Fat Mass Is Negatively Associated with Cortical Bone Size in Young Healthy Male Siblings

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Context: Body weight has been associated with bone mass and bone size through shared genetic determination and environmental influences. Whereas lean mass exerts a positive influence on bone size, the relationship between fat and bone remains unclear.

Objective: The objective of the present study was to investigate the individual influence of fat mass and lean mass on volumetric bone density and size in young healthy male siblings at age of peak bone mass.

Design: This was a cross-sectional, population-based sibling pair study.

Participants: A total of 677 men (25–45 yr) were included in this study with 296 independent pairs of brothers.

Main Outcome Measures: Areal and volumetric bone parameters were determined using dual-energy X-ray absorptiometry (DXA) and peripheral quantitative computed tomography (pQCT). Body composition was determined by DXA. Sex steroids, leptin, and adiponectin were determined by immunoassay.

Results: Total and regional fat mass were found to be inversely associated with areal bone mass and bone size, independent from lean mass (radius periosteal circumference β: -0.29 ± 0.04; $P < 0.001$). Lean mass was positively associated with bone size but inversely with cortical density at both tibia and radius ($P < 0.01$). The negative association between total fat mass and bone size was independent from sex steroid concentrations. Leptin but not adiponectin was inversely associated with bone size, but this was no longer significant after adjustment for body fat.

Conclusions: Increased fat mass is associated with smaller bone size, challenging the view of a high bone mass index as a protective factor for osteoporosis, whereas lean mass was a consistent positive determinant of bone size. (***J Clin Endocrinol Metab*** **94: 2325–2331, 2009)**

From epidemiological studies, body weight and body mass index (BMI) are well-described determinants of bone mass (1–4). A high BMI is considered protective against developing osteoporosis in both men and women, whereas thinness is a major risk factor for sustaining osteoporotic fractures (1, 5). Mechanical stress and strain are important in remodeling bone architecture and bone mass. In this view, a higher body weight is believed to increase the mechanical stress exerted on the skeleton through dynamic loads imposed by muscle and passive loads by whole body weight, ultimately leading to osteogenesis (6, 7).

In recent years, this view on the fat-bone relationship has been challenged by new findings, describing direct and indirect effects of adipose tissue on bone. Studies on the association of adipose tissue with bone mass reported inconsistent

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Abbreviations: aBMD, Areal bone mineral density; BMC, bone mineral content; BMI, body mass index; DXA, dual-energy X-ray absorptiometry; P1NP, procollagen type 1 amino-terminal propeptide; pQCT, peripheral quantitative computed tomography; S-CTX, serum C-terminal telopeptides of type I collagen; vBMD, volumetric bone mineral density.

results regarding the relationship fat mass, leptin, and bone (8–10), whereas recent publications suggested an absent or even negative influence of fat mass on bone after adjusting for adult body composition (11–13).

Apart from the mechanical loading, adipose tissue is hormonally active and both i*n vitro* and *in vivo* experiments proposed a prominent role for leptin as a local and central mediator between adipose tissue and bone (14). Adiponectin was found to stimulate both osteoclast and osteoblast formation in cell culture experiments (15, 16). Furthermore, lean, fat, and bone mass are all controlled by genetic factors and some kind of relationship can be expected through shared genetic determination (17).

Up until now, most of our understanding on the relationship between bone and body composition derives from observations obtained by bone measurements using dual-energy x-ray absorptiometry (DXA) with less evidence available using quantitative computed tomography (18–21), which allows assessment of both bone geometry and volumetric bone mineral density (vBMD).

In the present study, we aim at investigating the individual effect of fat and lean mass on vBMD and bone size in healthy male siblings at age of peak bone mass. Regional fat distribution, serum sex steroids, leptin, and adiponectin are studied in relation to bone parameters and genetic correlations between lean/fat mass and areal/volumetric bone parameters are determined in this population of young male siblings.

