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Lean mass and fat mass have differing associations with bone microarchitecture assessed by high resolution peripheral quantitative computed tomography in men and women from the Hertfordshire Cohort Study

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Abstract

Understanding the effects of muscle and fat on bone is increasingly important in the optimisation of bone health. We explored relationships between bone microarchitecture and body composition in older men and women from the Hertfordshire Cohort Study. 175 men and 167 women aged 72–81 years were studied. High resolution peripheral quantitative computed tomography (HRpQCT) images (voxel size 82µm) were acquired from the non-dominant distal radius and tibia with a Scanco XtremeCT scanner. Standard morphological analysis was performed for assessment of macrostructure, densitometry, cortical porosity and trabecular microarchitecture. Body composition was assessed using dual energy x-ray absorptiometry (DXA) (Lunar Prodigy Advanced). Lean mass index (LMI) was calculated as lean mass divided by height squared and fat mass index (FMI) as fat mass divided by height squared. The mean (standard deviation) age in men and women was 76 (3) years. In univariate analyses, tibial cortical area ($p < 0.01$), cortical thickness ($p < 0.05$) and trabecular number ($p < 0.01$) were positively associated with LMI and FMI in both men and women. After mutual adjustment, relationships between cortical area and thickness were only maintained with LMI [tibial cortical area, β (95% confidence interval (CI)): men 6.99 (3.97, 10.01), women 3.59 (1.81, 5.38)] whereas trabecular number and density were associated with FMI. Interactions by sex were found, including for the relationships of LMI with cortical area and FMI with trabecular area in both the radius and tibia ($p < 0.05$). In conclusion,

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LMI and FMI appeared to show independent relationships with bone microarchitecture. Further studies are required to confirm the direction of causality and explore the mechanisms underlying these tissue-specific associations.

Keywords

muscle; bone microarchitecture; fat; HRpQCT; mechanostat; epidemiology

1. Introduction

Sarcopenia is defined as the loss of skeletal muscle mass and strength that occurs with advancing age (1). As current demographic shifts are leading to an ageing population, the number at risk of sarcopenia is growing. Similarly, rates of obesity are also rising with dietary changes and more sedentary lifestyles. Previous work has suggested that both sarcopenia and obesity relate to bone mineral density (BMD) and hence fracture risk (2-5). Specifically, sarcopenia has been shown to be a risk factor for fracture through an increase in fall frequency (6, 7) and an association with poorer bone health (2, 3). Obesity is associated with greater total body fat mass. Interestingly, some studies have found areal BMD to be positively associated with fat mass (4, 5) whereas others did not (8, 9).

The relationship between muscle and bone is driven by multiple mechanisms. These include the hormones, such as growth hormone, with positive effects on both tissues and the mechanostat whereby forces from muscle contractions act to stimulate bone. Dietary factors and physical activity are also thought to play a role. Associations between fat and bone occur mainly through the action of oestrogen, which augments bone health, and adipokines, which can have both positive and negative effects. Other hormones and cytokines have also been implicated. Furthermore, increased loading from greater adiposity may also influence bone through the mechanostat (10).

Many epidemiological studies have assessed body composition using dual energy x-ray absorptiometry (DXA) (4, 8). DXA provides estimates of lean mass and fat mass but does have limitations. Although the majority of lean mass is made up of muscle, it also encompasses other tissues including tendon and ligament. Measurements can also be influenced by trunk thickness and level of tissue hydration, and ectopic fat is often significantly underestimated. Using other imaging modalities, fat can additionally be subdivided by its distribution. Studies have shown that subcutaneous adipose tissue (SAT) demonstrates different relationships with bone health than visceral adipose tissue (VAT) (11).

Relationships with bone structure have also been explored. Investigators have found consistent positive associations between lean mass and both bone geometry and estimates of bone strength (12-14). Conversely, although some positive relationships have been shown between adiposity and bone geometry, the specific compartments affected have varied, and relationships with volumetric BMD, particularly cortical, have been inconsistent (12, 13, 15). Furthermore, lean mass and fat mass are correlated; those with greater adiposity also tend to have larger muscles. Consequently, there is the potential for their relationships with

bone health to confound one another. Previous work, including from the Framingham study, has highlighted the importance of adjusting lean mass for total fat mass both in the assessment of sarcopenia prevalence and when assessing associations with adverse outcomes (16, 17).

