Adipose tissue, estradiol levels, and bone health in obese men with metabolic syndrome

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Abstract

Objective: Visceral adipose tissue (VAT) is associated with an increased risk of metabolic syndrome (MetS). Recent studies have suggested that VAT negatively affects bone. However, MetS has also been associated with higher estradiol (E₂) levels, which is bone protective. We therefore investigated the impact of VAT and E₂ levels on bone density, structural parameters, and strength estimates.

Design: A cross-sectional study was conducted in 72 obese men with MetS to investigate the impact of VAT and E₂ levels on bone.

Methods: Bone parameters were assessed by dual-energy X-ray absorptiometry (DXA), quantitative computed tomography (QCT), and high-resolution peripheral QCT (HRpQCT) at lumbar spine, proximal femur, radius, and tibia. VAT volume was measured by magnetic resonance imaging (MRI) and sexual hormones were measured in blood samples.

Results: Men with high VAT had a lower bone density at the hip ($P < 0.05$), lower cortical thickness, and higher buckling ratio at femoral neck (FN) ($P = 0.008$ and $P = 0.02$), compared with men with low VAT, despite a similar body weight and BMI. Generally, E₂ levels were low (median 43 pmol/l), and men with E₂ levels below median had reduced bone density at lumbar spine ($P = 0.04$), and impaired structural parameters at radius and tibia, compared with men with E₂ levels above median. At the hip, VAT volume and E₂ levels affected bone density independently and additively, and 50% of men with high VAT and low E₂ levels had osteopenia with significantly lower *T*-score at FN ($P = 0.004$).

Conclusions: High VAT and low E₂ negatively affect bone in obese men with MetS. VAT and E₂ affect bone density at the hip independently and additively, revealing an unexpected high prevalence of osteopenia in middle-aged men with MetS.

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Introduction

The prevalence of obesity and osteoporosis has rapidly increased over the last decades (1, 2). The bone–fat relationship has proven to be complex and published results in the field have been ambiguous. Body weight and BMI are positively correlated with bone mineral density (BMD) (3, 4, 5). However, obese individuals seem to have increased fracture risk regardless of a higher BMD (6, 7, 8),

and similarly for patients with type 2 diabetes (9, 10, 11). Obesity, metabolic syndrome (MetS), and type 2 diabetes are all associated with low-grade inflammation (12, 13). As visceral adipose tissue (VAT) produces inflammatory cytokines that are harmful to bone, research has focused on the role of VAT on bone (14, 15, 16, 17). At odds with this, a large VAT depot is also associated with an

increased aromatase activity and increased levels of estradiol (E_2) (18, 19), which is bone protective (20). Khosla *et al.* (21) found no association between bone density and sex steroid levels in eugonadal young men, but did find positive associations between bone density and low testosterone and E_2 levels in elderly men.

The effect of VAT on bone has previously been investigated in a number of studies. Gilsanz *et al.* (22) showed that, in young women, VAT was negatively correlated with BMD, whereas subcutaneous adipose tissue (SAT) was positively correlated with BMD. Yamaguchi *et al.* (23) showed that, in men with type 2 diabetes, both SAT and VAT were positively correlated with BMD. When data were adjusted for body weight, opposing results emerged, but VAT remained inversely related to the risk of vertebral fractures. Finally, Choi *et al.* (24) found that VAT was negatively correlated with BMD, whereas SAT was positively correlated with BMD in Korean men and women, after adjusting for body weight. These seemingly conflicting results may be due to the fact that SAT, VAT, waist circumference (WC), and BMI are highly correlated in most populations (25, 26) and this may potentially disturb statistical analyses due to collinearity.

We therefore investigated the impact of visceral fat volume and serum levels of E_2 on areal BMD (aBMD), volumetric BMD (vBMD), bone structure, and estimates of bone strength in a very homogeneous population of obese middle-aged men with MetS.

Subjects and methods

Study design and subjects

This was a cross-sectional study on baseline data from a randomized, placebo-controlled, double-blinded, single-center study (27). Seventy-two subjects were included in this study. Inclusion criteria were male sex, age between 30 and 60 years, and MetS. MetS was defined according to the International Diabetes Federation (28) as central obesity (WC ≥ 94 cm and/or BMI > 30 kg/m²) plus any two of the following: raised triglycerides (≥ 1.7 mmol/l), reduced HDL (≤ 1.03 mmol/l), raised blood pressure (systolic ≥ 130 mmHg or diastolic ≥ 85 mmHg), raised fasting plasma glucose (≥ 5.6 mmol/l), or drug treatment for the individual features. Exclusion criteria were other overt endocrine diseases, renal disease, hepatic disease, heart disease, malignant disease, anemia, alcohol abuse, planned lifestyle changes, and absolute or relative contraindications for magnetic resonance imaging (MRI).

Ethical aspects

The protocol was approved by the Regional Committee on Health Research Ethics (M-20110111) and the Danish Data Protection Agency, and the study was conducted in agreement with the Declaration of Helsinki II. Participants were given oral and written information about the purpose and nature of all procedures before informed consent was obtained. The protocol was registered at clinicaltrials.gov (NCT01412645) before recruitment was initiated.

Magnetic resonance imaging

VAT and SAT were determined with MRI using a fast spin echo sequence (Signa Excite 1.5 Tesla, Twin Speed; GE Medical Systems, Little Chalfont, Bucks, UK). Axial slices were obtained from the proximal border of the femoral heads to the upper pole of the most superior kidney, and Hippo Fat was used for data processing, providing estimates for SAT and VAT (29, 30).

