Lower bone turnover and relative bone deficits in men with metabolic syndrome: a matter of insulin sensitivity? The European Male Ageing Study

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ABSTRACT *(250 words)*

Introduction: Metabolic syndrome (MetS) has been associated with lower bone turnover and relative bone mass or strength deficits (i.e. not proportionate to body mass index, BMI), but the relative contributions of MetS components related to insulin sensitivity or obesity to male bone health remain unclear.

Methods: We determined cross-sectional associations of MetS, its components and insulin sensitivity (by homeostatic model assessment, HOMA-S) using linear regression models adjusted for age, center, smoking, alcohol and BMI. Bone turnover markers and heel broadband ultrasound attenuation (BUA) were measured in 3129 men aged 40-79. Two centers measured total hip, femoral neck and lumbar spine areal bone mineral density (aBMD, n=527) and performed radius peripheral quantitative computed tomography (pQCT, n=595).

Results: MetS was present in 975 men (31.2%). Men with MetS had lower β-CTX, PINP and osteocalcin ($P<0.0001$) and higher total hip, femoral neck and lumbar spine aBMD ($P\leq0.03$). Among MetS components, only hypertriglyceridemia and hyperglycemia were independently associated with PINP and β-CTX. Hyperglycemia was negatively associated with BUA, hypertriglyceridemia with hip aBMD and radius CSA and stress-strain index. HOMA-S was similarly associated with PINP and β-CTX, BUA and radius CSA in BMI-adjusted models.

Conclusions: Men with MetS have higher aBMD in association with their greater body mass, while their lower bone turnover and relative deficits in heel BUA and radius CSA are mainly related to correlates of insulin sensitivity. Our findings support the hypothesis that underlying metabolic complications may be involved in bone's failure to adapt to increasing bodily loads in men with MetS.

Keywords: Bone mineral density, bone turnover, male, metabolic syndrome, obesity, peripheral quantitative computed tomography

Summary: *(50 words)*

We examined cross-sectional associations of metabolic syndrome and its components with male bone turnover, density and structure. Greater bone mass in men with metabolic syndrome was related to their greater body mass, whereas hyperglycemia, hypertriglyceridemia or impaired insulin sensitivity were associated with lower bone turnover and relative bone mass deficits.

Introduction

The metabolic syndrome (MetS) encompasses several features –abdominal obesity, elevated blood pressure, dyslipidemia and hyperglycemia– which confer an increased risk of developing cardiovascular disease and type 2 diabetes mellitus (T2D) [1]. The usefulness of the MetS concept relies on the assumptions that (i) all components are important and treatable predictors of adverse cardiometabolic outcomes, (ii) MetS predicts these outcomes better than the sum of its individual components, and (iii) MetS predicts these outcomes better than simpler measures like body mass index (BMI). Indeed, instead of focusing on obesity, correlates (or consequences) of insulin resistance (hyperglycemia, hypertriglyceridemia) lie at the heart of the MetS concept [2]. Although MetS may be a useful construct to focus cardiovascular and T2D preventive strategies, the validity of this construct as well as its cut-off values remain debated. Nevertheless, all MetS components become increasingly prevalent with age, with around 25-35% of adults having MetS (depending on the definition and population studied) [3-7].

Osteoporosis is also a common age-related condition. With a male lifetime incidence of osteoporotic fractures as high as 20-25% in high-risk Caucasian populations, men contribute substantially to the overall fracture burden [8]. Contrary to the general belief that obesity is protective for the skeleton, a growing body of evidence suggests that the relationships between bone metabolism, obesity and insulin resistance are more complex. Both obesity and T2D have been associated with higher areal bone mineral density ($_{a}$BMD) [9, 10], which however does not seem to confer protection against fractures [11-13]. This paradox may be explained by higher falls risk and impact force, altered material properties [14] as well as relative deficits in cortical bone structure and strength [10, 14-17]. This relative bone deficit involves greater absolute $_{a}$BMD, cross-sectional bone area or volumetric BMD ($_{v}$BMD) with increasing BMI [10], whereas associations of MetS or T2D with bone outcomes become

negative in BMI-adjusted models [3-5, 7, 9] *i.e.* BMD or bone strength not being *as high* in MetS or T2D as would be expected based on BMI alone. In other words, bone strength adapts to increasing bodily loads, but this relationship becomes attenuated at higher levels of BMI. In a recent MrOS study for example, estimated hip bone strength increased linearly with BMI until it started to plateau around BMI 30 kg/m² [16]. Why the skeletal strength:load ratio flattens in obesity is incompletely understood, but metabolic complications may be involved since previous studies on MetS have consistently reported negative associations of MetS components with $_{a}$BMD in BMI-adjusted models [7]. Given the high prevalence of obesity and MetS, a deeper understanding of their relation to bone turnover, $_{a}$BMD and $_{v}$BMD, bone structure and bone strength can offer potentially important insights into male bone health. Using data from the observational European Male Ageing Study (EMAS), we examined associations of MetS, its components as well as insulin sensitivity (HOMA-S) with bone turnover markers (BTMs), heel quantitative ultrasound (QUS), hip and spine $_{a}$BMD and radius peripheral computed tomography (pQCT) measures.

Methods

Participants

The design, cohort profile and assessments of EMAS have been reported previously [18]. From 2003 to 2005, an age-stratified random population sample of men aged 40-79 was recruited by eight European centers: Manchester, United Kingdom; Leuven, Belgium; Malmö, Sweden; Tartu, Estonia; Łódź, Poland; Szeged, Hungary; Florence, Italy and Santiago de Compostela, Spain. Ethical approval was obtained according to local institutional requirements at all centers and all men provided written informed consent.