Although we are aware of some of the mechanisms through which muscle, fat and bone are interconnected, this area is not fully understood. Further analysis is required to unpick the individual associations of muscle with bone and fat with bone. These tissues are highly interdependent with mesenchymal stem cells having the capacity to differentiate into fat, bone or muscle. We know that fatty tissue may be deposited within muscle, particularly with advancing age, and this may also occur within bone (18). It is very important to understand these relationships as this may shed light on which mechanisms are most likely to be driving each of the independent associations identified.

To date, the independent relationships of muscle and fat with bone microstructure, including trabecular microarchitecture and cortical porosity, have not been well described. These bone parameters can now be assessed non-invasively, *in vivo* using high-resolution peripheral quantitative computed tomography (HRpQCT). The purpose of the current study is to extend current knowledge by investigating the relationships of bone geometry, volumetric BMD, and bone microarchitecture with both lean mass and fat mass in a well phenotyped cohort of older men and women from Hertfordshire.

2. Material and Methods

2.1. Participants

We recruited participants from the Hertfordshire Cohort Study (HCS) to perform an observational cross-sectional analysis. The HCS is a population-based cohort study in the United Kingdom (UK) designed to examine the relationships between growth in infancy and the subsequent risk of adult diseases, such as osteoporosis. Study design and recruitment have been described in detail previously (19). In brief, in conjunction with the National Health Service Central Registry and the Hertfordshire Family Health Service Association, we traced men and women who were born as singleton births between 1931 and 1939 in Hertfordshire and still lived there during the period 1998–2003. In 2011–2012, 570 men and women from the geographical area of East Hertfordshire were invited for a follow up study and 376 (66%) agreed to participate. Thirty four individuals were excluded due to the absence of full total body composition results: 2 declined scanning; 1 was unable to tolerate the procedure; 8 had artefacts in both upper limbs and 23 had artefacts in both lower limbs preventing inclusion of that region (most commonly arthroplasties). Whole body composition was therefore available in 342 (91%) participants who did not differ significantly from the overall recruited cohort (n=376) in terms of demographic and lifestyle factors ($p>0.05$ for age, sex, height, weight, calcium intake, vitamin D intake, physical activity, smoking status, alcohol consumption, and social class).

2.2. Body composition

Total lean mass (LM) (non-bone, non-fat mass) and fat mass (FM) were measured using dual-energy X-ray absorptiometry (DXA) (Lunar Prodigy Advanced Scanner, GE Medical Systems, UK). Total tissue mass was defined as the sum of total lean mass and total fat mass. Percentage lean mass was the proportion of total tissue mass made up by total lean mass. Percentage fat mass was the proportion of total tissue mass made up by total fat mass. In all cases, assessments were made excluding the head. Positioning for all scans was completed in accordance with manufacturer instructions. *In vivo* precision (root-mean square coefficient of variation (%CV)) for the DXA scanner has been determined for lean mass (1.87%) and fat mass (1.75%) by measuring 35 individuals twice on the same day with re-positioning.

On average, taller individuals have greater lean mass and bone size. Therefore, in definitions of sarcopenia, lean mass is often assessed as lean mass index (LMI) by dividing by height squared (20-22). The function of dividing by height squared has been shown to minimise the correlation between lean mass and height (21). Fat mass index (FMI) has also been developed akin to body mass index (BMI) (23). LMI and FMI were calculated in this way in the current study.

2.3. HRpQCT

Each participant had measurements of the non-dominant distal radius and tibia using HRpQCT (XtremeCT, Scanco Medical AG, Switzerland) except when the non-dominant limb had previously fractured in which case the dominant side was scanned. This allowed acquisition of a stack of parallel CT slices using a two-dimensional detector array. At both sites a total of 110 slices were obtained which represented a volume of bone 9mm in axial length with a nominal resolution (voxel size) of 82µm. The scanned limb was immobilized during the examination in a carbon fibre cast. Antero-posterior 2D scout views were performed to determine the region to be scanned. Positioning was in keeping with the manufacturer's guidelines and as described by Boutroy *et al* (24). All scans were acquired by one of two trained technicians using standard positioning techniques. Each scan was assessed for motion artefact, and if present a second scan was performed. A total of 7 radial images and 3 tibial images were excluded due to excessive motion artefact (25). Initial image analysis was carried out using the standard manufacturer's method and Image Processing Language (IPL, Version 6.1, ScancoMedical). Further analysis was performed using an automated segmentation algorithm (26). Each measure used has been validated against micro-CT imaging (27).

Quality control testing was performed on a weekly basis and quality assurance on a daily basis. Short term precision values (%CV) for cortical and trabecular BMD have been shown to range from 0.3 to 1.2 (28). The effective dose to the subject during each scan was <3µSv.