Dual-energy X-ray absorptiometry

aBMD at the lumbar spine (L1–L4) and hip was measured by dual-energy X-ray absorptiometry (DXA) using a Hologic Discovery scanner. Coefficient of variation (CV) of repositioning is 1.5% for lumbar spine BMD and 2.1% for femoral neck (FN) (31, 32). In addition, whole-body fat mass and lean body mass were measured by DXA.

Quantitative computed tomography

vBMD at lumbar vertebra 2 (LS vBMD) and the proximal femur was measured using a Philips Brilliance 40 multidetector helical CT scanner. CT scans were acquired from the distal endplate of L1 to the proximal endplate of L3, and from acetabulum directly above the femoral head to 2 cm below the lesser trochanter (TR), with 3 mm slice thickness and spacing as described previously (27). M J Orstrup analyzed all quantitative computed tomography (QCT) data, blinded to VAT distribution and E_2 levels, with a reanalysis precision of $0.6 \pm 0.6\%$ for lumbar spine (LS) vBMD_{trab}, and $0.6 \pm 0.7\%$ for total hip (TH) vBMD_{integral}.

We determined vBMD_{integral} in standard regions (TH, FN, TR, and intertrochanteric (IT)), along with separate trabecular estimates (vBMD_{trab}) and cortical estimates (vBMD_{cortical}). A fixed threshold for cortical separation of 0.350 g/cm³ was used. Geometric measures at the FN;

cross-sectional area (CSA_{total} , $CSA_{cortical}$, and cortical thickness (Ct.Th), along with estimates of bone strength (cross-sectional moment of inertia (CSMI) and buckling ratio) were determined (33, 34, 35).

High-resolution peripheral QCT

Assessment of geometry and microarchitecture of the non-dominant distal radius and tibia (or in case of a previous fracture in the non-fractured limb) were obtained using high-resolution peripheral QCT (HRpQCT) (XtremeCT, Scanco Medical, Brüttisellen, Switzerland) as described previously (27). The analysis was performed by M J Orstrup, who was blinded to VAT distribution and E_2 levels. CV of repositioning for radius and tibia density measures were 0.5–1.5% and the structural parameters 1–5% (36, 37). The following parameters were measured or calculated: trabecular bone volume fraction (BV/TV) is derived from the trabecular volume density, assuming a mineral density of fully mineralized bone of 1200 mg hydroxyapatite/cm³. Trabecular number (Tb.N) is a direct measure. Trabecular thickness (Tb.Th) and trabecular space (Tb.Sp) are calculated from Tb.N and BV/TV (38).

Biochemistry

We analyzed bone alkaline phosphatase (BAP), osteoprotegerin (OPG), osteocalcin (intact and N-terminal fragment), and procollagen 1 N-terminal propeptide (P1NP) as markers of bone formation. As markers of bone resorption, we analyzed C-terminal telopeptide of type 1 collagen (CTX), and cross-linked N-terminal telopeptide of type 1 collagen (NTx). In addition, parathyroid hormone (PTH) and 25-hydroxy vitamin D were measured. All analyses were performed by standard laboratory methods at the Department of Clinical Biochemistry (Aarhus University Hospital, Denmark), except OPG. OPG was measured using ELISA OPG Kit (Biomedica, Vienna, Austria). Intra- and inter-assay CV values were 4 and 5% respectively.

Markers reflecting metabolic state were measured; triglyceride, HDL, and alanine transaminase (ALT) were performed by standard laboratory methods at the Department of Clinical Biochemistry (Aarhus University Hospital, Denmark). Interleukin 6 (IL6), high-sensitivity C-reactive protein (hs-CRP), leptin, and adiponectin were analyzed using ELISA as described previously (39), and glucose using ELISA Kit from Roche with intra- and inter-assay CV values of 3 and 4% respectively. Sex steroid hormones (E_2 , estrone, testosterone, androstenedione,

and sex hormone-binding globulin (SHBG)) were measured by standard laboratory methods at the Danish National Health Laboratory.

Statistical analysis

Normality was checked by QQ-plots, and test for equal variance between groups was assessed by Levene's test for equal variances. The level of significance was 0.05. Analyses and graphs were performed with IBM SPSS Statistics (version 20), and SigmaPlot (version 12.5). Correlations between two variables were assessed by the Spearman's rank-order correlation. To determine the effect of VAT and E_2 on vBMD, structural bone parameters, and strength estimates, the study subjects were divided into two groups based on VAT median and E_2 median. Men with VAT below the median were grouped in VAT_{low}, and men with VAT above the median were grouped in VAT_{high}. Additionally, study subjects were divided into E_{2low} and E_{2high} according to E_2 levels below and above E_2 median.

To adjust for multiple comparisons, the multivariate *t*-test (Hotelling's *t*-test) was performed on biochemical markers of bone turnover and QCT-derived hip parameters. Variable dependency is accounted for within this multivariate test. Thereafter, differences between VAT groups and E_2 groups, respectively, were assessed by unpaired Student's *t*-test, or the Mann–Whitney rank sum test. Results are presented as mean $\pm$ s.d. or as median with interquartile (25%; 75%) range. Multiple linear regression analysis was used to investigate whether VAT group and E_2 group had independent impact on bone density at the hip. Finally, the population was divided according to VAT volume and E_2 levels into four groups (E_{2low} + VAT_{high}, E_{2low} + VAT_{low}, E_{2high} + VAT_{high}, and E_{2high} + VAT_{low}), and ANOVA with Student–Newman–Keuls *post hoc* adjustment was used to assess differences between groups.

