial s.c. fat were analyzed together in a stepwise linear regression model adjusted for above mentioned covariates (age, height, weight, per oral glucocorticoids, bisphosphonates, calcium intake, walking speed, smoking, and operator) to investigate which was most strongly associated with BMSi. In these analyses BMSi was associated with s.c. fat ($\beta = -0.34$, $p < 0.001$), but not with total body fat ($\beta = 0.03$, $p = 0.69$) or total leg fat ($\beta = 0.03$, $p = 0.76$). The independent association between total body lean and fat mass and HR-pQCT bone variables were also investigated using a linear regression model using the same covariates except for the operator covariate. In

Table 1. Group Characteristics

Subjects, *n*	202
Age (years)	78.2 ± 1.1
Height (cm)	161.1 ± 5.8
Weight (kg)	69.2 ± 12.6
Body mass index (kg/m^2)	26.6 ± 4.4
Calcium intake (mg/day)	751 ± 406
Walk speed over 10 meters (m/s)	2.1 ± 0.4
Bisphosphonates (current use), *n* (%)	18 (8.9)
Glucocorticoids (current use), *n* (%)	5 (2.5)
Duration since self-reported lower extremity fracture (years)[a]	8.6 (4.3, 18.8)
Smoking, *n*(%)	18 (8.9)
Lower leg fractures, *n*(%)	24 (11.9)
Whole body fat (kg)	27.0 ± 8.02
Whole body lean (kg)	42.3 ± 5.45
Android fat mass (kg)	2.0 ± 0.84
Gynoid fat mass (kg)	4.4 ± 1.3
Leg fat (kg)[b]	5.0 ± 1.7
Leg lean (kg)[b]	6.7 ± 1.0
Distal tibia subcutaneous fat (mm)	6.8 ± 4.5
BMSi[b]	75.6 ± 7.6
HR-pQCT[b]	
Distal tibia total bone area (mm^2)	437 ± 59.1
Distal tibia cortical area (mm^2)	136 ± 25.0
Distal tibia cortical vBMD (mg/cm^3)	970 ± 44.6
Distal tibia cortical thickness (mm)	1.9 ± 0.4
Distal tibia cortical porosity (%)	4.7 ± 2.2
Ultradistal tibia trabecular bone volume fraction (%)	12.3 ± 3.0
Ultradistal tibia trabecular number (mm^{-1})	1.8 ± 0.4
Ultradistal tibia trabecular thickness (μm)	702 ± 13.6

Results presented as mean with standard deviation for the continuous variables. Dichotomous variables are presented as number and percentage.

[a]Median ± interquartile range.

[b]Ipsilateral tibia of the nondominant radius.

this model only total body lean mass was associated with Ct.Ar ($\beta = 0.48$, $p < 0.001$) and BV/TV ($\beta = 0.26$, $p < 0.001$).

Associations between adipose tissue, bone microstructure, and BMSi

In order to investigate whether the observed associations between tibia s.c. fat, BMSi, and cortical porosity were nonlinear, these variables were investigated in women divided into quartiles of tibia s.c. fat. Higher quartiles were associated with lower BMSi (Fig. 2). Women in the highest quartile had lower BMSi (Fig. 2) and higher cortical porosity than women in the lowest quartile (Fig. 3).

Independent associations between tibia s.c. fat, BMSi, and bone microstructure

In multivariate regression models, tibia s.c. fat was, independently of covariates (age, height, weight, per oral glucocorticoids, bisphosphonates, calcium intake, walking speed, smoking, and operator), associated with BMSi ($\beta = -0.34$, $p < 0.001$), Ct.vBMD ($\beta = -0.32$, $p < 0.001$), and Ct.Po ($\beta = 0.18$, $p = 0.01$; Table 3). Tibia s.c. fat, adjusted for the same covariates, was associated with Tb.N and Tb.Th ($\beta = 0.19$, $p = 0.01$ and $\beta = -0.28$, $p < 0.001$, respectively) (Table 3). Tibia s.c. fat could explain 10.6% of the observed variation in BMSi in the fully adjusted model. Also, when Ct.Po or Ct.vBMD were included as covariates in the multivariate regression models, tibia s.c. fat was independently associated to BMSi ($\beta = -0.34$, $p < 0.001$ and $\beta = -0.31$ $p < 0.001$, respectively).