Subjects and Methods

Study design and population

Participants were recruited from the population registries of three semirural to suburban communities around Ghent, Belgium. Men (n = 12,446), 25–45 yr of age were contacted by direct mailing, briefly describing the study purpose and asking whether they had a brother within the same age range also willing to participate (maximal age difference between brothers was set at 12 yr). The overall response rate was 30.2%. Finally, a sample of 768 young healthy men agreed to participate who fulfilled the primary inclusion criterion of having a brother within the same age range. After exclusions, in total 677 men were included in the study. Two hundred ninety-six pairs of brothers (for a total of 592 men) were included in addition to 64 men as single participants, when their brother could not participate in the study; 19 men were included as third brother in a family and two as fourth brother. All participants were in good health and completed questionnaires about previous illness and medication use. Exclusion criteria were defined as illnesses or medication use affecting body composition, hormone levels, or bone metabolism (22). Physical activity was scored using the questionnaire as proposed by Baecke *et al.* (23). The study protocol was approved by the ethical committee of the Ghent University Hospital and informed consent was obtained from all participants.

Body composition and areal bone mineral density

Body weight and anthropometrics were measured in light indoor clothing without shoes. Whole body soft tissue composition, as well as bone mineral content (BMC) and areal bone mineral density (aBMD) at the lumbar spine and proximal femur (total hip region) were measured using DXA with a Hologic QDR-4500A device (software version 11.2.1; Hologic Inc., Bedford, MA). The coefficient of variation for both spine and whole-body calibration phantoms was less than 1% as calculated from daily and weekly measurements, respectively.

Volumetric and geometric bone parameters [peripheral quantitative computed tomography (pQCT)]

A pQCT device (XCT-2000; Stratec Medizintechnik, Pforzheim, Germany) was used to scan the dominant leg (tibia) and forearm (radius). The cortical volumetric bone mineral density (vBMD; milligrams per cubic centimeters), cortical cross-sectional area (square millimeters), endosteal and periosteal circumferences, and cortical thickness (millimeters) were measured at the midradius (66% of bone length from the distal end) and tibia (66%). Trabecular area and vBMD (milligrams per cubic centimeters) was measured using a scan through the metaphysis (at 4% of the radius length) at the nondominant arm. Adjusting cortical density to bone size (partial volume correction) was calculated according to previously published formulas (24).

Biochemical determinations

Venous blood samples were obtained between 0800 and 1000 h after overnight fasting. All serum samples were stored at −80 C until batch analysis. Commercial RIAs were used to determine serum levels of leptin (human leptin RIA; Linco Research Inc., St. Charles, MO), adiponectin (Diagnostic Systems Laboratories, Webster, TX), total testosterone, SHBG (Orion Diagnostica, Espoo, Finland), and estradiol (clinical assay; DiaSorin s.r.l., Saluggia, Italy) (22). C-terminal telopeptides of type I collagen (S-CTX) and procollagen type 1 amino-terminal propeptide (P1NP) were measured using an immunoelectrochemiluminescence technique (Modular; Roche Diagnostics, Mannheim, Germany). Free testosterone and free estradiol concentrations were calculated from serum total testosterone, estradiol, SHBG, and albumin concentrations using a previously validated equation derived from the mass action law (22).

Statistics

Descriptives are expressed as mean ± SD or median [first to third quartile] when criteria for normality were not fulfilled (Kolmogorov-Smirnov) and variables (bone parameters, body composition) were log transformed in subsequent linear models. Quantitative genetic analysis was used to partition the phenotypic variance into genetic and environmental variance components using the SOLAR 2.0 software (Southwest Foundation for Biomedical Research, San Antonio, TX). Linear mixed-effects modeling with random intercepts and a simple residual correlation structure was used to study the effect of body composition on volumetric and geometric bone parameters, with adjustment for the confounding effect of age, adult height and weight, or lean mass and taking into account the interdependence of measurements between brothers. Parameters of fixed effects were estimated via restricted maximum likelihood estimation and reported as estimates of effect size (β) with their respective SE. Associations were considered significant at $P < 0.05$. Statistical analyses were performed using S-Plus 7.0 (Insightful, Seattle, WA).