Results

Population characteristics

The population consisted of 72 men aged 49.2 ± 6.3 years. Baseline characteristics relevant to MetS were as follows: BMI of 33.6 ± 3.3 kg/m², WC 114 (108; 119) cm, triglyceride 1.8 (1.3; 2.3) mmol/l, HDL 1.2 (1.0; 1.4) mmol/l, systolic blood pressure 145.5 ± 15.5 mmHg, diastolic blood pressure 89.1 ± 9.9 mmHg, and fasting plasma glucose 5.8 (5.3; 6.0) mmol/l. In this homogeneous population, BMI and SAT (Fig. 1A) and WC and SAT (Fig. 1B) were positively correlated, but no correlations were found between BMI and VAT (Fig. 1C) or WC and VAT (Fig. 1D).

Figure 1

Correlations between BMI and SAT (A), WC and SAT (B), BMI and VAT (C), and WC and VAT (D). Open circle represents each trial subject ($n=72$). ρ and P value from the Spearman's rank-order correlation.

Clinical and metabolic characteristics across visceral fat mass median

Median VAT for VAT_{low} and VAT_{high} groups were 2239 and 3283 cm³ respectively ($P<0.001$). Clinical characteristics such as age, weight, BMI, and WC did not differ between groups (Table 1). Other measures of fat distribution, including whole-body fat mass (by DXA), and subcutaneous fat mass (MRI) did not differ between

groups either (Table 1). The level of ALT was significantly higher in the VAT_{high} group, IL6 borderline was significantly higher, and HDL was significantly lower in the VAT_{high} group (Table 1). Other metabolic markers, including insulin, adiponectin, and leptin, were not different between groups (Table 1).

Bone density and structural parameters across visceral fat median

vBMD_{integral} at TH was significantly lower in the VAT_{high} group compared with the VAT_{low} group ($P=0.01$). Similar results were found at FN, TR, and IT, although only borderline significant at TR (Table 2). In addition, the sub-analysis of vBMD_{trab} at TH was significantly lower in the VAT_{high} group ($P=0.02$), with similar site-specific effects at FN, TR, and IT (Table 2). vBMD_{cortical} at TH was higher in the VAT_{high} group compared with the VAT_{low} group ($P=0.01$), and similarly at IT. At FN and TR, vBMD_{cortical} did not differ between groups (Table 2). CSA_{total} at FN was similar across VAT groups (Table 2), while FN CSA_{cortical} and Ct.Th were significantly lower in the VAT_{high} group compared with the VAT_{low} group ($P=0.005$ and $P=0.008$ respectively). Furthermore, FN buckling ratio was significantly higher in the VAT_{high} group compared with the VAT_{low} group ($P=0.02$). Multivariate t -test on all the above-mentioned QCT-derived hip parameters across VAT groups revealed overall significant effects ($P=0.027$).

Table 1 Clinical and metabolic features across visceral fat median. Data are expressed as mean $\pm$ s.d. or median with interquartile range (25%; 75%).

	VAT _{low} ($n=36$)	VAT _{high} ($n=36$)	<i>P</i> value
Visceral fat (cm ³)	2239 (2021; 2400)	3283 (2941; 3798)	<0.001
Age (years)	48.3 $\pm$ 6.3	50.1 $\pm$ 6.2	0.22
Weight (kg)	107.7 (97.7; 117.7)	107.6 (103.5; 114.3)	0.83
BMI (kg/m ²)	33.9 (30.8; 36.3)	32.9 (31.9; 34.9)	0.57
Waist circumference (cm)	115 (105; 120)	114 (110; 117)	0.88
Whole-body fat mass (kg)	32.9 (27.9; 39.3)	33.7 (31.4; 37.1)	0.34
Subcutaneous fat (cm ³)	5917 (4462; 7580)	5466 (4530; 6915)	0.48
ALT (U/l)	35 (30; 43)	43 (35; 55)	0.04
Systolic blood pressure (mmHg)	143 $\pm$ 14	148 $\pm$ 17	0.13
Diastolic blood pressure (mmHg)	88 $\pm$ 9	90 $\pm$ 11	0.36
Glucose (mmol/l)	5.65 (5.30; 6.15)	5.87 (5.30; 6.01)	0.75
HDL (mmol/l)	1.25 (1.10; 1.40)	1.10 (0.95; 1.35)	0.04
Triglyceride (mmol/l)	1.60 (1.10; 2.30)	1.90 (1.43; 2.38)	0.13
IL6 (pg/ml)	1.12 (0.70; 1.78)	1.54 (1.07; 2.20)	0.07
hs-CRP (mg/l)	2.60 (1.45; 4.80)	2.75 (1.90; 5.20)	0.56
Leptin (ng/ml)	16.1 (11.3; 20.9)	14.3 (12.4; 17.8)	0.62
Adiponectin (ng/ml)	1.41 $\pm$ 0.58	1.33 $\pm$ 0.45	0.57
Insulin (pmol/l)	103.2 (54.6; 129.0)	83.9 (64.8; 100.4)	0.45

The P values were calculated by unpaired Student's t -test or the Mann-Whitney rank sum test, where appropriate. Significant results are highlighted in bold. VAT_{low}, visceral adipose tissue volume below median VAT; VAT_{high}, visceral adipose tissue volume above median VAT; ALT, alanine transaminase; IL6, interleukin 6; hs-CRP, high-sensitivity C-reactive protein.