Associations between BMSi and bone microstructure

BMSi was negatively correlated with Ct.Po ($r = -0.18$, $p = 0.01$; Fig. 4) and positively correlated with Ct.vBMD ($r = 0.23$, $p = 0.001$; Fig. 5), whereas no significant correlations with cortical ($r = 0.03$, $p = 0.64$) or total area ($r = -0.06$, $p = 0.42$) were observed at the distal tibial site. In addition, BMSi was positively correlated with Tb.Th at the ultradistal tibia ($r = 0.14$, $p = 0.04$), but not with Tb.N ($r = -0.04$, $p = 0.61$) or with BV/TV ($r = 0.09$, $p = 0.23$). BMSi did not differ significantly between women with and without prevalent lower extremity fracture (74.9 ± 7.6 versus 75.7 ± 7.9; $p = 0.89$).

BMSi is independently associated with cortical bone microstructure

After adjustment for covariates (age, height, weight, per oral glucocorticoids, bisphosphonates, calcium intake, walking speed, smoking, and operator), BMSi was still associated with Ct.Po ($\beta = -0.14$, $p = 0.04$) and Ct.vBMD ($\beta = 0.21$, $p = 0.02$) at the distal tibia. BMSi was not independently associated with any other bone parameter (Table 4).

Lower extremity fractures distribution over quartiles of s.c. fat

Self-reported prevalent fractures from the age of 50 years and onward located at the lower extremities did not differ between quartiles of s.c. fat (Q1 = 5, Q2 = 6, Q3 = 11, and Q4 = 2; $p = 0.058$). Tibial s.c. fat, adjusted for the covariates (age, height, walking speed, smoking, per oral glucocorticoids, bisphosphonates, and calcium intake), was not significantly associated to fracture prevalence of the lower extremities in a logistic regression (OR, 0.85; 95% CI, 0.51 to 1.41 per SD increase in s.c. fat, $p = 0.52$). When the same logistic regression model was applied, with the addition of Ct.vBMD as a covariate, similar results were obtained (OR, 0.85; 95% CI, 0.50 to 1.44; $p = 0.54$).

Discussion

A high body weight or BMI has been associated with high BMD[29] and has traditionally been described as a protective factor against fractures, but recent evidence has revealed that a significant proportion of fractures occur in the obese.[5] For any given BMD *T*-score, obesity is associated with increased fracture risk,[6] indicating that other factors, such as increased fall risk or poor bone quality, could mediate the negative effect of excessive adipose tissue. Fractures at sites with a large proportion of cortical bone, such the upper arm and ankle, are more common in obese women.[2,19] The association between adipose tissue and bone material strength has not previously been investigated in vivo in humans.

In the present study, we found that different adipose tissue depots were inversely and independently associated with BMSi, but only local adipose tissue (ie, s.c. tibia fat), was inversely associated with Ct.vBMD and positively associated with Ct.Po in our group of older women. These findings indicate that local

Table 2. Associations Between Parameters of Body Composition, BMSi and HR-pQCT–Derived Bone Variables