2.4. Anthropometry and Interviews

Height was measured to the nearest 0.1 cm using a wall-mounted SECA stadiometer on the day of scanning. Details regarding physical activity, dietary calcium intake, smoking status, alcohol consumption, socioeconomic status and, in women, years since menopause and use

of estrogen replacement therapy were collected from researcher-administered questionnaires. Physical activity was calculated as a standardised score ranging from 0–100 derived from frequency of gardening, housework, climbing stairs and carrying loads in a typical week based on the questionnaire of customary physical activity by Dallosso *et al.* (29). Higher scores indicated greater levels of activity. Dietary calcium intake and vitamin D intake (excluding supplements) were assessed using a food frequency questionnaire (30). Socioeconomic status was determined using current or most recent occupation of the participant in men and single women, and of the husband in ever-married women based on the Office of Population Censuses and Surveys Standard Occupational Classification Scheme for occupation and social class.

The East and North Hertfordshire Ethical Committees granted ethical approval for the study and all participants gave written informed consent in accordance with Governance Arrangements for NHS Research Ethics Committees Harmonised edition (September 2011) (31).

2.5. Statistical analysis

Due to differences in bone and body composition variables between men and women, and significant sex-interactions for relationships between body composition and HRpQCT parameters in the study cohort, we presented results separately for the two sexes. Study participant demographic and lifestyle characteristics, and body composition and HRpQCT measures were therefore described separately for men and women. Continuous variables were described as means [standard deviations (SDs)] and categorical and binary variables were summarised by counts and percentages. Differences between demographic and lifestyle characteristics in men and women were assessed using Student's t-tests for continuous variables and Pearson's X^2 test or Fisher's exact test for categorical variables. Multiple testing was then taken into consideration using a Bonferroni correction.

The primary analysis used multivariable linear regression to examine the associations between the measures of body composition (LMI and FMI) and HRpQCT bone parameters in the radius and tibia while examining sex interactions. To probe the significant interactions, the same relationships were then examined for men and women separately. To provide a graphical representation comparing regression coefficients across different bone parameters, anatomical regions of interests and between the sexes, relationships were reanalysed after standardization and differences were expressed as SD change in outcome per SD increase in predictor. These analyses were repeated with and without adjustment for a priori confounders: age, smoking status, alcohol consumption, calcium intake, vitamin D intake, physical activity, social class, and in women, years since menopause and estrogen replacement therapy.

Bone microarchitectural parameters were then regressed on both LMI and FMI within the same model. Due to the potential correlation between LMI and FMI, regression analysis of FMI against LMI was carried out to produce standardised FMI residuals – the difference between the observed FMI and the value that would be estimated purely as a function of LMI. The process of regression analysis was repeated to produce LMI residuals. HRpQCT outcomes were then regressed on LMI and FMI residuals, and vice versa. These analyses

were repeated with and without adjustment for a priori confounders as described above. Furthermore, as both LMI and FMI include height a further model using lean mass, fat mass and height was also produced to ensure over-adjustment for height had not occurred. Statistical significance was assessed at the $\alpha = 0.05$ level. Data were analysed using STATA version 12.

3. Results

The mean (SD) age of participants was 76 (3) years. On average, men were taller and heavier than women but BMI did not differ significantly by sex (table 1). 59.5% of men were current or ex-smokers whereas this figure was just 34.0% in women. Social class was similar in men and women. Table 2 describes components of body composition and bone microarchitecture in men and women.

With the exception of radial cortical thickness in women, positive relationships were found between both LMI and FMI with cortical area and thickness in both the radius and tibia (figure 1). Associations were stronger in the tibia than the radius and standardised regression coefficients tended to be greater for LMI than FMI. Radial and tibial trabecular number was positively associated with both LMI and FMI. FMI also showed a positive relationship with trabecular density. Adjustment for demographic and lifestyle covariates (age, smoking status, alcohol consumption, calcium intake, vitamin D intake, physical activity, social class, and in women, years since menopause and estrogen replacement therapy) did not materially affect the relationships described (table 3). Interactions by sex were found for the relationships of LMI with radial cortical area and tibial cortical area and thickness, and for the relationships of FMI with radial total area, radial trabecular area and tibial trabecular area.

When HRpQCT outcomes were regressed on LMI and FMI within the same model and again adjusted for demographic and lifestyle covariates, LMI was positively associated with cortical area and thickness in both men and women although this did not reach statistical significance for radial cortical thickness in women. Conversely, FMI was positively associated with trabecular number and inversely related to trabecular separation, although associations with trabecular separation did not reach statistical significance in the radius (table 4). These findings are similar to associations between bone microarchitecture and LMI residuals and FMI residuals respectively, and to the associations of bone microarchitecture with lean mass and fat mass when included in the same model and adjusted for height (results not shown).