Table 2 Bone characteristics across visceral fat median. Data are expressed as mean $\pm$ s.d. or median with interquartile range (25%; 75%).

	VAT _{low} (n=36)	VAT _{high} (n=36)	P value
Lumbar spine vBMD (g/cm ³)	0.149 $\pm$ 0.03	0.140 $\pm$ 0.03	0.22
Hip vBMD (g/cm ³)			
Total hip			
vBMD _{integral}	0.330 $\pm$ 0.05	0.303 $\pm$ 0.04	0.01
vBMD _{trab}	0.170 $\pm$ 0.02	0.158 $\pm$ 0.02	0.02
vBMD _{cortical}	0.811 $\pm$ 0.04	0.834 $\pm$ 0.04	0.01
Femoral neck			
vBMD _{integral}	0.315 $\pm$ 0.05	0.283 $\pm$ 0.04	0.003
vBMD _{trab}	0.191 $\pm$ 0.02	0.178 $\pm$ 0.03	0.03
vBMD _{cortical}	0.604 $\pm$ 0.03	0.598 $\pm$ 0.04	0.47
Trochanter			
vBMD _{integral}	0.248 $\pm$ 0.04	0.233 $\pm$ 0.03	0.08
vBMD _{trab}	0.164 $\pm$ 0.02	0.154 $\pm$ 0.02	0.05
vBMD _{cortical}	0.750 (0.72; 0.77)	0.770 (0.73; 0.82)	0.12
Intertrochanteric			
vBMD _{integral}	0.387 $\pm$ 0.06	0.354 $\pm$ 0.05	0.01
vBMD _{trab}	0.174 $\pm$ 0.02	0.160 $\pm$ 0.03	0.02
vBMD _{cortical}	0.820 (0.81; 0.85)	0.850 (0.82; 0.87)	0.01
Femoral neck geometry and strength			
CSA _{total} (cm ²)	11.75 $\pm$ 1.40	11.91 $\pm$ 0.25	0.65
CSA _{cortical} (cm ²)	3.23 (2.93; 3.93)	2.85 (2.52; 3.30)	0.005
Cortical thickness (mm)	0.305 (0.24; 0.36)	0.261 (0.22; 0.29)	0.008
CSMI (mm ⁴)	7.21 $\pm$ 1.56	6.82 $\pm$ 1.60	0.30
Buckling ratio (1)	6.17 (5.05; 8.53)	7.48 (6.47; 9.16)	0.02
Radius geometry			
BV/TV (1)	0.159 $\pm$ 0.03	0.153 $\pm$ 0.03	0.41
Tb.N (/mm)	2.15 $\pm$ 0.21	2.22 $\pm$ 0.24	0.20
Tb.Th (mm)	0.074 $\pm$ 0.01	0.069 $\pm$ 0.01	0.07
Tb.Sp (mm)	0.394 (0.37; 0.41)	0.374 (0.35; 0.41)	0.46
Cortical area (cm ²)	7.73 $\pm$ 1.72	7.75 $\pm$ 1.41	0.94
Ct.Th (mm)	0.889 $\pm$ 0.22	0.888 $\pm$ 0.16	0.99
Tibia geometry	(n=33)	(n=34)	
BV/TV (1)	0.167 $\pm$ 0.02	0.157 $\pm$ 0.02	0.11
Tb.N (/mm)	2.13 $\pm$ 0.26	2.10 $\pm$ 0.26	0.56
Tb.Th (mm)	0.078 $\pm$ 0.01	0.075 $\pm$ 0.01	0.12
Tb.Sp (mm)	0.399 (0.34; 0.44)	0.397 (0.36; 0.44)	0.54
Cortical area (cm ²)	17.30 $\pm$ 2.91	16.60 $\pm$ 2.49	0.30
Ct.Th (mm)	1.458 $\pm$ 0.30	1.397 $\pm$ 0.26	0.36

The *P* values were calculated by unpaired Student's *t*-test or the Mann-Whitney rank sum test where appropriate. Significant results are highlighted in bold. VAT_{low}, visceral adipose tissue volume below median VAT; VAT_{high}, visceral adipose tissue volume above median VAT; vBMD, volumetric bone mineral density; vBMD_{trab}, trabecular vBMD; CSA, cross-sectional area; CSMI, cross-sectional moment of inertia; BV/TV, bone volume/tissue volume; Tb.N, trabecular number; Tb.Th, trabecular thickness; Tb.Sp, trabecular space; Ct.Th, cortical thickness.

At LS, vBMD_{trab} was non-significantly lower in the VAT_{high} group compared with the VAT_{low} group (*P*=0.22). DXA-derived aBMD at FN was significantly lower in the VAT_{high} group compared with the VAT_{low} group (*P*=0.03), while no difference could be detected at LS (*P*=0.96).

HRpQCT-derived bone parameters across visceral fat median

HRpQCT-derived bone parameters revealed no significant differences between VAT groups in any structural parameters in peripheral bone (Table 2).

Bone biochemistry across visceral fat median

Multivariate *t*-test on bone biochemistry across VAT groups failed to show overall significant effects (*P*=0.60), thus markers of bone turnover, calcium metabolism, and sex hormone status did not differ between VAT groups.

Table 3 presents the uncorrected *P* values.

Distribution of fat and sex hormones across E₂ median

In order to investigate the effect of E₂ on bone, the population was divided into two groups for comparison

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Table 3 Bone-relevant biochemical markers across visceral fat median. Data are expressed as mean $\pm$ s.d. or median with interquartile range (25%; 75%).

