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CLINICAL STUDY

Influence of bone remodelling rate on quantitative ultrasound parameters at the calcaneus and DXA BMD_a of the hip and spine in middle-aged and elderly European men: the European Male Ageing Study (EMAS)

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Abstract

Objective: To assess the influence of sex hormones on markers of bone turnover and to explore the association between these markers and bone health in middle-aged and elderly European men.
Design: A cross-sectional population-based survey.
Methods: Men aged 40–79 years were recruited from population registers in eight European centres. Subjects completed a postal questionnaire which included questions concerning lifestyle and were invited to undergo quantitative ultrasound (QUS) of the calcaneus and to provide a fasting blood sample from which the bone markers serum N-terminal propeptide of type 1 procollagen (P1NP) and crosslinks (β C-terminal cross-linked telopeptide (β-cTX)), total testosterone, total oestradiol (E_2), sex hormone-binding globulin (SHBG) and insulin-like growth factor 1 (IGF1) were measured. Dual-energy X-ray absorptiometry (DXA) of the hip and lumbar spine was performed in two centres.
Results: A total of 3120, mean age 59.9 years (s.d. = 11.0) were included. After adjustment for centre, age, height, weight, lifestyle factors, season and other hormones, total and free E_2 were negatively associated with β-cTX but not P1NP while SHBG, IGF1 and parathyroid hormone (PTH) were positively associated with both β-cTX and P1NP. Total or free testosterone was not independently associated with either bone marker. After the same adjustments, higher levels of both bone markers were significantly associated with lower QUS parameters and lower DXA-assessed bone density at the total hip and lumbar spine.
Conclusions: E_2, SHBG, IGF1 and PTH contribute significantly to the regulation/rate of bone turnover in middle-aged and older European men. Higher rates of bone remodelling are negatively associated with male bone health.

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Introduction

In recent years, a number of cross-sectional studies (1–6) have assessed bone turnover markers in men, focusing on age-related variations, potential determinants of bone remodelling and the association between bone turnover and areal bone mineral density (BMD_a). These studies have shown serum levels of bone turnover markers to be high in young adult men followed by a prompt decline, at least until the age of 40 (1–6). From 40 years on, age-related patterns in men become less consistent. Average concentrations of bone turnover markers in older men have been shown to be either relatively stable, or to slightly decrease or increase (7, 8). These various age-related trends are probably due to the cumulative effects of several factors: real changes in bone remodelling, changes in the activity of enzymes involved in the metabolism of bone collagen, age-related decline in glomerular filtration and, for urinary markers, age-associated reduction in muscle mass leading to lower urinary creatinine excretion (9).

While age-related trends have been somewhat discordant, studies have consistently shown a large scatter of individual values of bone turnover marker levels. The biological significance of this scatter, however, remains unclear. One possibility is that it reflects the multiplicity of determinants of bone turnover in men. Hormonal factors, such as reduced exposure to free 17β-oestradiol (E_2) and free testosterone and lifestyle factors are amongst the candidates that may affect bone remodelling rate (10–12). Most analyses, however, have focused on just one or two selected potential determinants of bone turnover, with scant data on the cumulative interdependent effects of various factors and virtually no data on a number of specific potential confounders, such as serum insulin-like growth factor 1 (IGF1) (13).

Most reports assessing skeletal integrity according to levels of bone turnover have used dual-energy X-ray absorptiometry (DXA) measures of BMD_a (1, 3, 14–19). In older men, increased bone turnover markers have been shown to be associated with lower BMD_a and, more recently, poor bone microarchitecture (1, 3, 14–16). In line with these cross-sectional findings, prospective data have confirmed that higher levels of bone remodelling may be associated with increased rates of bone loss, although evidence for an increased risk of fracture is lacking (17–19). Data on the relationship between bone turnover rate and bone quantitative bone ultrasound (QUS) measurements are more limited, especially in men. In older women, bone turnover has been shown to be negatively related with calcaneal QUS in some but not all studies (20–25). In one study in men, QUS measurements were negatively associated with alkaline phosphatase, but the small sample size did not allow adjustment for potential confounding factors (26).

The objectives of this study were i) to characterise the distribution of bone turnover markers by age in middle-aged and elderly men, ii) to assess determinants of the bone turnover rate and iii) to explore the cross-sectional associations between bone turnover rate and male skeletal integrity as assessed by QUS and DXA in the context of the European Male Ageing Study (EMAS), a large population-based multinational cohort of men aged 40–79 years.