Study questionnaires and clinical assessments

Subjects completed a postal questionnaire which included questions about comorbidities, smoking and average number of days per week in which alcohol was consumed in the

previous month. Standardized measurements were taken for height to the nearest mm using a stadiometer (Leicester Height Measure, SECA UK Ltd) and body weight to the nearest 0.1 kg using an electronic scale (SECA, model 8801321009), with monthly calibrations in each center. BMI was calculated as weight in kilograms divided by height (in meters) squared. Waist circumference was measured using anthropometric tape midway between the iliac crest and lowest ribs, and the median of three measurements was recorded. Seated blood pressure (Omron 500I, Omron Healthcare Ltd, Milton Keynes, UK) was measured after a 5 min rest period. Reuben's Physical Performance Test (PPT) was assessed as time (in seconds) required for a 50-feet walk. Interviewer-assisted questionnaires included prescription and non-prescription medication, and the Physical Activity Scale for the Elderly (PASE).

Biological measurements

A fasting morning (before 10:00 a.m.) venous blood sample was obtained, from which serum was separated and stored at -80 °C until analysis. Methods of measurement for BTMs and hormones have been described in detail previously [19, 20]. Serum β C-terminal cross-linked telopeptide (β-CTX; β-Crosslaps, n=3018), N-terminal propeptide of type I procollagen (PINP, n=3020) and osteocalcin (stable N-MID fragment, n=1089 randomly selected subjects) were measured by electrochemiluminescence immunoassay (ECLIA) on the Elecsys 2010 automated analyser (Roche Diagnostics) [21]. The detection limits of these kits are 10 pg/ml, < 5 ng/ml and 0.5 ng/ml, and the intra-assay coefficient of variation (CV) < 5.0 %, <3.0 % and < 5.0 % for β-cTX, P1NP and osteocalcin, respectively. Glucose, cholesterol and triglyceride measurements were undertaken in each participating center. Insulin was assayed using quimioluminiscence at University of Santiago de Compostela. Indices of insulin resistance, sensitivity and bèta-cell mass were calculated using the homeostasis model assessment (HOMA-IR, HOMA-S, HOMA-B, respectively) [22]. The quantitative insulin sensitivity check index (QUICKI) was calculated as the inverse of the sum of the logarithms

of fasting glucose and insulin concentrations [23]. Methods for other hormone measurements in EMAS have been reported previously [20, 24, 25].

Definition of metabolic syndrome (MetS)

MetS was defined according to the 2009 harmonized criteria [1]. Subjects were classified as having MetS (MetS+) when ≥ 3 of the following criteria were present: waist circumference > 102 cm, triglycerides ≥ 1.7 mmol/l (150 mg/dl), HDL cholesterol < 1.03 mmol/l (40 mg/dl), systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg or use of antihypertensive drugs, and fasting glucose ≥ 5.6 mmol/l (100 mg/dl) or use of antidiabetic drugs. In the analyses comparing MetS+ to MetS- subjects, those with missing data were excluded when MetS could not be classified with certainty [25].

Quantitative ultrasound (QUS) of the heel

In all centers, QUS of the left heel was performed with the Sahara Clinical Sonometer (Hologic, Inc., Waltham, MA, USA) using a standardized protocol [19]. Each center calibrated the device daily with the physical phantom provided by the manufacturer. All quality control results were sent to Leuven and found stable throughout the study. Outputs included broadband ultrasound attenuation (BUA), speed of sound (SOS), estimated BMD (eBMD = 0.002592 × (BUA+SOS) − 3.687) and quantitative ultrasound index (QUI, a measure of stiffness calculated as QUI = 0.41[SOS] + 0.41[BUA] − 571). Short-term precision of the method was established by duplicate measurements performed in 20 randomly selected cohort members in Leuven. The *in vivo* CVs were 2.8, 0.3 and 2.3% for BUA, SOS and QUI, respectively. Ten repeat measurements were performed on a roving phantom at each center. Standardized CVs (root mean squared difference divided by range to mean ratio [26]) for within machine variability ranged by center: for SOS, from 1.0 to 5.6%, and BUA from 0.7 to 2.7%. Standardized CVs for between machine variability were 4.8% for BUA and 9.7% for SOS.

Dual-energy X-ray absorptiometry (DXA) and radius pQCT

Subjects in Leuven and Manchester had DXA and pQCT scans performed. For DXA, the same QDR 4500A Discovery scanners were used in both centers (Hologic Inc, Bedford, MA, USA). Lumbar spine (L1–4), femoral neck and total hip $_{a}$BMD were measured as described previously [19]. All scans and analyses were performed by trained and certified DXA technicians. The Hologic spine phantom was scanned daily to monitor the device performance and long-term stability. The precision errors (CV%) were 0.57% and 0.97% at L1-4, 1.28% and 2.04% at the femoral neck, and 0.56% and 0.97% at the total proximal femur in Leuven (n=20) and Manchester (n=31), respectively. Devices in Leuven and Manchester were cross-calibrated with the European spine phantom.