4. Discussion

LMI and FMI showed differing relationships with bone microarchitecture as assessed by HRpQCT at the distal radius and tibia. After LMI and FMI were mutually adjusted for one another, relationships with trabecular number were maintained for FMI whereas LMI was only found to be associated with cortical area and thickness. Sex interactions were found for the relationships between LMI and cortical area, and between FMI and trabecular area in both the radius and tibia.

In univariate analyses LMI and FMI were positively associated with cortical area, cortical thickness, and trabecular number. Mutual adjustment of LMI for FMI and vice versa, led to attenuation of relationships between FMI and cortical geometry whereas relationships with LMI remained robust. A similar pattern was shown for LMI residuals when included in a model with FMI. The relationship between LMI and cortical geometry is in keeping with the wider literature using pQCT which has shown analogous relationships between estimations of muscle mass and cortical geometry in 48 middle-aged (mean age 49.4 ± 2.4 years) adults (32) and cohorts of older men and women (59.4 ± 7.2 years (33) and >60 years (2)).

The independent relationship between lean mass and cortical geometry can be explained in several ways. The mechanostat hypothesis states that muscles apply forces to bones during contractions that cause deformations, or strains, within the bone tissue (10). These are sensed, predominantly by osteocytes, and provide stimuli for the bone to increase or maintain its strength; one such way it might do this is through an increase in cortical area and thickness. Clearly, the mechanostat may not be the sole mechanism of association as muscle size has been shown to be more strongly related to bone structure than muscle strength in older men and women (2) although this may partly be due to grip strength not accurately measuring the forces applied to bone by muscle contractions. Interestingly, an interaction by sex was identified for the relationship between LMI and cortical area in both the radius and tibia. In men, there was a significantly greater positive association. One possible explanation for this might be that muscle produces higher forces in men than women. Consequently, any increase in muscle size is likely to have a greater absolute change in the force applied to bone leading to a greater effect on bone structure.

Another reason why lean mass might correlate with bone health would include a common developmental component. In keeping with the developmental origins hypothesis, there is evidence that both muscle and bone strength are associated with early life environment (34, 35). Shared genetic and hormonal factors may also play a role. Both may positively impact muscle and bone growth, either directly or indirectly, resulting in a positive correlation between these two facets of musculoskeletal health (36). Lastly, exercise can affect both bone structure and muscle size in later life (37, 38). Although we attempted to adjust for the effect of contemporaneous physical activity, we were not able to account for exercise levels in childhood and early adulthood which may have had significant and enduring effects on lean mass and bone health.

In univariate analyses, both FMI and LMI were positively associated with trabecular number. The magnitude of this association was greater for FMI and this parameter was also positively related to trabecular density. Relationships between fat mass and trabecular density have been inconsistent in previous studies. In 140 postmenopausal women (mean age 68.4 years), assessed by Ng and colleagues using HRpQCT, a positive association was found between total body fat and radial trabecular density but, as in our study, it failed to reach statistical significance at this site in 169 older men (mean age 68.7 years). Positive relationships were also shown between fat mass and trabecular number in both men and women (11).

However, in a study of 50 slightly younger obese men and women with metabolic syndrome (mean age 34.6 years) significant associations between fat mass and bone microarchitecture were not found (39). This deviance from the results of the current study may be due to the younger age of the participants or related to the lack of underweight, normal and overweight individuals within the cohort.

In contrast to cortical parameters, in the current study relationships with trabecular number were fully attenuated for LMI after adjustment for FMI and an independent association with bone microarchitecture was only present for FMI. If the relationship between body composition and trabecular number was purely a weight-bearing effect then we would have expected a similar association with LMI. Therefore, this suggests that the univariate association between LMI and trabecular number occurs through confounding by FMI and the relationship between FMI and trabecular number is not purely a weight-bearing effect, instead inferring the importance of independent biological mechanisms relating to adipose tissue, which will now be discussed.