Results

Study population and patient characteristics

Six hundred seventy-seven subjects with a mean age of 34 ± 6 yr are included in the study. Mean height is 179 ± 6 cm and mean weight 81 ± 12 kg, with a BMI of 25.3 ± 3.5 kg/m^2. Body composition, DXA, and pQCT bone parameters are given in Tables 1 and 2. The plasma leptin concentration is 4.4 (2.8–7.0) μg/liter and adiponectin 8.4 (6.2–11.2) μg/ml. Bone markers P1NP [54 μg/liter (41–63)] and S-CTX [0.44 μg/liter (0.31–0.53) as well as testosterone [19 nmol/liter (16–23)] and estradiol concentrations [73 pmol/liter (62–84)] are within the expected range for young male subjects.

In this narrow age range, limited effects of age on bone parameters are observed [previously described in this cohort (22)].

TABLE 1. Descriptive areal bone parameters and body composition

Areal bone parameters	n = 677
Whole-body bone area (cm^2)	2353 ± 155
Whole-body BMC (kg)	2.88 ± 0.37
Whole-body aBMD (g/cm^2)	1.22 ± 0.099
Total hip bone area (cm^2)	45.3 ± 4.4
Total hip BMC (g)	48.9 ± 8.0
Total hip aBMD (g/cm^2)	1.080 ± 0.137
Lumbar spine bone area (cm^2)	71.6 ± 6.3
Lumbar spine BMC (g)	76.0 ± 12.6
Lumbar spine aBMD (g/cm^2)	1.058 ± 0.123
Whole-body lean mass (kg)	62.2 ± 6.6
Whole-body fat mass (kg)	16.4 ± 6.4
Appendicular fat mass (kg)	7.4 ± 2.7
Trunk fat mass (kg)	8.0 ± 3.9

Age is positively associated with the projected bone area (DXA) at the lumbar spine and total hip as well as with trabecular and cortical bone area and periosteal circumference at the radius (β: 0.001–0.004; $P < 0.05$), but not at the tibia after adjustment for body weight and height. Levels of physical activity are positively associated with periosteal circumference ($P < 0.01$) and cortical thickness at the tibia (β: 0.02 ± 0.003; $P < 0.001$) as described before (22), whereas physical activity was inversely associated with fat mass and leptin (β: −0.10 to −0.05; $P < 0.001$) and positively with lean mass (β: 0.01 ± 0.001; $P < 0.001$). All analyses are adjusted for age, height, and weight, unless otherwise indicated.

Areal bone parameters in relation to indices of body fat

Whole-body fat mass is negatively associated with aBMD, BMC, and projected bone areas at the hip, spine, and whole body when adjusted for lean mass or body weight (Table 3; all $P < 0.001$). Lean mass was found to be a consistent positive determinant of both areal bone area, BMC, and aBMD at all measured sites.

Correlations of lean and fat mass with bone size assessed by pQCT

Correlations between pQCT bone parameters and body composition are given in Table 4 (adjusted for height, weight, and age). Cortical and trabecular bone area at the radius and cortical bone area at the tibia are positively associated with lean mass.

TABLE 2. Descriptive volumetric pQCT bone parameters at the distal and midshaft radius and midshaft tibia

	Radius	Tibia
Trabecular bone area (mm^2) at 4%	187 ± 26	ND
Trabecular bone density (mg/cm^3) at 4%	228 ± 40	ND
Cortical bone density (mg/cm^3)	1101 ± 35	1112 ± 24
Cortical bone area (mm^2)	101 ± 14	364 ± 47
Cortical thickness (mm)	2.52 ± 0.32	4.52 ± 0.56
Periosteal circumference (mm)	48 ± 4	95 ± 6
Endosteal circumference (mm)	32 ± 4	66 ± 7

ND, Trabecular bone was not determined at the site of the distal tibia.