The pQCT protocol has been described previously [24]. In both centers, the non-dominant radius was measured using an XCT-2000 scanner (Stratec, Pforzheim, Germany) following the manufacturer's standard quality assurance procedures. Total and trabecular $_{v}$BMD (mg/mm3), trabecular area and total cross-sectional area (CSA) (mm2) were measured at the distal (4%) radius (voxel size 0.4 mm). Cortical $_{v}$BMD (mg/mm3), total CSA, cortical and medullary area (mm2), cortical thickness (mm), stress strain index (SSI) (mm3) and muscle CSA (mm2) were measured at the midshaft (50%) radius (voxel size 0.6 mm). The European Forearm Phantom (EFP) was measured in both centers; 10 repeat measurements were taken in slices 1–4. Differences were less than precision error for total, trabecular and cortical $_{v}$BMD, and cortical area; therefore, cross-calibration was omitted. The short term precision of 2 repeat measurements with repositioning were: total $_{v}$BMD 2.1% and 1.3%; trabecular $_{v}$BMD 1.27% and 1.42%; cortical $_{v}$BMD 0.77% and 0.71%; and cortical area 2.4% and 1.3% in Manchester (n = 22) and Leuven (n = 40), respectively.

Statistical analyses

Cross-sectional differences between MetS+ and MetS- groups were assessed by Mann-Whitney U-test and chi-square for continuous and categorical variables, respectively. Linear regression analysis of the association between metabolic (independent) and bone (outcome) variables were performed (i) unadjusted, (ii) adjusted for potential confounders (age, center, smoking and alcohol intake), (iii) adjusted for these confounders plus other MetS components (to examine whether individual MetS component demonstrate associations independent of other MetS components), or (iv) adjusted for confounders plus BMI. Associations are reported as standardized (z-score) β regression coefficients. Analyses were performed using Stata version 13.1 (StataCorp, College Station, TX, USA), and two-tailed $P < 0.05$ was considered significant. No adjustments for multiple testing were applied.

Results

Study population and characteristics

Of 3369 men in the baseline cohort, we excluded men taking glucocorticoids, drugs with possible hormonal effects (incl. sex steroids, gonadorelin analogues, strong opioids, and drugs for thyroid disorders), bone-active treatments (incl. bisphosphonates, calcium and/or vitamin D supplements), HIV drugs or men in whom MetS status could not be determined due to missing values. The total number of exclusion was 240 (7.12%), leaving 3129 men in the analytical sample. Of these, 975 (31.2%) were classified as having MetS, *i.e.* satisfying at least three MetS criteria. There were 257 (8.2%), 961 (30.7%), 936 (29.9%), 627 (20.0%), 284 (9.1%) and 64 (2.0%) men satisfying exactly 0, 1, 2, 3, 4 or 5 MetS criteria, respectively. Apart from having more MetS features, men with MetS were older, heavier, more often former smokers and less frequent drinkers (*Table 1*). They also had an altered endocrine profile, walked slower and reported less physical activity (*Supplemental Table 1*).

Associations between MetS, its components and bone turnover markers

Men with MetS had significantly lower levels of PINP, osteocalcin and particularly β-CTX (*Table 1*). In linear regression analyses, MetS (*Table 2*) was associated with lower BTMs, independent of confounders (age, center, smoking and alcohol; Model 1) and BMI (Model 3), except for osteocalcin. However, when MetS components were analyzed individually, only hypertriglyceridemia and hyperglycemia were inversely associated with PINP and β-CTX, independent from other MetS components (Model 2) or BMI (Model 3). Osteocalcin was independently and inversely associated only with hyperglycemia (Model 3). Also when analyzed as continuous variables, glucose and triglycerides showed independent inverse associations with β-CTX, PINP and osteocalcin, whereas blood pressure, HDL and waist circumference did not (*data not shown*). Insulin sensitivity (HOMA-S or QUICKI) was also associated with PINP and β-CTX, independently of age, center, smoking, alcohol and BMI (*Supplemental Table 2*). Adjustment for differences in either physical activity/performance, sex steroids, PTH, 25-OH-vitamin D, IGF-1 or CRP (*Supplemental Table 1*) did not alter the associations between MetS and BTMs (*data not shown*). Compared to the referent group of men satisfying exactly two MetS criteria, men with three, four or five MetS criteria had lower BTMs (*Suppl. Fig. 1A-B*). However, men with one or zero criteria also had higher BTMs, implying that there is no clear threshold at three MetS criteria above which BTMs are altered.

Associations between MetS, its components and heel QUS parameters

Following adjustment for age, center, smoking and alcohol, MetS was positively associated with BUA and QUI (*Table 3*) but not SOS or eBMD (*data not shown*). When adjusted for BMI however, these associations became non-significant. When individual MetS components were examined (*Table 3*), only waist >102 cm was positively associated with BUA and QUI (as well as SOS and eBMD, *not shown*), although not independently from BMI. In fact, BMI adjustment (Model 3) revealed a negative association of hyperglycemia with BUA. Also when analyzed as continuous variables, glucose and triglycerides where inversely associated

with BUA and QUI the BMI-adjusted model (*data not shown*). Fasting insulin levels and markers of insulin resistance were also negatively associated with BUA, SOS, QUI and eBMD, but again this was only evident following BMI adjustment (*Supplemental Table 2*). BMI itself was positively associated with QUS parameters (*Supplemental Table 2*). Adjustment for differences in either physical activity/performance, sex steroids, PTH, 25-OH-vitamin D, IGF-1 or CRP (*Supplemental Table 1*) did not affect the associations between MetS and QUS parameters (*data not shown*). Men with four MetS criteria had significantly higher BUA and QUI compared to the referent group of men satisfying two MetS criteria in unadjusted and confounder-adjusted analyses, but this was not the case for men meeting all five MetS criteria (*Suppl. Fig. 1C-D*). In fact, adjustment for BMI revealed significantly lower BUA and QUI in men with full MetS.