Materials and methods

Subjects

The subjects included in this analysis were recruited for participation in EMAS. Details concerning the study design and recruitment have been described previously (27). Briefly, men were recruited from population-based sampling frames in eight centres: Florence (Italy), Leuven (Belgium), Lodz (Poland), Malmö (Sweden), Manchester (UK), Santiago de Compostela (Spain), Szeged (Hungary) and Tartu (Estonia). Participating centres were selected to provide geographical and socioeconomic diversity within Europe, and their facilities to perform epidemiological surveys. Stratified random sampling was used with the aim of recruiting equal numbers of men in each of four 10-year age bands: 40–49, 50–59, 60–69 and 70–79 years. Subjects were invited by letter to complete a postal questionnaire and attend for an interviewer-assisted questionnaire. Ethics approval for the study was obtained in accordance with local institutional requirements in each centre. All subjects provided written informed consent.

Study questionnaires and clinical data

The postal questionnaire included questions concerning current smoking and alcohol consumption in the previous year (response set, every day/5–6 days per week/3–4 days per week/1–2 days per week/less than once a week/not at all). The subjects were also asked if they were currently being treated for a range of medical conditions that included diabetes and prostate disease. Information about medications was also collected. Height and weight were measured in a standardised fashion. Height to the nearest 1 mm using a stadiometer (Leicester height measure, SECA, Birmingham, UK Ltd) and body weight to the nearest 0.1 kg using an electronic scale (SECA, model no. 8801321009, SECA).

Hormone measurements

A single fasting morning (before 10:00) venous blood sample was obtained from all subjects. Serum was separated immediately after phlebotomy and stored at −80 °C until assay at the end of the baseline study.

Measurement of testosterone and E_2 were carried out by gas chromatography-mass spectrometry as described by Labrie *et al.* (28, 29). The lower limit of testosterone quantitation was 0.17 nmol/l and E_2 was 7.34 pmol/l. The coefficients of variation (CV) of testosterone measurements were 2.9% within runs and 3.4% between runs, and for E_2, were 3.5% within runs and 3.7% between runs. Sex hormone-binding globulin (SHBG) was measured by the Modular E170 platform electrochemiluminescence immunoassay (Roche Diagnostics) as described previously (30). Within- and between-assay CV for SHBG measurements were 1.70 and 3.18% respectively. The free and bioavailable (non-SHBG-bound) testosterone and E_2 levels were derived from total hormone, SHBG and albumin concentrations using mass action equations and association constants as described by Vermeulen *et al.* (31) and Van Pottelbergh *et al.* (32). In addition, samples were transported in frozen state to a single laboratory for measurement of IGF1 and parathyroid hormone (PTH; University of Santiago de Compostela). Serum was assayed for IGF1 using chemiluminescence. Within- and between-assay CV for IGF1 were 7.4 and 2.9% respectively. The detection limit of the assay was 20 ng/ml. Serum was assayed for PTH using a chemiluminescence immunoassay (Nichols Advantage Bio-Intact PTH assay, Quest Diagnostics, Madison, NJ, USA). Intra- and inter-assay CV for PTH were 6 and 2.8% respectively. The detection limit of the chemiluminescence immunoassay was 1.6 pg/ml.

Bone marker measurements

To assess bone resorption, serum β C-terminal cross-linked telopeptide (β-cTX) was measured on the Elecsys 2010 automated analyser (Roche Diagnostics GmbH) using the β-Crosslaps/serum reagents (33). This assay is specific for cross-linked β-isomerised type I collagen C-telopeptide fragments and uses two MABs, each recognising the Glu-Lys-Ala-His-βAsp-Gly-Gly-Arg peptide (Crosslaps antigen). The intra-assay CV evaluated by repeated measurements of several serum samples was $<5.0\%$. The detection limit was 10 pg/ml. To evaluate bone formation, measurements were performed on the Elecsys 2010 with a two-site assay using MABs raised against intact human N-terminal propeptide of type 1 procollagen (P1NP) purified from human amniotic fluid. This assay detects both intact mono- and trimeric forms (total P1NP), as described previously (34). The inter-assay CV was $<3.0\%$ and the lower detection limit <5 ng/ml.