	VAT _{low} (n=36)	VAT _{high} (n=36)	P value
BAP (U/l)	28.6 $\pm$ 7.6	28.8 $\pm$ 6.7	0.91
Osteocalcin (μ g/l)	21.6 (17.9; 26.2)	20.1 (16.3; 24.6)	0.22
P1NP (μ g/l)	48.4 $\pm$ 14.8	43.0 $\pm$ 12.3	0.10
OPG (pmol/l)	4.8 $\pm$ 1.6	4.7 $\pm$ 1.4	0.86
CTx (ng/ml)	0.32 (0.26; 0.44)	0.30 (0.23; 0.45)	0.53
NTx (nmol/l)	19.0 $\pm$ 4.3	18.7 $\pm$ 4.5	0.76
PTH (pmol/l)	4.64 (3.78; 6.60)	5.11 (4.38; 6.01)	0.18
25-Hydroxy vitamin D (nmol/l)	47.3 (28.6; 75.2)	45.2 (30.6; 65.4)	0.82
Estradiol (pmol/l)	36.3 (14.5; 68.6)	50.5 (31.3; 75.0)	0.18
Testosterone (nmol/l)	11.4 $\pm$ 3.2	11.5 $\pm$ 3.3	0.91
SHBG (nmol/l)	31.4 $\pm$ 9.9	32.5 $\pm$ 11.3	0.66

The *P* values were calculated by unpaired Student's *t*-test or the Mann-Whitney rank sum test where appropriate. VAT_{low}, visceral adipose tissue volume below median VAT; VAT_{high}, visceral adipose tissue volume above median VAT; BAP, bone alkaline phosphatase; P1NP, procollagen 1 N-terminal propeptide; OPG, osteoprotegerin; CTx, C-terminal telopeptide of type 1 collagen; NTx, cross-linked N-terminal telopeptide of type 1 collagen; PTH, parathyroid hormone; SHBG, sex hormone-binding globulin.

based on E₂ median (E_{2low} and E_{2high}). Median E₂ for E_{2low} and E_{2high} were 20.0 and 70.5 pmol/l respectively (*P* < 0.001). In addition, estrone was different between E₂ groups (Table 4). Testosterone and androstenedione, which are converted by aromatase to E₂ and estrone, respectively, and SHBG and insulin were comparable between groups (Table 4). Clinical characteristics such as age, weight, BMI, and WC did not differ between groups, nor did measures of whole-body fat mass, VAT, and SAT (Table 4).

Table 4 Clinical features, fat distribution, and sex hormones across estradiol median. Data are expressed as mean $\pm$ s.d. or median with interquartile range (25%; 75%).

	E _{2low} (n=36)	E _{2high} (n=36)	P value
Estradiol (pmol/l)	20.0 (12.1; 33.8)	70.5 (57.6; 93.5)	<0.001
Estrone (pmol/l)	60.0 (47.0; 80.3)	94.0 (66.3; 126.5)	<0.001
Testosterone (nmol/l)	10.9 $\pm$ 3.0	11.9 $\pm$ 3.4	0.22
Androstenedione (nmol/l)	1.95 (1.38; 2.48)	1.71 (1.44; 2.14)	0.45
SHBG (nmol/l)	30.6 $\pm$ 10.1	33.3 $\pm$ 10.9	0.28
Insulin (pmol/l)	81.3 (52.2; 111.1)	98.2 (64.9; 113.9)	0.30
Age (years)	49.0 $\pm$ 6.6	49.4 $\pm$ 6.1	0.76
Weight (kg)	107.1 $\pm$ 13.6	110.7 $\pm$ 10.9	0.21
BMI (kg/m ²)	33.1 $\pm$ 3.3	34.1 $\pm$ 3.2	0.21
Waist circumference (cm)	113 (104; 120)	115 (110; 118)	0.26
Whole-body fat mass (kg)	32.6 (28.6; 38.9)	33.3 (31.9; 37.6)	0.31
Visceral fat (cm ³)	2638 (2141; 3186)	2824 (2277; 3372)	0.24
Subcutaneous fat (cm ³)	5437 (4471; 7119)	5786 (4589; 7222)	0.54

The *P* values were calculated by unpaired Student's *t*-test or the Mann-Whitney rank sum test where appropriate. Significant results are highlighted in bold. E_{2low}, estradiol below median E₂; E_{2high}, estradiol above median E₂; SHBG, sex hormone-binding globulin.

Bone density and structural parameters across E₂ median

At the lumbar spine, vBMD_{trab} was significantly lower in the E_{2low} group compared with the E_{2high} group (*P* = 0.04). Multivariate *t*-test on all QCT-derived hip parameters failed to show overall significant effects of E₂ at the hip (*P* = 0.25). The uncorrected *P* values are presented in Table 5.

DXA-derived aBMD at FN was significantly lower in the E_{2low} group compared with the E_{2high} group (*P* = 0.02), and a similar yet only borderline significant difference was found at LS (*P* = 0.058).

HRpQCT-derived bone parameters across E₂ median

BV/TV and Tb.N were significantly lower and Tb.Sp significantly higher in the E_{2low} group compared with the E_{2high} group at both distal radius and distal tibia (Table 5).

Bone biochemistry across E₂ median

Multivariate *t*-test on bone biochemistry across E₂ groups failed to show overall significant effects (*P* = 0.16), thus markers of bone turnover and vitamin D levels did not differ between groups (Table 6).