Associations of MetS and its components with $_a$BMD and pQCT outcomes

A subgroup of men from Manchester and Leuven underwent DXA (n=527) and radius pQCT (n=595). Men with MetS had higher $_a$BMD at the lumbar spine, total hip and femoral neck (*Table 1*). MetS was positively associated with $_a$BMD at all three sites independent of confounders, but not following BMI adjustment (*Table 4*). Among MetS components, waist > 102 cm and hyperglycemia were independently associated with $_a$BMD at all three sites, but not independent from BMI (*Table 4*). Interestingly, hypertriglyceridemia was inversely associated with femoral neck $_a$BMD when adjusted for other MetS components or BMI.

Men with MetS had higher muscle area, with a trend towards greater cortical bone area (P=0.053) and lower muscle density (P=0.06) (*Table 1*). In linear regression analyses, MetS as a whole was not associated with skeletal pQCT parameters (*data not shown*). Among MetS components, waist circumference was independently associated with greater CSA at the ultradistal (*not shown*) and midcortical site, cortical thickness and bone area, SSI and muscle area (*Table 4*). Hypertriglyceridemia (or triglycerides as a continous variable, *data not*

shown) was negatively associated with CSA and SSI independent from other MetS components or BMI, and with cortical bone area when adjusted for BMI (*Table 4*). Both at the ultradistal and mid-radius, MetS or its components were not associated with $_{v}$BMD (*data not shown*). Cortical bone area, CSA and SSI were also associated with HOMA-S and QUICKI, but only in BMI-adjusted models (*Supplemental Table 2*).

Discussion

MetS is fairly common in the general population; in line with previous studies [3-7, 9], almost one-third of our 40-79 year-old European men qualified under recent international criteria [1]. Our main findings are that in men with MetS, the lower bone turnover and greater bone mass at loaded sites (as reflected by heel BUA and total hip, femoral neck and lumbar spine $_{a}$BMD) are not uniformly associated with all MetS components. The lower bone turnover was mainly associated with MetS components related to insulin sensitivity (hyperglycemia, hypertriglyceridemia) or indices thereof (HOMA-S, QUICKI). On the other hand, the association of MetS with greater bone mass was determined by greater body mass (either by waist circumference or BMI) and not present at the radius, despite greater forearm muscle area. The associations of MetS with lower BTMs and superior QUS parameters in the overall cohort did not appear to be explained by higher free/bioavailable E2 levels (*data not shown*) and occurred in spite of an otherwise adverse endocrine/biochemical profile in men with MetS (*Supplementary Table 1*).

There is agreement in the literature that MetS is not an overall valid construct in relation to bone health because not all components of MetS have similar associations with skeletal outcomes [3, 5, 9]. Previous studies in older men have shown that MetS or hyperglycemia are inversely associated with BTMs as the outcome [5, 6, 27]. However, these studies have not reported association of individual MetS components with BTMs independent from other MetS components. Our finding that not only hyperglycemia but also hypertriglyceridemia

was independently associated with BTMs is of interest given that triglycerides were strongly related to $_{a}$BMD and fracture risk in two previous studies [3, 5]. We reasoned that insulin sensitivity could explain the association with hyperglycemia and hypertriglyceridemia, and indeed found that indices like HOMA-S and QUICKI were independently associated with bone turnover (*Supplementary Table 2*). The finding that men with greater waist circumference or BMI have higher absolute heel QUS parameters, hip and spine $_{a}$BMD and mid-radius cortical bone and muscle area are also in accordance with previous studies [3, 9, 10, 12, 13, 16] and consistent with the hypothesis that bone mass in obesity is adapted to greater bodily loads.

Importantly however, adjusting for BMI revealed *inverse* associations of hyperglycemia with heel BUA, and of hypertriglyceridemia with $_{a}$BMD at the total hip, femoral neck as well as radius cortical bone area, CSA and SSI. This is in agreement with previous observations in T2D [11, 15] and several studies on MetS and male bone health [3-5, 9] which also found $_{a}$BMD, bone width or strength to be increased in absolute terms, but not in BMI-adjusted models i.e. not *as much* as could have been expected for body weight. Similarly, we found that men with MetS had greater forearm muscle area (*Table 1*) which was entirely related to their higher waist circumference or BMI (*Table 4*). However, this should not be taken to imply superior muscle mass (let alone strength) in obese people, given the limitations of pQCT in assessing adipose infiltration which occurs interstitially, inter- and intracellularly in muscle (as suggested by the trend towards lower muscle density; *Table 1*).

What exactly drives the non-linear relationship or plateau in the bone strength-BMI relationship remains unknown [16, 28]. Among the possibilities we examined, the relative skeletal deficits in men with MetS did not appear to be associated with adverse biochemical/endocrine factors or decreased physical activity/ performance. Instead, our data suggest that the greater bone mass was strongly determined by obesity, but obese subjects are

also prone to higher fasting glucose and triglyceride levels and reduced insulin sensitivity (*Supplementary Table 2*) which may in turn play a negative role and mitigate the stimulatory effect of body mass. Further research is however needed to investigate whether detrimental skeletal effects derive directly from high glucose or triglyceride levels, indirectly from impaired insulin signaling, or both.

The negative association of hypertriglyceridemia with cortical bone area and strength (as reflected by the SSI) was explained by decreased bone width (as reflected by CSA, a measure of periosteal bone expansion). Even when cortical thickness or $_{v}$BMD were unaffected in our population-based study, bone size is known to be a major determinant of bone strength. A recent study also reported that bone width at the femoral neck (estimated by DXA) was lower in MetS, although the results in men were only borderline significant [9]. In contrast, Szulc *et al*. suggested that MetS affects mainly BMC rather than bone size [5]. However, these previous DXA-based findings are more likely to be confounded by projectional artifacts than our pQCT results.