An epidemiological study exploring data from twins and their first degree relatives has shown that fat mass and BMD in both the hip and lumbar spine and likely to be influenced by common genes, suggesting these genes may have pleiotrophic effects related to both adiposity and bone health (4). There is evidence of a link between poorer trabecular bone health and greater marrow adiposity (40) which may be found in individuals with greater FMI (41). Several endocrine factors can also influence the fat-bone relationship. In particular, adipokines, such as leptin and adiponectin, are thought to play an important role (42, 43). Leptin is produced by adipocytes and its level is proportional to total body fat mass; it is therefore elevated in obesity (44). Osteoblasts and chondrocytes have leptin receptors and leptin may increase osteoblast differentiation and proliferation, and through the RANKL-OPG axis, inhibit osteoclastogenesis (42, 45). In this way leptin may increase skeletal mass to support the greater fat mass. Adiponectin's mechanism of action is not fully understood but is likely to relate to direct effects on osteoblasts and osteoclasts (46). Although, like leptin, it is produced in fat cells, levels are inversely related to BMI (47). Adiponectin levels are negatively associated with BMD (43) and therefore as individuals with higher FMI would have lower levels of adiponectin they would be expected to have a higher BMD.

Adipocytes also produce estrogen, under the regulation of several paracrine and autocrine factors (48), and in postmenopausal women this is the main source. FM has also been shown to increase the levels of bioavailable estradiol by directly inhibiting production of sex hormone binding globulin in the liver (49). Estrogen is well established to have beneficial effects on bone and may therefore play a role in the relationship found. It can also have positive effects on muscle function, for example through improved repair and regeneration (50). This may further augment the univariate relationship between fat and bone.

Insulin also influences bone health. It is co-secreted with amylin and preptin and these hormones are found at higher levels in obesity. Insulin, amylin and preptin have all been shown to stimulate osteoblast growth. Amylin additionally inhibits osteoclastic function and

thus bone resorption. Epidemiological studies are consistent with these effects showing hypoinsulinaemia to be associated with low BMD (51).

The cohort in which this study was completed is unique and important to discuss. It comprises a group of men and women born as singleton births between 1931 and 1939 that were traced through their birth records. Its main strengths are that it is well phenotyped which allows adjustment for potential covariates. It is also community based, includes both men and women, and has been shown to be fairly representative of the UK population by means of a comparison with the Health Survey for England (19). The results are therefore likely to be generalizable to this population. However, those studied were entirely Caucasian and recruited one specific region of Hertfordshire. Thus, extrapolation of the findings to other ethnic groups and regions is uncertain. Furthermore, body composition data was only available on a subset (91%) of participants and therefore current analyses were limited to this group. Although it is possible that this might affect external validity, this group was not found to differ significantly from the overall study cohort. The cohort also has a narrow age range and associations may vary across different stages of adulthood, particularly in women. Consequently, the study is likely to provide useful information about older individuals but this may not be as applicable to other demographic groups. Other limitations of the study include its cross-sectional design which makes attribution of causality difficult. Furthermore, bone microarchitecture was studied in the extremities whereas components of body composition were assessed within the whole body minus head. This clearly limits evaluation of direct local associations.

5. Conclusions

We have demonstrated that LMI was positively associated with radial and tibial cortical geometry independent of FMI. Whereas, FMI was positively associated with trabecular number independent of LMI. Furthermore, interactions by sex were also found, including for the relationships of LMI with cortical area and FMI with trabecular area in both the radius and tibia. To our knowledge, this study is the first to demonstrate these findings. Components of body composition therefore clearly display important relationships with bone structure. However, longitudinal analyses are required to confirm the direction of causality. The differing associations identified may reflect predominantly physical elements relating muscle to cortical bone, and endocrine factors from adipose tissue affecting trabecular bone with its greater rate of turnover. However, an understanding of the precise mechanisms involved will require further investigation.

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Figure 1.

Regression coefficients and 95% confidence intervals for HRpQCT parameters by lean mass index (black boxes) and fat mass index (white boxes); all variables expressed as z-scores

Table 1

Summary of participant characteristics

Variable	Women		Men		p value ^e
	Total N	Mean (SD)	Total N	Mean (SD)	
Age ^a	167	76.4 (2.6)	175	76.0 (2.6)	0.135
Height (cm)	167	160.2 (6.3)	175	173.6 (6.5)	<0.001
Weight (kg)	167	71.1 (12.6)	175	81.8 (11.7)	<0.001
BMI (kg/cm ²)	167	27.7 (4.7)	175	27.2 (3.7)	0.227
Weekly calcium intake (mg)	167	7906 (2685)	175	8503 (2039)	0.021
Vitamin D intake (ug)	167	25.7 (10.3)	175	25.8 (11.4)	0.902
Physical activity score	167	61.9 (13.2)	175	66.0 (13.1)	0.005
	Total N	n (%)	Total N	n (%)	
Smoker status	165		175		
Never		109 (66.1)		71 (40.6)	
Ex		48 (29.1)		92 (52.6)	
Current		8 (4.8)		12 (6.9)	<0.001
Alcohol intake	166		175		
None		33 (19.9)		9 (5.1)	
Recommend ^b		130 (78.3)		136 (77.7)	
>Recommend		3 (1.8)		30 (17.1)	<0.001
Social status ^c	167		169		
I-IIIINM		72 (43.1)		77 (45.6)	
IIIM-V		95 (56.9)		92 (54.4)	0.651
Years since menopause ^d	164		NA	NA	
<5 yrs		4 (2.4)			
5 to <10 yrs		18 (11.0)			
10 to <15 yrs		37 (22.6)			
15 to <20 yrs		32 (19.5)			
>20 yrs		30 (18.3)			
Hysterectomy		43 (26.2)			NA
HRT (ever use) ^d	167		NA	NA	
Yes		69 (41.3)			
No		98 (58.7)			NA