Impact of testosterone on bone parameters

Thirty-six percent of the study population had testosterone deficiency with a testosterone level below 10.4 nmol/l (8.0 $\pm$ 1.6 nmol/l). The remaining 64% of the population

Table 5 Bone characteristics across estradiol median. Data are expressed as mean $\pm$ S.D. or median with interquartile range (25%; 75%).

	E₂ low (n=36)	E₂ high (n=36)	P value
Lumbar spine vBMD (g/cm ³)	0.137 $\pm$ 0.03	0.152 $\pm$ 0.03	0.04
Hip vBMD (g/cm ³)			
Total hip			
vBMD _{integral}	0.307 $\pm$ 0.05	0.327 $\pm$ 0.04	0.07
vBMD _{trab}	0.159 $\pm$ 0.02	0.168 $\pm$ 0.02	0.07
vBMD _{cortical}	0.830 $\pm$ 0.04	0.815 $\pm$ 0.03	0.10
Femoral neck			
vBMD _{integral}	0.294 $\pm$ 0.05	0.304 $\pm$ 0.04	0.34
vBMD _{trab}	0.179 $\pm$ 0.03	0.190 $\pm$ 0.02	0.07
vBMD _{cortical}	0.605 $\pm$ 0.04	0.597 $\pm$ 0.04	0.34
Trochanter			
vBMD _{integral}	0.234 $\pm$ 0.04	0.247 $\pm$ 0.03	0.12
vBMD _{trab}	0.155 $\pm$ 0.02	0.162 $\pm$ 0.02	0.16
vBMD _{cortical}	0.770 (0.73; 0.83)	0.755 (0.71; 0.78)	0.04
Intertrochanteric			
vBMD _{integral}	0.356 $\pm$ 0.05	0.385 $\pm$ 0.06	0.03
vBMD _{trab}	0.162 $\pm$ 0.03	0.172 $\pm$ 0.02	0.11
vBMD _{cortical}	0.840 (0.82; 0.86)	0.840 (0.81; 0.86)	0.73
Femoral neck geometry and strength			
CSA _{total} (cm ²)	11.64 $\pm$ 1.60	12.02 $\pm$ 1.27	0.27
CSA _{cortical} (cm ²)	3.08 (2.50; 3.32)	3.15 (2.78; 3.69)	0.20
Cortical thickness (mm)	0.272 (0.22; 0.32)	0.281 (0.24; 0.33)	0.37
CSMI (mm ⁴)	6.80 $\pm$ 1.67	7.22 $\pm$ 1.47	0.26
Buckling ratio (1)	7.58 $\pm$ 2.44	7.08 $\pm$ 1.80	0.33
Radius geometry			
BV/TV (1)	0.149 $\pm$ 0.03	0.163 $\pm$ 0.03	0.04
Tb.N (/mm)	2.12 $\pm$ 0.24	2.25 $\pm$ 0.20	0.02
Tb.Th (mm)	0.070 $\pm$ 0.01	0.073 $\pm$ 0.01	0.37
Tb.Sp (mm)	0.398 (0.36; 0.44)	0.374 (0.35; 0.41)	0.04
Cortical area (cm ²)	7.63 $\pm$ 1.69	7.85 $\pm$ 1.43	0.57
Ct.Th (mm)	0.871 $\pm$ 0.20	0.906 $\pm$ 0.20	0.44
Tibia geometry	(n=34)	(n=33)	
BV/TV (1)	0.155 $\pm$ 0.03	0.169 $\pm$ 0.02	0.02
Tb.N (/mm)	2.04 $\pm$ 0.27	2.20 $\pm$ 0.22	0.01
Tb.Th (mm)	0.076 $\pm$ 0.01	0.077 $\pm$ 0.01	0.62
Tb.Sp (mm)	0.406 (0.37; 0.47)	0.380 (0.34; 0.42)	0.02
Cortical area (cm ²)	16.87 $\pm$ 2.90	17.02 $\pm$ 2.54	0.83
Ct.Th (mm)	1.416 $\pm$ 0.29	1.439 $\pm$ 0.26	0.73

The *P* values were calculated by unpaired Student's *t*-test or the Mann-Whitney rank sum test where appropriate. Significant results are highlighted in bold. E₂low, estradiol below median E₂; E₂high, estradiol above median E₂; vBMD, volumetric bone mineral density; vBMD_{trab}, trabecular vBMD; CSA, cross-sectional area; CSMI, cross-sectional moment of inertia; BV/TV, bone volume/tissue volume; Tb.N, trabecular number; Tb.Th, trabecular thickness; Tb.Sp, trabecular space; Ct.Th, cortical thickness.

had a relatively low mean testosterone level of 13.3 $\pm$ 2.1 nmol/l. Testosterone below (T_{low}) and above population mean (T_{high}) did not influence lumbar spine vBMD_{trab} or hip vBMD_{integral} (neither TH nor site-specific). In addition, the structural parameters in peripheral bone were not significantly different between testosterone groups (data not shown).

Impact of lean body mass on bone parameters

Lean body mass below (lean_{low}) and above population mean (lean_{high}) did not affect lumbar spine vBMD_{trab}

or hip vBMD_{integral} (neither TH nor site-specific). The structural parameters in radius were not significantly different between lean mass groups either. However, men with Lean_{high} had significantly higher tibia BV/TV, Tb.N, and lower Tb.Sp (data not shown).