Quantitative ultrasound of the heel

QUS of the left heel was performed with the Sahara Clinical Sonometer (Hologic, Inc., Waltham, MA, USA) using a standardised protocol in all centres. Each centre calibrated the device daily with the physical phantom provided by the manufacturer and the performances of the devices was found to be stable. Outputs included the rate of loss of ultrasonic intensity with frequency (broadband ultrasound attenuation (BUA) measured in decibels per megahertz using Fourier transformation of the recorded signal) and the velocity of ultrasound transmission through bone (speed of sound (SOS) measured in meters per second from the sound propagation time between the transducers). In addition, estimated heel bone mineral density ($eBMD_a$) in grams per square centimetre, was derived from the BUA and SOS measures: $eBMD_a = 0.002592 \times (BUA + SOS) - 3.687$. Short-term precision of the method was established by duplicate measurements performed in 20 randomly selected cohort members in Leuven. The *in vivo* CV were 2.8 and 0.3% for BUA and SOS, respectively, and 3.4% for $eBMD_a$. Repeat measurements were performed on a roving phantom at each of the eight centres (35). Standardised CV (SCV) for within machine variability ranged by centre: for SOS, from 1.0 to 5.6%, and BUA from 0.7 to 2.7%. SCV for between machine variability were 4.8% for BUA and 9.7% for SOS (35).

Dual-energy X-ray absorptiometry

BMD_a scans were carried out in the Manchester and Leuven subsets of EMAS ($n=676$). Both sites used DXA QDR 4500A devices from the same manufacturer (Hologic, Inc.). BMD_a was measured at the lumbar spine (L1–L4) and proximal femur (total region). All scans and analysis were performed by trained and certified DXA technicians. The Hologic Spine Phantom was scanned daily to monitor the device performance and long-term stability. With our equipment, the precision errors of these measurements in Leuven were 0.57 and 0.56% at the lumbar spine and total femur region respectively. In Manchester, these precision errors were 0.97 and 0.97% respectively. Both devices were cross-calibrated with the European Spine Phantom (36).

Statistical analysis

Descriptive statistics were used to summarise subject characteristics including the distribution of bone turnover markers (P1NP and β-cTX), heel QUS parameters (BUA, SOS and $eBMD_a$), DXA BMD_a at the total hip and lumbar spine, sex hormone levels and IGF1. The association between age and bone marker levels, and also sex hormones and bone markers was assessed visually using scatter plots, superimposing linear lines and also locally-weighted scatter plot smooth (LOWESS) curves to examine potential nonlinearity. The strength of the associations was assessed using linear regression (with the bone turnover marker as dependent variable) and results expressed as β coefficients. In subsequent analyses for ease of interpretation and comparison we

standardised hormone measures and bone turnover markers into Z scores. To examine potential nonlinear/threshold effects we categorised these variables into tertiles and quintiles. Multivariable linear regression was then used to determine the association between hormone levels (separate models for each of the sex hormones, SHBG and IGF1) and bone turnover markers, adjusting for potential confounders including age, height, weight, centre, season of measurement and lifestyle characteristics – with the bone turnover marker as dependent variable. We then used a model that included all measured hormones to determine their independent associations with bone marker levels. Multivariable linear regression was then used to determine the association between bone turnover markers and QUS/DXA parameters with adjustments initially for centre, age, height, weight, lifestyle factors and season of measurement and then with further adjustments for the sex hormones and IGF1, to determine whether or not the associations between bone turnover makers and bone health were influenced by these factors. For all linear regression models, the distribution of the residuals was assessed by plotting quantiles of the standardised residuals against quantiles of a normal distribution, visually assessing if the plot deviated from a straight line and then statistically testing for deviation from normality by the Shapiro–Wilk test. There was no important deviation from the normality assumption in any of the reported results. For all multivariable models, the variance inflation factor was calculated to quantify the severity of any potential multicollinearity and only models where the multicollinearity was low were included. Results of all linear regression analyses are expressed as β coefficients or standardised β coefficients and 95% confidence intervals (CIs). Statistical analysis was performed by STATA version 9.2 (http://www.stata.com).

Results

Subjects

A total of 3120 men with a mean age of 59.9 years (S.D. = 11.0) had complete bone marker and QUS data. Characteristics of the subjects are shown in Table 1. In addition, 21% reported that they currently smoke, while 56% of the men reported consuming alcohol at least 1 day/week, 8% reported currently being treated for diabetes, 12% for prostate disease and 4% reported currently taking corticosteroids.