^aAt time of scans;^bRecommended maximum weekly consumption of alcohol (14 units for women, 21 units for men);^cI-IIIINM (I to III non-manual), IIIM-V (III manual to V);^dYears since menopause and Hormone Replacement Therapy as measured at HCS baseline (1998-2003);^eBonferroni correction used to adjust significance level to p<0.005

Table 2

Summary of body composition by dual energy X-ray absorptiometry and bone microarchitecture by high resolution peripheral quantitative computed tomography in men and women.

Variable		Women Mean (SD)	Men Mean (SD)
Whole body DXA ¹			
Tissue Mass (kg)		68.24 (12.24)	78.84 (11.55)
Fat Mass (kg)		29.63 (9.28)	25.12 (8.35)
Fat Mass Index (kg/m ²)		11.55 (3.59)	8.35 (2.76)
Fat Mass (% of tissue)		42.48 (6.79)	31.18 (6.78)
Lean Mass (kg)		38.62 (4.41)	53.72 (5.27)
Lean Mass Index (kg/m ²)		15.06 (1.55)	17.82 (1.44)
Lean Mass (% of tissue)		57.51 (6.79)	68.83 (6.78)
HRpQCT ²			
Total area (mm ²)	R	286.2 (42.8)	427.0 (78.5)
	T	716.0 (101.5)	904.1 (145.3)
Cortical area (mm ²)	R	47.2 (8.5)	72.0 (14.2)
	T	93.1 (16.3)	142.2 (27.3)
Cortical thickness (microm)	R	682.0(165.1)	838.2(195.6)
	T	917.3(192.2)	1224.2(260.9)
Cortical density (mg/cm ³)	R	902.7 (59.4)	908.2 (48.9)
	T	810.1 (64.9)	874.3 (59.8)
Cortical porosity (%)	R	3.6 (1.4)	4.1 (1.5)
	T	10.0 (2.6)	8.9 (2.5)
Trabecular area (mm ²)	R	237.3 (44.3)	350.4 (74.8)
	T	621.8 (108.0)	761.5 (149.5)
Trabecular density (mg/cm ³)	R	142.0 (43.1)	178.9 (37.1)
	T	170.2 (40.7)	188.2 (34.9)
Trabecular number(cm ⁻¹)	R	20.6(4.1)	23.4(2.5)
	T	22.6(3.6)	24.3(3.0)
Trabecular thickness (microm)	R	56.7(10.6)	63.5(10.3)
	T	62.8(12.0)	64.8(9.8)
Trabecular separation (microm)	R	454.9(145.4)	370.2(62.6)
	T	392.8(88.1)	354.0(54.3)

¹ n=167 for women and 175 for men.

² In the radius, n=164 for women and 171 for men; in the tibia, n=164 for women and 175 for men. R, radius; T, tibia.

Table 3

Lean mass index and fat mass index individually regressed on HRpQCT outcomes by sex with adjustment for covariates*.