Whole-body fat mass is inversely associated with cortical bone size at both radius and tibia due to a negative genetic correlation.

Associations of lean and fat mass with bone size using mixed-effects linear models

The correlations reported in Table 4 as determined by SOLAR (Southwest Foundation for Biomedical Research) were replicated in this cohort using linear mixed-effects models. Figure 1 illustrates the estimates of these mixed-effects models (adjusted for age, height, and lean or fat mass), confirming the described associations in Table 4. At both radius and tibia, lean mass is a consistent positive predictor of bone area and size (all $P < 0.01$), whereas fat mass is an independent negative determinant. Cortical thickness is positively associated with lean mass at both radius and tibia, whereas a negative association with fat mass is observed at the tibia (Fig. 1). Adjusting for body weight in place of lean mass and physical activity did not alter the negative associations between fat mass and bone parameters (data not shown).

pQCT vBMD in relation to indices of body fat

Using mixed-effects models, no significant association between fat mass and cortical or trabecular vBMD is observed (Fig. 1), whereas cortical vBMD is inversely associated with lean mass at the radius and tibia (Fig. 1). Adjusting cortical vBMD to bone size (partial volume correction) or adjustment for physical activity and body weight leads to similar conclusions (data not shown).

Regional fat distribution in relation to pQCT bone parameters

Trunk fat and appendicular fat mass are highly correlated and the negative associations described above between whole-body fat and cortical bone size are also observed with both trunk fat and appendicular fat mass (data not shown). When trunk fat and appendicular fat are studied in the same statistical model, trunk fat remained inversely associated with bone size (tibia cortical bone area, β: −0.23 ± 0.06; $P < 0.001$), whereas the association with appendicular fat is no longer significant or strongly reduced [tibia cortical bone area, β: −0.06 ± 0.06; $P = 0.33$ (standardized estimates to allow comparison)].

Serum leptin, adiponectin, and bone marker concentrations in relation to pQCT volumetric bone parameters

Leptin is found to have an inverse association with periosteal and endosteal circumference and cortical bone area at radius and tibia and with cortical thickness at the tibia (Fig. 2). Trabecular area is negatively associated with leptin whereas no relationship between leptin and trabecular density is observed. Adjustment for physical activity did not alter the associations between leptin and bone parameters (data not shown). At the tibia and radius, no associations between adiponectin and cortical bone parameters are observed (data not shown), whereas a positive association is observed between serum adiponectin and trabecular bone area (β: 0.042 ± 0.01; $P < 0.001$). After adjustment for physical activity, this association remained significant.

TABLE 3. Estimates (unstandardized) predicting areal bone parameters by fat and lean mass, adjusted for body weight or in the same mixed-effects model

Areal bone parameters	Fat mass, adjusted for body weight Estimate ± SE	Lean mass, adjusted for body weight Estimate ± SE	Fat mass, adjusted for lean mass Estimate ± SE	Lean mass, adjusted for fat mass Estimate ± SE
Whole-body bone area (cm^2)	−0.09 ± 0.01	0.49 ± 0.03	−0.03 ± 0.004	0.36 ± 0.02
Whole-body BMC (g)	−0.24 ± 0.02	1.07 ± 0.07	−0.09 ± 0.01	0.81 ± 0.04
Whole-body BMD (g/cm^2)	−0.14 ± 0.01	0.58 ± 0.06	−0.05 ± 0.01	0.45 ± 0.03
Total hip bone area (cm^2)	−0.12 ± 0.01	0.58 ± 0.06	−0.06 ± 0.01	0.35 ± 0.04
Total hip BMC (g)	−0.32 ± 0.02	1.46 ± 0.99	−0.12 ± 0.01	1.10 ± 0.06
Total hip BMD (g/cm^2)	−0.19 ± 0.02	0.88 ± 0.09	−0.05 ± 0.01	0.75 ± 0.05
Lumbar spine bone area (cm^2)	−0.10 ± 0.01	0.48 ± 0.05	−0.08 ± 0.01	0.19 ± 0.03
Lumbar spine BMC (g)	−0.23 ± 0.03	0.98 ± 0.12	−0.12 ± 0.02	0.61 ± 0.07
Lumbar spine BMD (g/cm^2)	−0.13 ± 0.01	0.50 ± 0.09	−0.04 ± 0.01	0.42 ± 0.06