Our study has several strengths including its large, geographically diverse random sample of European men. The age range was broad, but the associations of MetS and its components with BTMs and QUS were similar across 10-year age bands or in subgroups aged < 60 vs. ≥ 60 years (*data not shown*). This is the first study of bone health in MetS with pQCT data. Limitations include lack of prospective analyses or fracture outcomes and, like any observational study, we cannot confirm causality (nor exclude reverse causation). Although mounting evidence supports the assumptions that obesity and insulin signaling affect the skeleton [17, 29, 30], our findings remain hypothesis-generating. Furthermore, measurements of glucose and lipids were not centralized, although these measurements are generally well standardized. There is considerable interest in the role of undercarboxylated osteocalcin [29], but only total osteocalcin was available for a randomly selected subsample of men in our

study. The lack of association of MetS or hypertriglyceridemia with osteocalcin (*Table 2*) likely resulted from lack of statistical power compared to PINP or β-CTX. In recent studies however, T2D or MetS in older men were not only associated with lower undercarboxylated osteocalcin but also lower total osteocalcin [27], PINP as well as β-CTX [31, 32], indicating that lower bone turnover in human insulin resistant states is not uniquely associated with undercarboxylated osteocalcin. The associations reported with dichotomized MetS components were confirmed in sensitivity analyses with continuous variables (*data not shown*). Thus, the lack of associations of skeletal outcomes with e.g. hypertension was not explained by the fact that according to the MetS cut-off, almost 80% of MetS− men still had a blood pressure ≥ 130/85 mmHg (Supplementary Table 1). Finally, obesity or T2D may be associated with spinal osteoarthritis [28] or increased cortical porosity [14, 15], but vertebral X-rays or high-resolution pQCT were unavailable in this study.

In summary, MetS is associated with lower bone turnover and higher bone mass at the heel, hip and spine. In line with previous studies however, MetS does not seem to be a useful unifying construct in relation to bone health, because (i) not all components were individual predictors of skeletal outcomes and (ii) there was no clear cut-off for number of MetS criteria above which BTMs or bone mass were dose-dependently affected (*Suppl. Fig. 1*). Instead, we found differential associations of lower bone turnover mainly with correlates of insulin resistance, and of body mass (either by waist circumference or BMI) with higher heel BUA, hip and spine $_{a}$BMD and radius CSA. Importantly, BMI adjustment revealed negative associations between markers of insulin resistance and bone mass, suggesting that the positive effects of bodily loads on bone may be partially offset by concomitant metabolic derangements. In terms of clinical implications, these findings offer a note of caution against false reassurance by low BTMs or absence of low BMD in men with obesity, MetS and/or insulin resistance.

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Tables

Table 1: Characteristics of men without and with metabolic syndrome.

		MetS – *n*=2154	**MetS + *n* = 975**	***P* value**
Age (years)		58.3 (49.4, 68.8)	60.7 (52.0, 70.4)*	0.0001
Weight (kg)		78.6 (71.8, 86.0)	91.7 (82.9, 101.1)*	< 0.0001
Height (cm)		173.4 (168.6, 178.5)	173.9 (168.8, 179.0)	0.20
BMI (kg/m^2)		26.2 (24.1, 28.2)	30.4 (28.1, 32.8)*	< 0.0001
Current smoker		474 [22.2%]	193 [20.0%]	0.16
Ever smoker		1446 [68.4%]	718 [74.6%]*	0.001
Alcohol	every day	356 [16.6 %]	132 [13.6 %]	0.06
	5-6 days/week	163 [7.6 %]	54 [5.6 %]	
	3-4 days/week	265 [12.4 %]	128 [13.2 %]	
	1-2 days/week	444 [20.8 %]	204 [21.1 %]	
	< once/week	578 [27.0 %]	285 [29.4 %]	
	none	334 [15.6 %]	166 [17.1 %]	
Waist circumference (cm)		94.5 (88.4, 99.8)	106.5 (102.2, 113.0)*	< 0.0001
Systolic BP (mmHg)		142.0 (128.5, 155.0)	150.0 (139.0, 164.0)*	< 0.0001
Diastolic BP (mmHg)		85.0 (78.0, 93.0)	90.0 (82.0, 98.0)*	< 0.0001
HDL cholesterol (mmol/l)		1.4 (1.2, 1.7)	1.2 (1.0, 1.4)*	0.0001
Triglycerides (mmol/l)		1.1 (0.8, 1.5)	2.0 (1.4, 2.7)*	< 0.0001
Fasting glucose (mmol/l)		5.2 (4.8, 5.5)	5.9 (5.5, 6.7)*	< 0.0001
Bone turnover markers				
PINP (µg/l)		40.0 (31.0, 51.0)	35.2 (27.4, 47.2)*	< 0.0001
Osteocalcin (µg/l)		21.6 (18.0, 26.4)	19.1 (15.3, 24.6)*	< 0.0001
β-CTX (ng/l)		339.8 (245.3, 470.4)	279.1 (183.3, 406.1)*	< 0.0001
QUS parameters		***n*=2106**	***n*=936**	
BUA (dB/MHz)		78.9 (67.5, 92.1)	80.0 (69.1, 92.6)	0.10
SOS (m/s)		1548.1 (1527.9, 1571.2)	1547.5 (1527.7, 1567.8)	0.73
eBMD (g/cm^2)		0.531 (0.452, 0.618)	0.536 (0.454, 0.618)	0.64
QUI		96.1 (83.6, 109.9)	96.8 (83.9, 109.9)	0.60
DXA: $_a$BMD (g/cm^2)		***n*=401**	***n*=126**	
Lumbar spine (L 1-4)		1.029 (0.927, 1.126)	1.076 (0.970, 1.234)*	0.0004
Total hip		1.002 (0.925, 1.105)	1.049 (0.950, 1.160)*	0.006
Femoral neck		0.796 (0.724, 0.890)	0.821 (0.734, 0.927)*	0.03
pQCT: 50% radius		***n*=458**	***n*=137**	
Cortical bone area (mm^2)		105.3 (97.0, 115.0)	108.8 (99.8, 117.0)	0.05
Cortical thickness (mm)		3.2 (3.0, 3.5)	3.3 (3.1, 3.6)	0.17
Cross-sectional area (mm^2)		147.2 (133.6, 161.8)	149.0 (137.5, 164.2)	0.34
Stress strain index (mm^3)		328.9 (288.9, 381.5)	344.9 (295.4, 390.8)	0.10
Medullary area (mm^2)		39.9 (32.3, 49.8)	40.0 (32.5, 46.3)	0.70
Muscle area (mm^2)		3612.5 (3184.2, 4045.0)	3782.0 (3448.8, 4229.8)*	0.0002
Muscle density (mg/cm^3)		82.9 (81.2, 84.2)	82.4 (80.4, 84.1)	0.06
4% radius				
Trabecular density (mg/cm^3)		205.7 (178.1, 233.3)	205.8 (170.7, 234.7)	0.65