Association between age, bone turnover and sex hormones

Neither P1NP nor β-cTX was linearly associated with age (Fig. 1). When mean levels were compared between subjects stratified into 5-year age bands a slight U-shaped pattern emerged (data not shown). Compared with subjects aged 60–65 (mean = 39.0 ng/ml; 95% CI = 37.4–40.7), those aged 40–45 had higher P1NP levels (mean = 45.5 ng/ml; 95% CI = 43.5–47.5; $P<0.001$). Similarly, compared with those aged 60–65, those aged 75–79 had higher P1NP levels (mean = 42.8 ng/ml; 95% CI = 40.8–44.8; $P<0.01$). The same pattern was observed for β-cTX. However, the magnitude of these associations was small and age accounted for <1% of the variation in bone turnover markers. As expected, levels of free testosterone decreased with age while total E_2 increased with age (Fig. 1). Age was positively associated with SHBG and PTH levels and negatively associated with IGF1 (Fig. 1).

Table 1 Subject characteristics. Data are presented as mean (S.D.)

Variables	Values
n	3120
Age at interview (years)	59.9 (11.0)
Height (cm)	173.6 (7.3)
Weight (kg)	83.2 (13.6)
Body mass index (kg/m^2)	27.6 (4.0)
Testosterone (nmol/l)	16.4 (6.0)
Free testosterone (pmol/l)	289.9 (88.5)
Bioavailable testosterone (nmol/l)	7.1 (2.2)
Oestradiol (E_2; pmol/l)	73.3 (24.5)
Free E_2 (pmol/l)	1.3 (0.4)
Bioavailable E_2 (pmol/l)	50.7 (17.0)
SHBG (nmol/l)	42.8 (19.6)
Parathyroid hormone (pg/ml)	28.4 (11.9)
IGF1 (ng/ml)	133.0 (42.9)
QUS	
Estimated bone mineral density (g/cm^2)	0.541 (0.135)
Broadband ultrasound attenuation (dB/MHz)	80.1 (18.9)
Speed of sound (m/s)	1550.5 (34.0)
DXA	
Total hip (g/cm^2)	1.013 (0.145)
Lumbar spine (g/cm^2)	1.053 (0.174)
Bone markers	
P1NP (ng/ml)	41.7 (17.6)
β-cTX (pg/ml)	352.1 (179.9)

Association between sex hormones and bone turnover

In bivariate analyses, higher levels of total E_2 were not significantly associated with β-cTX (β coefficient (β) = −0.201; 95% CI = −0.458, 0.056; $P=0.13$); however, free E_2 was associated with lower β-cTX (Fig. 2). Higher levels of SHBG were associated with higher β-cTX (Fig. 2). Total/free E_2 was not associated with P1NP, but higher SHBG was significantly associated with higher P1NP (data not shown). Free testosterone was not associated with either β-cTX or P1NP (data not shown). In a multivariable model including age, centre, height, weight, current smoking, alcohol consumption and season, higher levels of both total and free E_2 were associated with lower β-cTX, and higher levels of SHBG remained associated with higher

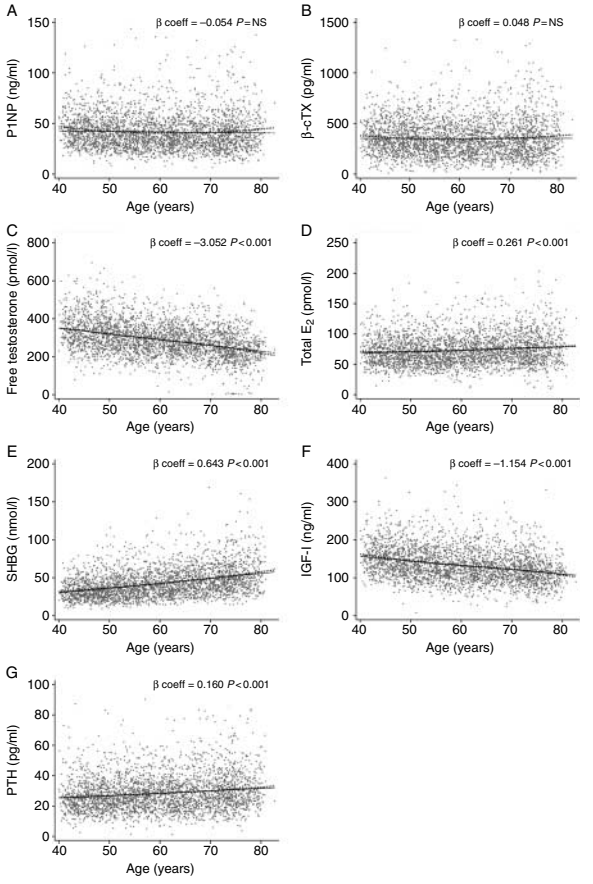

Figure 1 Association between age and (A) N-terminal propeptide of type 1 procollagen, (B) β C-terminal cross-linked telopeptide, (C) free testosterone, (D) total oestradiol, (E) sex hormone-binding globulin, (F) insulin-like growth factor 1 and (G) parathyroid hormone. The solid lines represent the linear relationship, the dashed lines represent locally weighted scatterplot smoothing.