All analyses were corrected for age and height. All estimates presented in the table: $P \leq 0.001$ and were corrected additionally for age and height. BMD, Bone mineral density.

When whole-body fat mass and leptin concentrations are analyzed in the same multivariate model as explanatory variables (Fig. 2, *filled black squares*), whole-body fat predominates over leptin as determinant of cortical bone size. Cortical bone area, periosteal circumference, and cortical thickness at radius and tibia are no longer associated with leptin after adjustment for body fat, whereas a small (residual) effect of leptin is observed on the endosteal circumference at tibia and radius (Fig. 2; $P = 0.03$–0.06).

Both bone formation marker P1NP (β: −0.14 ± 0.03; $P < 0.001$) and resorption marker S-CTX (β: −0.20 ± 0.04; $P < 0.001$) are negatively associated with fat mass, even after adjustment for current age, height, and weight. However, the negative association between bone size and fat mass is unaffected by introduction of bone markers in the statistical model (data not shown).

Sex steroids in relation to areal and pQCT bone parameters

Sex steroids as determinants of areal and volumetric bone parameters in this cohort have been reported previously (22). In brief, both testosterone and estradiol are positively associated with BMC and aBMD at hip and spine, although the association with estradiol is markedly stronger. Regarding the pQCT measurements, estradiol is positively associated with vBMD and cortical thickness and inversely with endosteal circumference at the radius (22).

TABLE 4. Phenotypic (ρ_P), genetic (ρ_G), and environmental (ρ_E) correlations between indices of body composition and volumetric bone density and size at the radius and tibia, adjusted for age, current height, and weight

	Radius		Tibia	
	Whole-body lean mass	Whole-body fat mass	Whole-body lean mass	Whole-body fat mass
Trabecular bone area (mm^2) at 4%	$\rho_P = 0.34^a$	$\rho_P = -0.32^a$		
	$\rho_G = 0.41^a$	$\rho_G = -0.39^a$		
	$\rho_E = -0.14$	$\rho_E = -0.01$		
Trabecular bone density (mg/cm^3) at 4%	$\rho_P = 0.10^b$	$\rho_P = -0.12^b$		
	$\rho_G = 0.22$	$\rho_G = -0.23$		
	$\rho_E = -0.47$	$\rho_E = 0.23$		
Cortical bone area (mm^2)	$\rho_P = 0.39^a$	$\rho_P = -0.42^a$	$\rho_P = 0.47^a$	$\rho_P = -0.47^a$
	$\rho_G = 0.49^a$	$\rho_G = -0.55^a$	$\rho_G = 0.51^a$	$\rho_G = -0.54^a$
	$\rho_E = 0.05$	$\rho_E = -0.08$	$\rho_E = 0.31$	$\rho_E = -0.26$
Cortical bone density (mg/cm^3)	$\rho_P = -0.11^b$	$\rho_P = 0.07$	$\rho_P = -0.15^b$	$\rho_P = 0.09$
	$\rho_G = -0.17$	$\rho_G = 0.08$	$\rho_G = -0.25$	$\rho_G = 0.19$
	$\rho_E = -0.004$	$\rho_E = 0.07$	$\rho_E = -0.41$	$\rho_E = 0.48$
Periosteal circumference (mm)	$\rho_P = 0.42^a$	$\rho_P = -0.39^a$	$\rho_P = 0.46^a$	$\rho_P = -0.42^a$
	$\rho_G = 0.55^a$	$\rho_G = -0.50^a$	$\rho_G = 0.56^a$	$\rho_G = -0.52^a$
	$\rho_E = -0.03$	$\rho_E = -0.13$	$\rho_E = -0.10$	$\rho_E = -0.12$
Endosteal circumference (mm)	$\rho_P = 0.31^a$	$\rho^p = -0.25^a$	$\rho_P = 0.22^a$	$\rho_P = -0.18^a$
	$\rho_G = 0.40^a$	$\rho_G = -0.30^b$	$\rho_G = 0.32^b$	$\rho_G = -0.24^b$
	$\rho_E = 0.002$	$\rho_E = -0.15$	$\rho_E = -0.29$	$\rho_E = -0.22$
Cortical thickness (mm)	$\rho_P = 0.11^b$	$\rho^p = -0.16^b$	$\rho_P = 0.25^a$	$\rho_P = -0.27^a$
	$\rho_G = 0.15$	$\rho_G = -0.28^b$	$\rho_G = 0.22^b$	$\rho_G = -0.29^b$
	$\rho_E = 0.05$	$\rho_E = 0.02$	$\rho_E = 0.42$	$\rho_E = -0.21$