		Tibia distal					Tibia ultradistal		
	OsteoProbe BMSi[a]	Ct.Po (%)	Ct.vBMD (mg/cm^3)	Ct.Th (mm)	Ct.Ar (mm^2)	Tt.Ar (mm^2)	BV/TV (%)	Tb.N (mm^{-1})	Tb.Th (μm)
BMI	**–0.17 (0.01)**	0.02 (0.76)	0.01 (0.93)	**0.26 (<0.001)**	**0.34 (<0.001)**	0.11 (0.12)	**0.29 (<0.001)**	**0.44 (<0.001)**	–0.12 (0.08)
Whole body fat mass	**–0.16 (0.02)**	–0.04 (0.55)	0.03 (0.67)	**0.16 (0.02)**	**0.26 (<0.001)**	**0.16 (0.02)**	**0.28 (<0.001)**	**0.41 (<0.001)**	–0.10 (0.16)
Whole body lean mass	–0.11 (0.13)	–0.07 (0.34)	0.04 (0.57)	**0.14 (0.04)**	**0.31 (<0.001)**	**0.40 (<0.001)**	**0.29 (<0.001)**	**0.40 (<0.001)**	–0.08 (0.28)
Android fat mass	–0.14 (0.053)	–0.08 (0.28)	0.11 (0.14)	**0.24 (<0.001)**	**0.30 (<0.001)**	0.06 (0.39)	**0.34 (<0.001)**	**0.38 (<0.001)**	0.003 (0.96)
Gynoid fat mass	–0.12 (0.08)	–0.04 (0.55)	0.21 (0.84)	0.11 (0.14)	**0.21 (0.003)**	**0.21 (0.003)**	**0.26 (<0.001)**	**0.37 (<0.001)**	–0.08 (0.26)
Leg, fat mass[a]	**–0.19 (0.01)**	0.02 (0.73)	–0.07 (0.32)	0.06 (0.44)	**0.18 (0.01)**	**0.24 (<0.001)**	**0.17 (0.02)**	**0.35 (<0.001)**	**–0.18 (0.01)**
Leg, lean mass[a]	**–0.16 (0.03)**	0.03 (0.71)	–0.05 (0.47)	0.11 (0.12)	**0.29 (<0.001)**	**0.42 (<0.001)**	**0.21 (0.003)**	**0.38 (<0.001)**	**–0.14 (0.046)**
Tibia, subcutaneous fat[a]	**–0.33 (<0.001)**	**0.19 (0.01)**	**–0.23 (0.001)**	0.05 (0.51)	0.13 (0.06)	0.13 (0.07)	0.09 (0.19)	**0.37 (<0.001)**	**–0.27 (<0.001)**

Pearson correlation coefficients with p values in parentheses. Non-normally distributed variables were log-transformed. For BMS measurements, $n = 202$; for HR-pQCT measurements, $n = 200$. Values of $p < 0.05$ are regarded as significant and are shown in bold. Tibia distal = 14% of the bone length; tibia ultradistal = manufacturer's recommended standard analyses. Cortical evaluation results in Ct.Po, Ct.vBMD, Ct.Th, Ct. Ar, and Tt.Ar. Standard analysis results in BV/TV, Tb.N, and Tb.Th.

BMSi = bone material strength index; Ct.Po = cortical porosity; Ct.vBMD = cortical volumetric bone mineral density; Ct.Th = cortical thickness; Ct.Ar = cortical area; Tt.Ar = total area; BV/TV = trabecular bone volume fraction; Tb.N = trabecular number; Tb.Th = trabecular thickness; BMI = body mass index.

[a]On the HR-pQCT–measured leg.

Fig. 2. Mean values of bone material strength index with 95% confidence intervals in 202 older women divided into quartiles of tibia subcutaneous fat. $^{*}p < 0.05$ and $^{***}p < 0.001$.

adipose tissue could exert a negative effect on cortical bone quality and microstructure, suggesting that a possible adverse effect of adipose tissue on bone is local or paracrine, rather than systemic. In agreement with this hypothesis, a previous study reported impaired bone microstructure in transiliac crest biopsies in women with higher trunk fat mass.[30] Women in the highest tertile of trunk fat had lower trabecular bone volume fraction, fewer and thinner trabeculae, and higher cortical porosity[30] than women in the lowest tertile. We also found thinner trabeculae at the distal tibia in women with higher s.c. tibia fat, but the trabecular number was increased, resulting in absence of an association between tibia s.c. fat and bone volume fraction in our group. In agreement with the results from the present study, an inverse association was observed between fat area and cortical vBMD of the tibia[18] in a cohort of 211 women

Fig. 3. Mean values of cortical porosity (%) with 95% confidence interval in 200 older women divided into quartiles of tibia subcutaneous fat. $^{**}p < 0.01$.