Relation between effects of VAT and E₂ at the hip

Multiple linear regression analysis revealed that VAT volume and E₂ levels were significantly and independently associated with THvBMD_{integral} (VAT: standardized β = -0.316, *P* = 0.007 and E₂: standardized β = 0.261,

Table 6 Bone-relevant biochemical markers across estradiol median. Data are expressed as mean $\pm$ s.d. or median with interquartile range (25%; 75%).

	E ₂ low (n=36)	E ₂ high (n=36)	P value
BAP (U/l)	28.2 $\pm$ 7.5	29.1 $\pm$ 6.8	0.60
Osteocalcin (μ g/l)	20.6 (16.3; 26.8)	20.5 (18.0; 24.2)	0.56
P1NP (μ g/l)	41.3 (29.6; 58.8)	46.3 (39.4; 53.6)	0.16
OPG (pmol/l)	4.6 $\pm$ 1.4	4.9 $\pm$ 1.6	0.43
CTx (ng/ml)	0.30 (0.24; 0.51)	0.32 (0.27; 0.41)	0.97
NTx (nmol/l)	18.7 $\pm$ 4.9	18.9 $\pm$ 3.8	0.84
PTH (pmol/l)	4.75 (4.00; 5.68)	5.75 (4.22; 6.81)	0.07
25-Hydroxy vitamin D (nmol/l)	48.7 (34.5; 72.7)	40.2 (28.6; 69.0)	0.22

The *P* values were calculated by unpaired Student's *t*-test or the Mann-Whitney rank sum test where appropriate. E₂low, estradiol below median; E₂high, estradiol above median; BAP, bone alkaline phosphatase; P1NP, procollagen 1 N-terminal propeptide; OPG, osteoprotegerin; CTx, C-terminal telopeptide of type 1 collagen; NTx, cross-linked N-terminal telopeptide of type 1 collagen; PTH, parathyroid hormone.

$P=0.029$), whereas testosterone and lean body mass were not associated with THvBMD_{integral} (testosterone: $P=0.87$ and lean mass: $P=0.70$). Results were unchanged when the metabolic markers (IL6, HDL, and ALT) were included in the regression analysis. Then, the population was grouped according to VAT volume and E₂ levels into four groups (E₂low + VAT_{high} ($n=16$), E₂low + VAT_{low} ($n=20$), E₂high + VAT_{high} ($n=20$), and E₂high + VAT_{low} ($n=16$)). Integral vBMD at TH differed between groups (ANOVA overall $P=0.011$) and *post hoc* testing showed significantly lower TH vBMD_{integral} in the E₂low + VAT_{high} group compared with the E₂high + VAT_{low} group ($P=0.006$) (Fig. 2A). FN *T*-score was significantly lower in the E₂low + VAT_{high} group compared with the E₂high + VAT_{low} group (-1.04 ± 0.78 vs -0.13 ± 0.70 , $P=0.004$), and the prevalence of osteopenia was 50.0 and 6.3% respectively (Fig. 2B).

Discussion

In this cross-sectional study of 72 middle-aged obese men with MetS, we found that men with VAT volume above population median had lower integral and trabecular vBMD at the hip, and lower Ct.Th and higher buckling ratio at FN, indicating a more fragile bone. E₂ levels seemed more important at lumbar spine and the peripheral bones, as men with E₂ below population median had lower lumbar spine vBMD_{trab}, reduced BV/TV and Tb.N, and increased Tb.Sp at tibia and radius, while effects were less pronounced at the hip.

This population was very homogeneous and no correlations between VAT and BMI or WC were found. This allows us to interpret data without adjustments for BMI or WC that may confound results due to collinearity. Other studies have shown correlations between VAT and BMI or WC (40, 41), which is probably due to inclusion of populations with a large variability in age and BMI. Participants in our study all had relatively high amounts of VAT regardless of their BMI and WC, which explains the lack of correlation. Dividing the study subjects in two groups based on population-median VAT yielded no differences in age, BMI, WC, or SAT between groups, confirming that the impaired bone-specific outcomes observed in the VAT_{high} group are not biased by these characteristics. The use of QCT and HRpQCT provides measures of not only bone density, but also bone geometry and strength. The QCT technique reduces noise from excess soft tissue (42), which is why QCT-derived vBMD may predict bone strength better in this category of patients, compared with DXA (43, 44). At the hip, QCT estimates integral bone density, trabecular bone density, and cortical bone density. The latter should be cautiously interpreted as cortex is very thin, and the resolution in scans with 3 mm slice thickness is sub-optimal when discriminating cortical and trabecular compartments (45, 46). We found lower integral and trabecular vBMD at all measured hip sites, and lower Ct.Th and higher buckling ratio at FN in the VAT_{high} group. Furthermore, vBMD_{cortical} was significantly higher in the VAT_{high} group at TH and IT region. vBMD_{cortical} primarily

Figure 2

Total hip integral volumetric BMD (A) and femoral neck *T*-score (B) across combined E₂ and VAT groups (E₂low + VAT_{high} ($n=16$), E₂low + VAT_{low} ($n=20$), E₂high + VAT_{high} ($n=20$), and E₂high + VAT_{low} ($n=16$)). Dashed line distinguishes men with normal BMD (FN *T*-score > -1) and men with osteopenia (FN *T*-score ≤ -1 and > -2.5). Open circle represents each trial subject and solid line represents group mean. The overall *P* values were calculated by one-way ANOVA. * $P<0.01$.

depends on cortical mineralization and porosity, without taking cortical area into account. However, bone size also determines resistance to load, and therefore CSA was measured at FN (34). FN CSA_{total} was comparable between groups, while the VAT_{high} group had lower CSA_{cortical} and Ct.Th, indicating that VAT promotes cortical thinning at the FN, primarily due to endocortical bone loss. This could be a result of excess endocortical remodeling leading to transition of cortical bone to trabecular bone and a change in edge detection when analyzing the QCT scans. We speculate that the endocortical zone was detected as trabecular bone compartment in the VAT_{high} group, and would therefore expect the trabecular vBMD to be slightly overestimated. Indeed, we found a significantly lower trabecular bone density in the VAT_{high} group. Thus, the true trabecular bone density may be even lower than our results indicate.