The phenotypic correlation (ρ_P) between two traits can be expressed terms of genetic (ρ_G) and environmental correlations (ρ_E). A genetic correlation indicates pleiotropy, meaning that the two traits are influenced to some extent by the same genes or sets of genes.

[a] $P < 0.001$.

[b] $P < 0.05$.

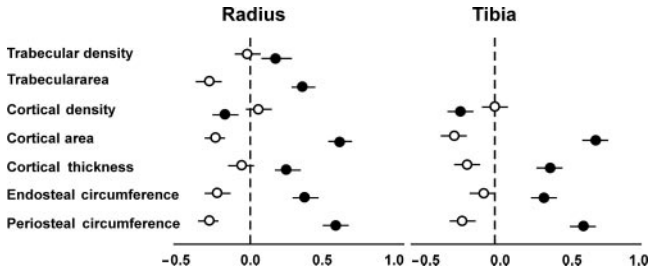

FIG. 1. Standardized estimates representing associations between whole-body fat and lean mass with volumetric bone density and geometric bone parameters in the same mixed-effects model, adjusted for age and height (●, lean mass; ○, fat mass; *bar*, 95% confidence limit).

At both radius and tibia, total estradiol (radius β: 0.02 ± 0.006; $P = 0.004$; tibia β: 0.01 ± 0.004; $P = 0.005$) and free estradiol (radius β: 0.02 ± 0.005; $P = 0.005$; tibia β: 0.01 ± 0.003; $P = 0.004$) were associated with cortical vBMD, independent of lean and fat mass, whereas (free) testosterone was not associated with cortical vBMD (data not shown). No interactions between fat mass or lean mass are observed with testosterone or estradiol in determining vBMD (P = NS).

Regarding bone size, fat mass remains inversely associated with periosteal circumference (both radius and tibia, $P < 0.001$) independent from (free) testosterone or estradiol, whereas lean mass remains positively associated. At the radius, fat mass (β: −0.07 ± 0.01; $P < 0.001$) remains inversely associated with endosteal circumference, adjusting for free estradiol (β: −0.06 ± 0.02; $P = 0.009$) or (free) testosterone (P = NS).

Discussion

Our findings demonstrate that fat mass is inversely associated with bone size after correction for current weight, whereas lean mass has a strong positive influence on bone mass. This is the first study to report the relationship between fat mass and bone parameters, using bone measurements by pQCT, allowing to distinguish between bone geometry and vBMD and assessing body composition independently by DXA. In this regard, inaccurate determination of bone mass due to interposition of a variable amount of soft tissue cannot be held responsible for our findings. Moreover, differences in the amount of exercise cannot explain our findings because after adjustment for current physical activity, fat mass remained inversely associated with bone mass. Bone markers were within the expected range in our young men, together with limited effects of age indicates a steady state in bone mass in our population (25).