levels of both β-cTX and P1NP (Table 2). Higher levels of free testosterone in this analysis were associated with lower β-cTX but not P1NP. After the multivariable model was further adjusted for other hormone levels (free E_2 was included rather than total), free E_2 remained negatively associated with β-cTX and SHBG remained positively associated with both β-cTX and P1NP (Table 2). IGF1 and PTH were positively associated with both bone turnover markers (Table 2). There was no evidence of threshold effects when any of the hormones were included in the models categorised into either tertiles or quintiles. Overall, age, lifestyle and key hormones regulating bone metabolism accounted for 20 and 8% of the variability in β-cTX and P1NP respectively.

Association between bone turnover, ultrasound parameters and DXA-assessed bone density

After adjustment for age, centre, height, weight, current smoking, alcohol consumption and season, bone turnover markers were negatively associated with the QUS parameters (Table 3). Higher levels of P1NP were associated with lower BUA, SOS and $eBMD_a$. Likewise, higher levels of β-cTX were associated with lower BUA, SOS and $eBMD_a$. Similar results were observed with DXA BMD_a at both the total hip and lumbar spine sites (Table 3). There was no evidence of any threshold effect when the bone turnover markers were included in the models categorised into either tertiles or quintiles.

Association between bone turnover, ultrasound parameters and DXA-assessed bone density: influence of sex hormones, IGF1 and PTH

After the multivariable model had been further adjusted for free testosterone, free E_2, SHBG, IGF1 and PTH, higher levels of β-cTX remained associated with lower BUA, SOS and $eBMD_a$ (Table 3). Similarly, higher levels of P1NP remained associated with lower SOS and $eBMD_a$, however, the association with BUA became non-significant. In similar models, higher levels of both bone markers remained associated with significantly lower DXA-measured BMD_a at the total hip and lumbar spine. Overall, age, lifestyle, hormones and bone markers accounted for 11–25% of the variability in QUS/DXA parameters. Excluding subjects taking corticosteroids or reporting being treated for diabetes or prostate disease did not influence the results.

Discussion

In the large population-based EMAS cohort of ageing men, age, lifestyle and key hormones regulating bone metabolism jointly accounted for between 8 and 20% of the variability in bone turnover rate. E_2, SHBG, IGF1 and PTH (but not testosterone, total or free) were identified as independent predictors of bone remodelling. In multi-adjusted models, which included hormone levels as covariates, higher levels of bone remodelling were significantly and negatively associated with QUS parameters and DXA-assessed BMD_a.

Overall, bone turnover marker levels were relatively stable with age in the investigated age range. Several studies have provided evidence that levels of bone

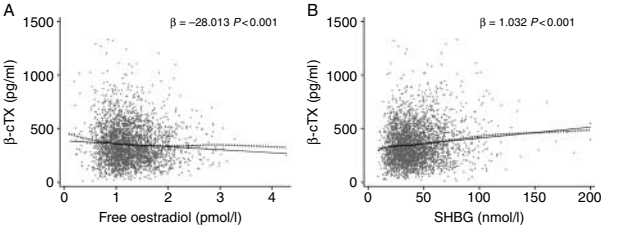

Figure 2 Association between β C-terminal cross-linked telopeptide and (A) free oestradiol and (B) sex hormone-binding globulin. The solid lines represent the continuous relationship, the dashed lines represent locally weighted scatterplot smoothing.

Table 2 Association between hormones and bone turnover markers. Results expressed as β coefficients (95% CI). Statistically significant values are presented in boldface.