Table 3. Independent Associations Between Tibia Subcutaneous Fat and Bone Measurements (ie, BMSi and HR-pQCT–derived Bone Variables)

Dependent variables	β for tibia s.c. fat as independent variable	*p*
BMSi	**–0.34 (–0.46 to –0.21)**	**<0.001**
Tibia ultradistal		
Trabecular bone volume fraction (%)	–0.10 (–0.24 to 0.05)	0.22
Trabecular number (mm^{-1})	**0.19 (0.05 to 0.33)**	**0.01**
Trabecular thickness (μm)	**–0.28 (–0.42 to –0.14)**	**<0.001**
Tibia distal		
Cortical volumetric BMD (mg/cm^3)	**–0.32 (–0.48 to –0.16)**	**<0.001**
Cortical thickness (mm)	–0.12 (–0.28 to 0.04)	0.15
Cortical porosity (%)	**0.18 (0.04 to 0.32)**	**0.01**
Cortical area (mm)	–0.07 (–0.23 to 0.09)	0.37
Total area (mm)	0.11 (–0.01 to 0.24)	0.08

Stepwise linear regression model ($n = 202$) investigating the associations between bone variables, as dependent variables, and tibia s.c. fat and covariates (age, height, log weight, bisphosphonates, per oral glucocorticoids, current smoking, daily calcium intake, walk speed, and operator [only for the BMSi analysis]) as independent variables. The results are presented as standardized beta coefficients (SD change in the dependent variable per SD change in the independent variable) with 95% confidence interval and *p* values. Significance was defined by a *p* value <0.05 and significant values are in bold.

BMSi = bone material strength index.

(25 to 71 years old). In contrast, to these associations found between local fat and bone variables, a general increase in adipose tissue (and in lean tissue) seen in obese subjects, has been positively associated with bone size and density. It was recently reported in a case-control study, that older obese women ($n = 30$) had lower tibia cortical porosity, higher Ct.vBMD, increased trabecular BV/TV, and greater calculated tibia failure load than normal weight women ($n = 30$).[31] However, the obese women also had considerably greater lean mass than the controls (46.3 ± 4.6 kg versus 35.2 ± 3.7 kg), which most likely also influenced the observed differences in bone parameters. In the present group, we found that total body lean mass, but not fat mass, was independently associated to Ct.Ar and BV/TV, parameters important for bone strength, whereas no associations were seen between any of these body composition variables and cortical vBMD or porosity.

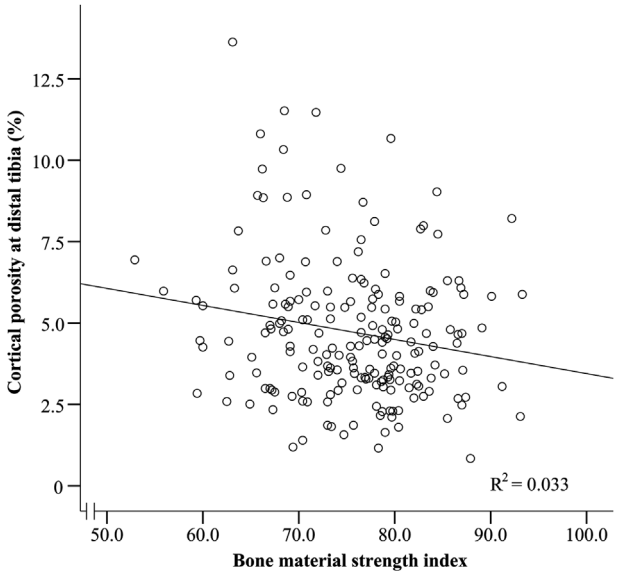

Fig. 4. Cortical porosity (%) measured at the distal tibia versus crude (not adjusted for operator) bone material strength index in 200 older women.