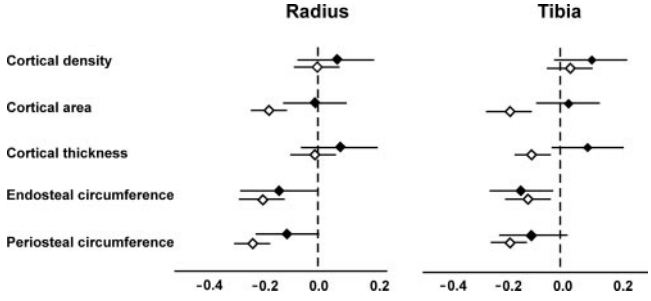

FIG. 2. Standardized estimates derived from mixed-effects models representing associations between leptin with volumetric bone density and geometric bone parameters with and without adjustment for whole-body fat mass (◇, leptin; ◆, leptin adjusted for fat mass; *bar*, 95% confidence limit). All analyses are adjusted for age and height.

The inverse relationship between fat mass and bone markers can be explained through the smaller bone area and size with increasing fat mass, whereas no arguments for increased bone loss in this population could be established.

The finding of an unfavorable effect of increasing fat mass on bone, together with significant positive associations with lean mass, corroborates the well-described mechanostat theory. Bone geometry is mainly adapted to the dynamic load imposed by muscle force and less to passive loading as imposed by fat mass (26).

Previous reports on the relationship between fat and bone mass used DXA for both the determination of body composition and areal bone parameters. In line with our findings, Hsu *et al.* (12) reported a negative association between whole-body and hip BMC and fat mass in a large sample of Chinese men and women. Across the whole range of body weight, a linear decrease in BMC was observed per quartile increase of fat mass in both men and women. Travison *et al.* (27) described a nonlinear association between fat mass and bone and suggested a threshold above which no beneficial effect of obesity on bone mass could be observed. Zhao *et al.* (28), studying both Chinese and Caucasian populations, found that body fat mass was negatively correlated with bone mass when the mechanical loading effect of body weight was statistically removed. In men 30–79 yr old of diverse ethnicity (black, Hispanic, and white), Travison *et al.* (29) reported that geometric bone parameters at the hip (assessed by DXA) were positively related to lean mass. Similar to our results, controlling for lean mass eliminated the effect of fat mass on bone size or even rendered this association negative (29). In adolescent boys, Janicka *et al.* (13) observed negative associations between fat mass and aBMD at the spine and leg, together with negative associations with vertebral trabecular bone density and femoral cortical bone area. In females, these effects were less pronounced or nonsignificant.

The mechanisms underlying these and our observations whereby adiposity can influence bone mass remain incompletely understood. Four mechanisms can be proposed explaining this inverse relationship.

First, bone, muscle and fat mass have a shared genetic and epigenetic determination.

Adipocytes, myoblasts, and bone-forming osteoblasts derive from the same mesenchymal stem cells (14). During differentiation, specific gene expression determines the fate of these precursor cells. Adipogenesis is guided by the nuclear hormone receptor peroxisome proliferator activated receptor-γ. In contrast, expression of the major osteogenic transcription factors (runt-related transcription factor-2) guide differentiation toward osteoblasts through the Wnt pathway (30). However, the control and signaling mechanisms that finally lead to an adipogenic or osteogenic cell lineage decision remain largely elusive. In our cohort, we observed a negative genetic correlation between bone and fat mass, which could indicate that during development shared molecular pathways with opposing effects on bone and fat could play a role.