We also investigated the effects of E₂ levels on bone. Dividing the study subjects based on population-median E₂ yielded no between-group differences in age, fat distribution, testosterone, androstenedione, or SHBG levels. This confirms that the impaired bone-specific outcomes we found in the E_{2low} group are not biased by these characteristics. The E_{2low} group was estrogen deficient with a median E₂ of 20 pmol/l and the E_{2high} group was estrogen sufficient with a median E₂ of 71 pmol/l (reference range for men between 20 and 70 years: 48–170 pmol/l (<http://www.ssi.dk/English.aspx> assessed 4th July 2014)). Men in the E_{2low} group had lower lumbar spine trabecular bone density and impaired geometric measures at tibia and radius. Khosla *et al.* have previously described that rate of bone loss at the radius was associated with total and bioavailable E₂ levels in elderly men but not in middle-aged men. Khosla *et al.* (47) also reported that bioavailable E₂ levels below 40 pmol/l were associated with significantly higher rates of bone loss and higher levels of bone resorption markers in elderly men than bioavailable E₂ levels above 40 pmol/l. Although steroid levels are difficult to compare between laboratories, there is a striking difference between total E₂ levels in our population and the population that Khosla *et al.* described. Median E₂ level in this study was 43 pmol/l vs 103 pmol/l (middle-aged men) and 110 pmol/l (elderly men) described by Khosla *et al.* This lower level of E₂ in our population could explain why we, in contrary to Khosla *et al.*, found that E₂ is associated with bone density in middle-aged obese men. However, we did not find any differences in the bone resorption markers between groups, but the inter-individual variations in markers of bone resorption and bone formation are considerable.

The cross-sectional design may not be strong enough to demonstrate a possible difference.

Surprisingly, fat distribution was unaffected by E₂ level. We would have expected to find a positive association between levels of E₂ and fat volume, due to increased aromatase activity (19, 20). Maggio *et al.* (48) investigated men with and without MetS and found higher E₂ levels in men with MetS. Our population had low levels of testosterone and as testosterone is the substrate for adipose tissue aromatase to produce E₂, low testosterone levels could explain why adipose tissue volume did not affect E₂ levels.

Multiple linear regression analysis revealed that VAT volume and E₂ levels were independently associated with THvBMD_{integral}. Results presented in Fig. 2A clearly suggest an additive negative effect of VAT and low E₂ levels on bone in men. To ensure that these findings are relevant in a clinical perspective, we investigated the FN T-scores across groups. Interestingly, we found a clinically relevant difference of more than 1 in FN T-scores between the E_{2low} + VAT_{high} group compared with the E_{2high} + VAT_{low} group. Comparing osteopenia prevalence in the E_{2low} + VAT_{high} group (50.0%) with the E_{2high} + VAT_{low} group (6.3%), the clinical importance of VAT and E₂ levels certainly becomes evident. Nielson *et al.* (49) reported a prevalence of osteopenia of 11.9% in a population of Caucasian men aged between 45 and 74 years with a BMI of 30+. In comparison, our population comprises Caucasian men, average age 49 years (between 30 and 60 years) and average BMI 34 kg/m². The prevalence of osteopenia in our population is higher (29.2%), which could be explained by the fact that all participants had MetS.

Testosterone and lean body mass did not influence BMD at the spine or hip in our population. However, lean body mass positively affected structural parameters at the tibia. A recent study on 40 younger women and ten men with MetS has demonstrated that lean mass was positively associated with HRpQCT-derived bone parameters at both tibia and radius (50). Unfortunately, they did not perform QCT at the hip or spine and did not have the opportunity to investigate effects of VAT and SAT separately.

Strengths of this study are the homogeneous population allowing us to interpret data without adjustments for BMI, WC, and testosterone levels, in addition to the use of several distinct methods to characterize bone. The limitations to this study are: first, the cross-sectional design does not allow us to determine causality. The differences we found in bone parameters across VAT and E₂ groups could be caused by an overall worse metabolic status. However, when including metabolic markers in a

multiple linear regression analysis, VAT and E_2 are still the only markers significantly associated with $THvBMD_{integral}$. Secondly, we included a limited number of subjects in this cohort. This study grounds on baseline data from another study (27) in which the sample size was determined. *Post hoc* power calculations suggested more than 90% power to detect a 10% difference between groups in $THvBMD_{integral}$. However, the risk of type II errors is relatively high in relation to the biochemical bone turnover markers, as the inter-individual variation is considerable. This may explain the lack of difference in bone turnover markers in this study.

In summary, we found that an increased amount of VAT in obese middle-aged men with MetS is associated with harmful effects on BMD, bone geometry, and bone strength at the hip, whereas the harmful effects of low estrogen levels primarily unfolds at the lumbar spine, radius, and tibia. In addition, we found that VAT volume and E_2 levels affect bone density at the hip independently and additively. The reduction in BMD in men with high VAT and low E_2 has a magnitude implying clinical importance for future fracture risk in men suffering from MetS.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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