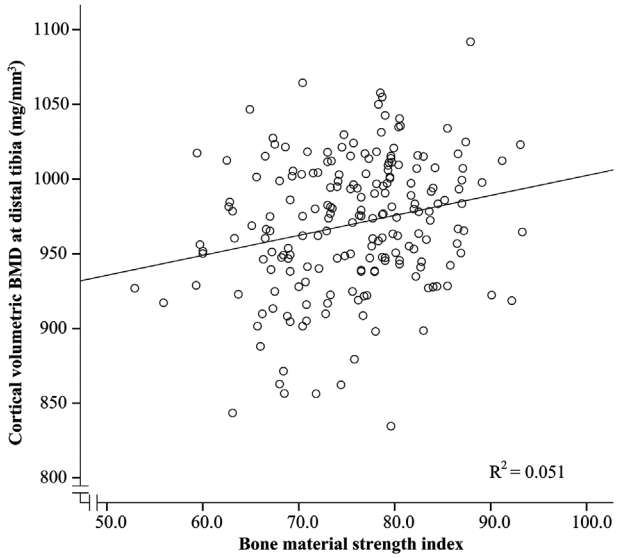

Fig. 5. Cortical volumetric BMD (mg/mm^3) measured at the distal tibia versus crude (not adjusted for operator) bone material strength index in 200 older women.

We speculate that a possible direct negative influence of local adipose tissue on cortical BMSi, density, and porosity could be a contributing factor as to why overweight patients are more prone to fractures in extremities (in which cortical bone accounts for a large proportion of the bone strength) such as the upper arm and ankle. However, it should be acknowledged that increased weight in obesity induces higher loads affecting the bone of the ankle to greater repeated stresses over time, which could also contribute to the increased fracture risk at this site.[32] We investigated frequency of prevalent lower extremity fracture in regard to different amount of s.c. fat, but we could not observe any significant association; however, the analyses contained few fracture cases, limiting the statistical power. In addition, fractures were self-reported and occurred over 11 years, on average, prior to study inclusion. Therefore, the role of tibia s.c. fat, bone microstructure, and BMSi, in relation to incident fractures would be highly relevant to investigate.

The mechanisms behind the fat and bone interaction and how they differ in the cortical and trabecular bone compartments are not clear. Osteoblasts and adipocytes both originate from the same mesenchymal stem cells (MSCs) and it has been reported that osteoporotic patients have increased marrow adipocyte infiltration,[33] suggesting that a shifted pattern in MSC differentiation contributes to the pathogenesis of osteoporosis.

Table 4. Linear Regression Model for BMSi Measured at the Mid-Tibia and HR-pQCT–Derived Bone Variables

HR-pQCT–independent variables	β for BMSi as dependent variable	*p*
Tibia ultradistal		
Trabecular bone volume fraction (%)	0.13 (–0.01 to 0.26)	0.07
Trabecular number (mm^{-1})	0.02 (–0.13 to 0.17)	0.77
Trabecular thickness (μm)	0.11 (–0.02 to 0.24)	0.10
Tibia distal		
Cortical volumetric BMD (mg/cm^3)	**0.21 (0.08 to 0.34)**	**0.02**
Cortical thickness (mm)	0.08 (–0.05 to 0.22)	0.21
Cortical porosity (%)	**–0.14 (–0.27 to –0.01)**	**0.04**
Cortical area (mm)	0.04 (–0.09 to 0.17)	0.53
Total area (mm)	–0.08 (–0.22 to 0.06)	0.23

Linear regression model ($n = 202$) with BMSi as the dependent variable and HR-pQCT–derived bone variables adjusted for covariates (age, height, log weight, bisphosphonates, per oral glucocorticoids, current smoking, daily calcium intake, walk speed, and operator). Tibia distal = 14% of the bone length; tibia ultradistal = manufacturer's recommended standard analyses. The results are presented as standardized beta coefficients (SD change in the dependent variable per SD change in the independent variable) with 95% confidence intervals and *p* values. Significance was defined by a *p* value <0.05 and significant values are in bold.

BMSi = bone material strength index.