Values are expressed as median (IQR) or *n* [%]

Table 2: Associations of MetS and its components with bone turnover markers.

		Univariate	Model 1†	Model 2‡	Model 3 §
β-CTX	MetS	-0.35 (-0.42, -0.27)*	-0.34 (-0.42, -0.26)*	-	-0.26 (-0.35, -0.17)*
	Waist >102 cm	-0.24 (-0.31, -0.16)*	-0.24 (-0.32, -0.17)*	-0.16 (-0.24, -0.08)*	-0.09 (-0.19, 0.02)
	Triglycerides >150 mg/dL	-0.24 (-0.32, -0.17)*	-0.23 (-0.31, -0.16)*	-0.16 (-0.24, -0.08)*	-0.17 (-0.25, -0.09)*
	HDL <40 mg/dL	-0.12 (-0.22, -0.01)*	-0.14 (-0.24, -0.03)*	-0.03 (-0.14, 0.08)	-0.07 (-0.18, 0.04)
	Hypertension	-0.13 (-0.23, -0.03)*	-0.10 (-0.20, 0.00)	-0.02 (-0.12, 0.09)	-0.02 (-0.12, 0.08)
	Hyperglycemia	-0.30 (-0.37, -0.22)*	-0.30 (-0.37, -0.22)*	-0.25 (-0.32, -0.17)*	-0.25 (-0.33, -0.17)*
PINP	MetS	-0.19 (-0.26, -0.11)*	-0.18 (-0.26, -0.11)*	-	-0.20 (-0.29, -0.11)*
	Waist >102 cm	-0.10 (-0.17, -0.02)*	-0.10 (-0.17, -0.02)*	-0.04 (-0.12, 0.04)	-0.07 (-0.18, 0.03)
	Triglycerides >150 mg/dL	-0.16 (-0.24, -0.08)*	-0.16 (-0.24, -0.08)*	-0.13 (-0.21, -0.05)*	-0.16 (-0.24, -0.08)*
	HDL <40 mg/dL	-0.03 (-0.14, 0.08)	-0.05 (-0.16, 0.06)	0.00 (-0.12, 0.11)	-0.03 (-0.14, 0.08)
	Hypertension	-0.07 (-0.17, 0.02)	-0.04 (-0.14, 0.06)	0.02 (-0.09, 0.12)	-0.02 (-0.12, 0.09)
	Hyperglycemia	-0.21 (-0.29, -0.14)*	-0.21 (-0.28, -0.13)*	-0.19 (-0.27, -0.11)*	-0.21 (-0.29, -0.14)*
Osteocalcin	MetS	-0.26 (-0.39, -0.12)*	-0.29 (-0.43, -0.15)*	-	-0.15 (-0.31, 0.00)
	Waist >102 cm	-0.25 (-0.38, -0.12)*	-0.27 (-0.40, -0.14)*	-0.21 (-0.35, -0.08)*	-0.08 (-0.26, 0.10)
	Triglycerides >150 mg/dL	-0.20 (-0.34, -0.07)*	-0.23 (-0.37, -0.09)*	-0.16 (-0.31, -0.02)*	-0.13 (-0.27, 0.02)
	HDL <40 mg/dL	0.01 (-0.15, 0.17)	-0.06 (-0.23, 0.11)	0.07 (-0.11, 0.24)	0.02 (-0.15, 0.19)
	Hypertension	-0.19 (-0.34, -0.04)*	-0.13 (-0.29, 0.03)	-0.04 (-0.20, 0.12)	-0.03 (-0.20, 0.13)
	Hyperglycemia	-0.25 (-0.38, -0.12)*	-0.26 (-0.39, -0.12)*	-0.20 (-0.34, -0.07)*	-0.19 (-0.33, -0.06)*

*P<0.05. Results are expressed as z-score β-coefficients (95% CI). † Model 1: Adjusted for age, centre, smoking, alcohol ‡ Model 2: Adjusted for age, centre, smoking, alcohol and other MetS components § Model 3: Adjusted for age, center, smoking, alcohol and BMI

Table 3: Associations of MetS and its components (dichotomized) with QUS parameters.