		Lean mass index				Fat mass index			
		Men		Women		Men		Women	
		Beta (95% CI)	p-value	Beta (95% CI)	p-value	Beta (95% CI)	p-value	Beta (95% CI)	p-value
Total area (mm ²)	R	4.02 (−5.11,13.15)	0.386	4.63 (−0.05,9.32)	0.053	−4.47 (−9.32,0.38)	0.071 [†]	1.41 (−0.61,3.42)	0.171 [†]
	T	6.38 (−10.08,22.83)	0.445	4.41 (−6.15,14.96)	0.411	−5.26 (−14.01,3.49)	0.237	2.19 (−2.38,6.76)	0.344
Cortical area (mm ²)	R	2.90 (1.35,4.44)	<0.001[†]	1.38 (0.48,2.28)	0.003[†]	0.72 (−0.13,1.57)	0.098	0.48 (0.09,0.86)	0.017
	T	7.91 (5.10,10.72)	<0.001[†]	4.49 (2.93,6.05)	<0.001[†]	2.70 (1.12,4.29)	0.001	1.56 (0.85,2.26)	<0.001
Cortical thickness (μm)	R	33.47 (11.70,55.24)	0.003	9.28 (−8.75,27.32)	0.311	12.48 (0.67,24.29)	0.038	3.40 (−4.33,11.13)	0.386
	T	60.34 (32.78,87.90)	<0.001[†]	33.16 (13.74,52.58)	0.001[†]	22.40 (7.24,37.57)	0.004	9.50 (0.90,18.10)	0.031
Cortical density (mg/cm ³)	R	2.58 (−2.91,8.06)	0.355	−3.21 (−9.63,3.22)	0.325	−0.73 (−3.71,2.25)	0.630	−0.52 (−3.28,2.23)	0.707
	T	4.41 (−2.27,11.09)	0.194	6.52 (−0.32,13.36)	0.061	0.63 (−2.96,4.22)	0.731	3.43 (0.49,6.38)	0.023
Cortical porosity (%)	R	0.06 (−0.09,0.22)	0.419	0.06 (−0.09,0.22)	0.414	0.07 (−0.01,0.15)	0.097	0.00 (−0.06,0.07)	0.956
	T	0.08 (−0.19,0.36)	0.559	0.03 (−0.26,0.32)	0.832	0.11 (−0.04,0.25)	0.158	0.02 (−0.11,0.14)	0.788
Trabecular area (mm ²)	R	1.78 (−6.93,10.50)	0.686	3.50 (−1.35,8.35)	0.156	−4.17 (−8.79,0.45)	0.076 [†]	1.11 (−0.97,3.19)	0.294 [†]
	T	−2.38 (−19.24,14.49)	0.781	0.39 (−10.80,11.57)	0.946	−8.72 (−17.63,0.18)	0.055 [†]	0.88 (−3.97,5.72)	0.721 [†]
Trabecular density (mg/cm ³)	R	1.98 (−2.21,6.17)	0.352	3.16 (−1.34,7.67)	0.167	1.79 (−0.44,4.02)	0.115	2.18 (0.27,4.09)	0.025
	T	1.97 (−1.93,5.88)	0.320	1.44 (−2.80,5.69)	0.503	2.50 (0.45,4.55)	0.017	1.50 (−0.32,3.33)	0.105
Trabecular number (cm ^{−1})	R	0.23 (−0.05,0.51)	0.108	0.40 (−0.03,0.83)	0.070	0.19 (0.04,0.33)	0.014	0.29 (0.11,0.47)	0.002
	T	0.54 (0.23,0.86)	0.001	0.59 (0.22,0.96)	0.002	0.44 (0.28,0.60)	<0.001	0.34 (0.18,0.49)	<0.001
Trabecular thickness (μm)	R	0.06 (−1.10,1.21)	0.925	0.01 (−1.13,1.14)	0.992	0.13 (−0.49,0.75)	0.684	−0.00 (−0.49,0.48)	0.993
	T	−0.78 (−1.87,0.30)	0.154	−0.96 (−2.24,0.32)	0.141	−0.27 (−0.85,0.31)	0.362	−0.38 (−0.94,0.17)	0.177
Trabecular separation (μm)	R	−5.59 (−12.74,1.56)	0.124	−9.08 (−24.50,6.34)	0.246	−3.45 (−7.27,0.37)	0.076	−7.43 (−13.95,−0.92)	0.026
	T	−7.75 (−13.62,−1.89)	0.010	−12.05 (−21.25,−2.85)	0.011	−6.79 (−9.81,−3.77)	<0.001	−6.43 (−10.37,−2.49)	0.002

Key: In bold if p<0.05. R, radius; T, tibia.

* Covariates are age at HRpQCT scan, smoking status, alcohol consumption, calcium intake, vitamin D intake, physical activity and social class; with additional adjustment for years since menopause and HRT use in women.

[†] Significant sex interaction (p<0.05).

Table 4

Lean mass index and fat mass index within mutually adjusted model, regressed on HRpQCT outcomes by sex with adjustment for covariates*.