Microindentation, using the OsteoProbe device, is a recently introduced method to assess BMSi in human. Associations between BMSi and parameters of bone microstructure have not yet been reported in humans in vivo. In the present study we found a positive association between BMSi and Ct.vBMD, and an inverse relationship between BMSi and Ct.Po. These associations were still significant after adjustment for a wide variety of covariates, including anthropometrics, physical function, medications, calcium intake, and BMSi operator. Lean mass was strongly positively associated with total and cortical area, but negatively associated with BMSi, which could indicate opposing actions of lean mass on cortical bone size and material properties. Although we found that BMSi was associated with cortical microstructure, the variation of BMSi explained by Ct.Po and Ct.vBMD was only 3.3% and 5.1%, respectively. Furthermore, the independent association between tibia s.c. fat and BMSi remained unaltered when Ct.Po or Ct.vBMD were included as covariates in the regression analyses. Therefore, we draw the conclusion that the associations between tibia s.c. fat and BMSi are largely independent of the cortical microstructure assessed with HR-pQCT.

Recently, a microindentation study performed on cadaveric tibial bones revealed a positive correlation between cortical tissue mineral density and BMSi. Also, a negative correlation between cortical porosity and BMSi[34] was found. The present study constitutes the first in vivo study in humans corroborating the findings based on cadaveric bones. Previous studies have reported that BMSi could discriminate between osteoporotic fracture cases and controls and that BMSi is independent of aBMD.[15,17]

Our study has some limitations. First, four different operators performed the measurements for BMSi and their mean value differed when compared with an analyses of variance (ANOVA), although the interobserver CV of 5.2% was acceptable. This study only investigated the associations between bone structure and bone material strength in women and may not be transferrable to men. Furthermore, s.c. fat was measured as the amount of tissue between the anterior tibia and the outside of the skin, encompassing various thickness of the skin and small amounts of other soft tissues. To ensure that this method actually measured s.c. fat, we performed a calibration study that revealed that our method was highly correlated ($r = 0.95$) to a previously published fat measuring method[28] using pQCT images. Our data on fractures is also limited by the use of self-reported fractures, which are often incorrect or misclassified.[35] The performed study also has strengths. We have investigated the so-far-largest population-based sample of older women and analyzed associations between adipose tissue, BMSi, and bone microstructure, in vivo. The findings of the present study shed new light on the possible mechanism by which adipose tissue could affect material properties of the cortical bone.

Indentation distance increase (IDI) obtained with RPI on the tibia, using the Biodent equipment, was recently shown to predict human femur failure load.[36] It has also been reported that IDI and a combination of IDI and loading slope predicted ultimate stress and toughness in human femurs, subjected to three-point bending.[37] The total indentation distance (TID, also obtained using the Biodent) when indented through the periosteum was correlated ($r = –0.44$) to critical stress intensity required to initiate cracks and that TID and average loading slope (an RPI measurement related to bone matrix hardness) could contribute to different properties of fracture toughness in human cadaveric bones.[38] OsteoProbe-derived BMSi was, in another study, found to be weakly correlated to total indentation distance (TID; $r = –0.36$) and unloading slope ($r = –0.41$), the latter parameters obtained using the Biodent technique.[34] However, it should also be acknowledged that there is no direct evidence available concerning to what extent BMSi reflects the actual biomechanical properties of bone.

In conclusion, we found that local adipose tissue is inversely and independently associated with BMSi even when adjusting for multiple covariates including cortical bone microstructure variables such as Ct.vBMD and Ct.Po, indicating a possible adverse effect of adipose tissue on bone quality. Independent associations between local adipose tissue, Ct.Po, and Ct.vBMD suggest that adipose tissue could possibly also have a negative impact on the cortical bone microstructure.

Disclosures

All authors state that they have no conflicts of interest.

Acknowledgments

This study was supported by the Swedish Research Council, the Lundberg Foundation, and the ALF/LUA grant from the Sahlgrenska University Hospital and Gustaf V's and Queen Victoria's Freemason Foundation.

Authors' roles: Made substantial contributions to conception and design, acquisition of data, or analyses and interpretation of data: DS, ML, RR, MZ, AD, and AGN. Participated in drafting the manuscript or revising it critically for important intellectual content: DS, ML, RR, MZ, AD, and AGN. Approved the final version of the submitted manuscript: DS, ML, RR, MZ, AD, and AGN. The following authors agree to be accountable for all

aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved: DS, ML, RR, MZ, AD, and AG.

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