		Univariate	Model 1†	Model 2‡	Model 3§
BUA	MetS	0.10 (0.02, 0.17)*	0.11 (0.03, 0.19)*	-	-0.05 (-0.14, 0.03)
	Waist >102 cm	0.18 (0.11, 0.25)*	0.20 (0.13, 0.28)*	0.22 (0.14, 0.30)*	0.00 (-0.11, 0.10)
	Triglycerides >150 mg/dL	0.01 (-0.07, 0.09)	0.01 (-0.07, 0.08)	-0.02 (-0.10, 0.06)	-0.07 (-0.15, 0.01)
	HDL <40 mg/dL	0.04 (-0.06, 0.15)	0.05 (-0.06, 0.15)	0.01 (-0.10, 0.12)	-0.01 (-0.12, 0.10)
	Hypertension	-0.02 (-0.12, 0.08)	0.02 (-0.08, 0.12)	0.00 (-0.10, 0.10)	-0.06 (-0.16, 0.04)
	Hyperglycemia	-0.03 (-0.10, 0.05)	-0.01 (-0.08, 0.07)	-0.04 (-0.12, 0.04)	-0.09 (-0.16, -0.01)*
QUI	MetS	0.06 (-0.01, 0.14)	0.08 (0.01, 0.16)*	-	-0.05 (-0.13, 0.04)
	Waist >102 cm	0.12 (0.04, 0.19)*	0.14 (0.07, 0.22)*	0.16 (0.08, 0.24)*	-0.02 (-0.13, 0.08)
	Triglycerides >150 mg/dL	0.00 (-0.08, 0.08)	0.00 (-0.08, 0.07)	-0.02 (-0.10, 0.06)	-0.07 (-0.15, 0.01)
	HDL <40 mg/dL	0.03 (-0.08, 0.13)	0.03 (-0.08, 0.14)	0.00 (-0.11, 0.11)	-0.01 (-0.12, 0.09)
	Hypertension	-0.06 (-0.16, 0.04)	0.00 (-0.10, 0.10)	-0.01 (-0.11, 0.09)	-0.07 (-0.17, 0.04)
	Hyperglycemia	-0.04 (-0.11, 0.03)	-0.01 (-0.08, 0.06)	-0.03 (-0.11, 0.05)	-0.07 (-0.15, 0.00)

*P<0.05. Results are expressed as z-score β-coefficients (95% CI). † Model 1: Adjusted for age, centre, smoking, alcohol ‡ Model 2: Adjusted for age, centre, smoking, alcohol and other MetS components § Model 3: Adjusted for age, center, smoking, alcohol and BMI

Table 4: Associations of MetS and its components (dichotomized) with $_{a}$BMD and pQCT parameters.

		Univariate	Model 1†	Model 2‡	Model 3§
$_{a}$BMD (g/cm^2) L1-4:	MetS	0.41 (0.21, 0.61)*	0.36 (0.16, 0.57)*	-	0.07 (-0.15, 0.29)
	Waist >102 cm	0.48 (0.29, 0.67)*	0.45 (0.26, 0.64)*	0.42 (0.22, 0.62)*	0.07 (-0.19, 0.33)
	Triglycerides >150 mg/dL	0.04 (-0.15, 0.23)	0.03 (-0.16, 0.22)	-0.10 (-0.30, 0.09)	-0.12 (-0.30, 0.07)
	HDL <40 mg/dL	0.15 (-0.15, 0.45)	0.11 (-0.20, 0.42)	0.02 (-0.29, 0.33)	-0.04 (-0.34, 0.26)
	Hypertension	0.27 (0.03, 0.51)*	0.25 (0.00, 0.49)	0.15 (-0.10, 0.40)	0.10 (-0.15, 0.34)
	Hyperglycemia	0.38 (0.18, 0.58)*	0.32 (0.12, 0.52)*	0.24 (0.04, 0.44)*	0.19 (-0.01, 0.39)
$_{a}$BMD (g/cm^2) FN:	MetS	0.24 (0.04, 0.44)*	0.25 (0.06, 0.45)*	-	-0.12 (-0.33, 0.09)
	Waist >102 cm	0.37 (0.18, 0.55)*	0.44 (0.25, 0.62)*	0.43 (0.23, 0.62)*	-0.06 (-0.31, 0.19)
	Triglycerides >150 mg/dL	-0.03 (-0.22, 0.16)	-0.10 (-0.29, 0.09)	-0.23 (-0.42, -0.04)*	-0.26 (-0.44, -0.08)*
	HDL <40 mg/dL	0.13 (-0.17, 0.42)	0.10 (-0.20, 0.41)	0.06 (-0.24, 0.37)	-0.06 (-0.34, 0.23)
	Hypertension	-0.04 (-0.28, 0.20)	0.10 (-0.15, 0.34)	-0.01 (-0.25, 0.23)	-0.07 (-0.31, 0.16)
	Hyperglycemia	0.26 (0.07, 0.46)*	0.33 (0.13, 0.52)*	0.28 (0.08, 0.48)*	0.18 (-0.01, 0.37)
$_{a}$BMD (g/cm^2) Total hip:	MetS	0.31 (0.11, 0.51)*	0.34 (0.13, 0.54)*	-	-0.09 (-0.30, 0.12)
	Waist >102 cm	0.49 (0.30, 0.68)*	0.55 (0.36, 0.74)*	0.54 (0.35, 0.74)*	0.02 (-0.22, 0.27)
	Triglycerides >150 mg/dL	-0.01 (-0.20, 0.18)	-0.04 (-0.23, 0.15)	-0.19 (-0.38, 0.00)	-0.23 (-0.41, -0.05)*
	HDL <40 mg/dL	0.12 (-0.18, 0.43)	0.16 (-0.15, 0.47)	0.08 (-0.23, 0.39)	-0.03 (-0.32, 0.26)
	Hypertension	0.04 (-0.20, 0.28)	0.11 (-0.13, 0.36)	-0.01 (-0.25, 0.24)	-0.10 (-0.34, 0.13)
	Hyperglycemia	0.31 (0.11, 0.50)*	0.35 (0.15, 0.55)*	0.28 (0.08, 0.48)*	0.17 (-0.02, 0.36)
pQCT: 50% radius					
CSA					
	Waist >102 cm	0.36 (0.18, 0.53)*	0.34 (0.16, 0.53)*	0.41 (0.22, 0.60)*	0.22 (-0.03, 0.47)
	Triglycerides >150 mg/dL	-0.18 (-0.36, 0.00)*	-0.18 (-0.37, 0.00)	-0.21 (-0.40, -0.02)*	-0.26 (-0.44, -0.07)*
	HDL <40 mg/dL	-0.17 (-0.44, 0.10)	-0.18 (-0.46, 0.10)	-0.20 (-0.48, 0.08)	-0.23 (-0.51, 0.05)
	Hypertension	-0.12 (-0.34, 0.11)	-0.16 (-0.40, 0.07)	-0.22 (-0.45, 0.01)	-0.28 (-0.52, -0.04)*
	Hyperglycemia	0.12 (-0.07, 0.30)	0.09 (-0.10, 0.28)	0.07 (-0.13, 0.26)	0.03 (-0.17, 0.23)