Second, events during puberty, when the majority of bone acquisition occurs, might have persisting effects on bone mass and body composition during adulthood. Indeed, at this age there is a critical convergence of hormonal and environmental influences, interacting with the (epi)genetic background to enhance linear growth and bone expansion. Data from the Gothenburg Osteoporosis and Obesity Determinants (GOOD)-cohort learn that pubertal timing in men is related to central adiposity, bone mineral density, and previous fractures (31, 32) and that previous sport activity during adolescence has persistent effects on adult bone size (33).

Third, sex steroids could mediate the interplay between fat mass and bone size or density because testosterone as well as estradiol are important determinants of peak bone mass in men (22). In our cohort, the association between fat mass and bone size was independent from testosterone or estradiol concentrations, whereas estradiol was significantly associated with vBMD and endosteal circumference. These analyses provide arguments that adipose tissue can influence bone mass through other mechanisms than by altering sex steroid profiles through intraadipose sex steroid metabolism. However this does not exclude an interaction between adipose tissue, sex steroid metabolism, and bone accretion during puberty, leading to the described associations at adult age.

Finally, adipose tissue is metabolically active, and both direct and indirect effects of leptin on bone have been described from *in vitro* and *in vivo* experiments (34). In leptin-deficient mice, a high bone mass was observed, which was mediated through a central effect by altering the activity of the sympathetic nervous system, but also a direct effect of leptin on osteoblasts and bone marrow stromal cells has been described. In our population, leptin was found to have an inverse relationship with bone size after controlling for current weight and height. However, in multivariate statistical models, the influence of fat mass on bone was stronger than that of leptin on bone, suggesting that adipose tissue can influence bone through other mechanisms than leptin. Recent animal data provide evidence that increasing body weight trough high fat diet can effectively increase bone mass in mice, but the effect was independent from leptin signaling (35). Moreover, in the GOOD cohort leptin was also found to be inversely associated with cortical bone size, albeit in younger men (36).

The role of adiponectin on bone remains controversial. *In vitro* data suggest that adiponectin promotes both osteoclast and osteoblast formation (15, 16), although in humans no major role of adiponectin was found in determining bone mineral density or fracture risk (37, 38).

In our cohort, we found that both appendicular and trunk fat mass are negatively associated with bone size at both the upper and lower limbs. When introduced into the same statistical model, trunk fat remained inversely associated with bone size, whereas appendicular fat mass lost significance, providing arguments that trunk fat has a stronger influence on bone than appendicular fat. Data on fat distribution and bone parameters are scarce, although in pubertal girls (mean age 14.4 yr) but not males, sc fat was found to be inversely associated with cortical density and bone strength (39).

Lean mass was inversely associated with cortical vBMD, which could be explained through a higher cortical remodeling in subjects with a higher muscle mass. Cortical vBMD can be regarded as an integrated parameter, determined by both cortical porosity and mean material density. Intracortical remodeling decreases cortical vBMD because remodeling increases porosity due to incompletely filled osteonal canals and by replacement of old (and therefore higher density) material with new (lower density) bone material (20, 21). A partial volume effect (24) leading to an apparent lower vBMD in smaller bones could not explain our observations because this correction did not alter our associations with vBMD at the tibia or radius.

The strength of our study is the well-described large population-based cohort of male siblings and the use of independent methods to determine bone mass and body composition, although some limitations need to be discussed. In our cohort of sibling pairs, the observations within brothers cannot be considered fully independent from each other. However, we used linear mixed-effects modeling with random intercepts in our statistical analyses to account for this familial interdependence. Furthermore, our design is a cross-sectional, population-based study and does not allow to establish causative relationships.

In conclusion, we have shown unfavorable effects of fat mass on bone size, independent of sex steroids, in a large cohort of healthy young men, challenging the view of a high BMI as a protective factor for osteoporosis. Lean mass was the major determinant of bone size, providing further evidence that bone size is adapted to the dynamic load imposed by muscle force rather than to passive loading.

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