		Lean mass index				Fat mass index			
		Men		Women		Men		Women	
		Beta (95% CI)	p-value	Beta (95% CI)	p-value	Beta (95% CI)	p-value	Beta (95% CI)	p-value
Total area (mm ²)	R	8.45 (−1.29,18.19)	0.089	3.99 (−1.45,9.43)	0.149	−6.20 (−11.42,−0.98)	0.020	0.54 (−1.79,2.87)	0.645
	T	12.10 (−5.55,29.75)	0.178	2.50 (−9.72,14.71)	0.687	−7.70 (−17.12,1.73)	0.109	1.65 (−3.64,6.94)	0.538
Cortical area (mm ²)	R	2.77 (1.10,4.44)	0.001	1.10 (0.06,2.14)	0.038	0.15 (−0.74,1.05)	0.741	0.24 (−0.21,0.68)	0.291
	T	6.99 (3.97,10.01)	<0.001	3.59 (1.81,5.38)	<0.001	1.30 (−0.31,2.91)	0.114	0.78 (0.01,1.55)	0.048
Cortical thickness (μm)	R	27.92 (4.39,51.45)	0.020	7.08 (−13.87,28.02)	0.505	6.75 (−5.85,19.36)	0.292	1.87 (−7.10,10.84)	0.680
	T	51.30 (21.61,80.99)	0.001	29.59 (7.12,52.06)	0.010	12.08 (−3.77,27.93)	0.134	3.09 (−6.64,12.83)	0.531
Cortical density (mg/cm ³)	R	3.32 (−2.70,9.34)	0.278	−3.47 (−10.94,3.99)	0.360	−1.41 (−4.63,1.82)	0.390	0.22 (−2.97,3.42)	0.890
	T	4.50 (−2.75,11.75)	0.222	3.41 (−4.45,11.27)	0.392	−0.28 (−4.15,3.59)	0.887	2.70 (−0.71,6.10)	0.120
Cortical porosity (%)	R	0.02 (−0.15,0.19)	0.798	0.08 (−0.10,0.26)	0.362	0.07 (−0.02,0.16)	0.152	−0.02 (−0.09,0.06)	0.680
	T	0.01 (−0.29,0.31)	0.940	0.02 (−0.32,0.35)	0.929	0.10 (−0.06,0.26)	0.202	0.01 (−0.13,0.16)	0.851
Trabecular area (mm ²)	R	5.86 (−3.46,15.17)	0.216	2.95 (−2.69,8.58)	0.303	−5.37 (−10.37,−0.38)	0.035	0.47 (−1.94,2.89)	0.699
	T	4.85 (−13.21,22.90)	0.597	−0.84 (−13.79,12.12)	0.899	−9.70 (−19.34,−0.06)	0.049	1.06 (−4.55,6.67)	0.710
Trabecular density (mg/cm ³)	R	0.77 (−3.75,5.30)	0.736	0.80 (−4.38,5.97)	0.762	1.63 (−0.79,4.06)	0.186	2.01 (−0.21,4.23)	0.075
	T	0.22 (−3.94,4.38)	0.917	−0.39 (−5.28,4.49)	0.874	2.46 (0.23,4.68)	0.030	1.59 (−0.52,3.70)	0.139
Trabecular number (cm ^{−1})	R	0.12 (−0.18,0.42)	0.437	0.08 (−0.42,0.57)	0.760	0.16 (0.00,0.32)	0.048	0.27 (0.06,0.49)	0.011
	T	0.28 (−0.04,0.60)	0.089	0.27 (−0.15,0.68)	0.208	0.38 (0.21,0.56)	<0.001	0.28 (0.10,0.46)	0.002
Trabecular thickness (μm)	R	−0.08 (−1.34,1.18)	0.905	0.01 (−1.31,1.33)	0.986	0.14 (−0.53,0.82)	0.674	−0.00 (−0.57,0.56)	0.987
	T	−0.71 (−1.88,0.46)	0.232	−0.69 (−2.17,0.79)	0.357	−0.13 (−0.75,0.50)	0.693	−0.23 (−0.87,0.41)	0.477
Trabecular separation (μm)	R	−3.76 (−11.48,3.97)	0.338	−0.43 (−18.13,17.26)	0.961	−2.68 (−6.81,1.46)	0.203	−7.34 (−14.92,0.24)	0.057
	T	−3.51 (−9.61,2.59)	0.258	−6.17 (−16.66,4.31)	0.246	−6.09 (−9.34,−2.83)	<0.001	−5.09 (−9.63,−0.55)	0.028

Key: In bold if p<0.05. R, radius; T, tibia.

* Covariates are age at HRpQCT scan, smoking status, alcohol consumption, calcium intake, vitamin D intake, physical activity and social class; with additional adjustment for years since menopause and HRT use in women