Cortical thickness				
Waist >102 cm	0.21 (0.03, 0.38)*	0.28 (0.10, 0.47)*	0.27 (0.08, 0.46)*	0.13 (-0.12, 0.38)
Triglycerides >150 mg/dL	0.06 (-0.12, 0.24)	0.03 (-0.15, 0.21)	-0.02 (-0.22, 0.17)	-0.06 (-0.24, 0.13)
HDL <40 mg/dL	0.08 (-0.21, 0.36)	0.11 (-0.18, 0.39)	0.06 (-0.24, 0.35)	0.03 (-0.25, 0.32)
Hypertension	-0.04 (-0.27, 0.18)	0.08 (-0.15, 0.31)	0.03 (-0.21, 0.26)	-0.01 (-0.25, 0.22)
Hyperglycemia	-0.02 (-0.22, 0.17)	0.04 (-0.15, 0.24)	0.01 (-0.19, 0.20)	-0.04 (-0.23, 0.16)
Cortical bone area				
Waist >102 cm	0.40 (0.23, 0.58)*	0.45 (0.27, 0.63)*	0.48 (0.29, 0.67)*	0.25 (0.00, 0.49)
Triglycerides >150 mg/dL	-0.08 (-0.26, 0.10)	-0.10 (-0.28, 0.08)	-0.17 (-0.36, 0.02)	-0.23 (-0.41, -0.05)*
HDL <40 mg/dL	-0.03 (-0.31, 0.25)	-0.01 (-0.30, 0.28)	-0.05 (-0.34, 0.24)	-0.12 (-0.40, 0.17)
Hypertension	-0.06 (-0.28, 0.17)	0.00 (-0.23, 0.23)	-0.08 (-0.31, 0.15)	-0.14 (-0.38, 0.09)
Hyperglycemia	0.04 (-0.15, 0.23)	0.07 (-0.13, 0.26)	0.03 (-0.16, 0.22)	-0.04 (-0.24, 0.15)
Stress strain index				
Waist >102 cm	0.40 (0.23, 0.58)*	0.42 (0.24, 0.60)*	0.48 (0.30, 0.67)*	0.30 (0.05, 0.55)*
Triglycerides >150 mg/dL	-0.15 (-0.33, 0.03)	-0.15 (-0.34, 0.03)	-0.19 (-0.38, -0.01)*	-0.26 (-0.44, -0.07)*
HDL <40 mg/dL	-0.20 (-0.48, 0.07)	-0.20 (-0.49, 0.08)	-0.23 (-0.51, 0.06)	-0.29 (-0.58, -0.01)*
Hypertension	-0.13 (-0.35, 0.10)	-0.11 (-0.34, 0.12)	-0.18 (-0.40, 0.05)	-0.23 (-0.47, 0.00)
Hyperglycemia	-0.01 (-0.20, 0.17)	0.00 (-0.19, 0.19)	-0.02 (-0.21, 0.17)	-0.08 (-0.28, 0.11)
Muscle area				
Waist >102 cm	0.56 (0.38, 0.73)*	0.68 (0.51, 0.84)*	0.61 (0.44, 0.79)*	-0.23 (-0.44, -0.03)*
Triglycerides >150 mg/dL	0.17 (-0.01, 0.35)	0.13 (-0.05, 0.30)	-0.06 (-0.23, 0.11)	-0.13 (-0.29, 0.02)
HDL <40 mg/dL	0.30 (0.02, 0.59)*	0.32 (0.04, 0.59)*	0.20 (-0.07, 0.47)	0.08 (-0.16, 0.32)
Hypertension	0.07 (-0.15, 0.30)	0.32 (0.11, 0.54)*	0.21 (-0.01, 0.42)	0.00 (-0.19, 0.20)
Hyperglycemia	0.07 (-0.12, 0.26)	0.22 (0.04, 0.41)*	0.09 (-0.08, 0.27)	-0.05 (-0.21, 0.12)

*P<0.05. Results are expressed as z-score β-coefficients (95% CI). † Model 1: Adjusted for age, centre, smoking, alcohol ‡ Model 2: Adjusted for age, centre, smoking, alcohol and other MetS components § Model 3: Adjusted for age, center, smoking, alcohol and BMI