Independent variables (per S.D.)	Dependent variables			
	Adjusted for age, centre, height, weight, current smoking, alcohol consumption and season		Further adjusted for FT, free E_2, SHBG, IGF1 and PTH	
	P1NP (per S.D.)	β-cTX (per S.D.)	P1NP (per S.D.)	β-cTX (per S.D.)
FT	−0.036 (−0.077, 0.004)	**−0.073 (−0.111, −0.034)***	−0.030 (−0.079, 0.020)	−0.028 (−0.075, 0.018)
Total E_2	0.017 (−0.019, 0.053)	**−0.060 (−0.094, −0.026)***	–	–
Free E_2	−0.024 (−0.060, 0.013)	**−0.083 (−0.118, −0.049)***	−0.009 (−0.053, 0.035)	**−0.075 (−0.116, −0.034)***
SHBG	**0.135 (0.095, 0.175)***	**0.083 (0.045, 0.121)***	**0.169 (0.128, 0.209)***	**0.116 (0.079, 0.154)***
IGF1	**0.070 (0.032, 0.107)***	**0.088 (0.052, 0.123)***	**0.116 (0.078, 0.153)***	**0.128 (0.093, 0.163)***
PTH	**0.114 (0.078, 0.150)***	**0.178 (0.144, 0.211)***	**0.126 (0.090, 0.161)***	**0.193 (0.160, 0.226)***

*$P<0.05$. FT, free testosterone.

turnover are high in young adult men – higher in fact than in women of similar age which most likely reflects active bone remodelling during consolidation after growth arrest, which occurs with some delay in men compared with women – and then gradually decline (9, 37). This decrease is mostly observed before the age of 40 (1, 4, 6) and could not be assessed in our cohort of men who were aged between 40 and 79 years. When we compared mean levels of bone turnover between subjects stratified into 5-year age bands, a slight U-shaped pattern emerged, with levels declining until age 60–65 years and then slightly rising. A more marked resurgence of bone turnover has been previously observed in older men for some, but not all, bone turnover markers – mainly free and total deoxypyridinoline, and in some, but not all, cohorts – mainly in men over the age of 80 (1–4). Such a significant increase has been primarily observed in elderly men with impaired mobility and hormonal or nutritional insufficiencies (38–40), and may therefore not apply to the present cohort of relatively healthy community-dwelling men.

Most of the variability in bone turnover rate could not be accounted for, even when combining age, lifestyle and key hormones of bone metabolism data. This suggests a major role for other determinants such as genetic background, nutritional status, underlying (occult) comorbidities as well as normal biological short- and long-term variability in bone turnover rate (41). In line with the inhibitory effect of E_2 on bone resorption (42, 43), low free E_2 was negatively associated with levels of β-cTX (3, 44, 45). The association between P1NP and free E_2, however, was not significant, possibly reflecting the divergent effects of an oestrogen-induced reduction in the overall rate of bone remodelling, on the one hand, and an oestrogen-mediated increase in bone formation locally at the level of the individual bone remodelling units, on the other hand (42).

Free testosterone was not significantly related to any of the bone turnover markers, suggesting that age-associated changes in androgen status is unlikely to drive bone turnover independently in men. However, it should be noted that, in our cohort, most participants had testosterone levels within the normal range. Higher levels of some bone markers (mainly indices of bone resorption) have been shown in hypogonadal men (38, 46–48).

We observed a highly significant positive correlation between SHBG and bone turnover, in line with previous studies in men and consistent with evidence that SHBG

Table 3 Association between bone markers, DXA and QUS parameters. Results expressed as β coefficients (95% CI). Statistically significant values are presented in boldface.

Dependent variables	Independent variables			
	Adjusted for age, centre, height, weight, current smoking, alcohol consumption and season		Further adjusted for free testosterone, free E_2, SHBG, IGF1 and PTH	
	P1NP (per S.D.)	β-cTX (per S.D.)	P1NP (per S.D.)	β-cTX (per S.D.)
QUS ($n=3120$)				
BUA (per S.D.)	**−0.040 (−0.073, −0.006)***	**−0.073 (−0.109, −0.037)***	−0.030 (−0.066, 0.005)	**−0.064 (−0.102, −0.026)***
SOS (per S.D.)	**−0.066 (−0.100, −0.032)***	**−0.098 (−0.134, −0.062)***	**−0.063 (−0.098, −0.027)***	**−0.093 (−0.131, −0.055)***
$eBMD_a$ (per S.D.)	**−0.055 (−0.089, −0.021)***	**−0.089 (−0.125, −0.053)***	**−0.049 (−0.085, −0.014)***	**−0.082 (−0.120, −0.044)***
DXA BMD_a ($n=676$)				
Total hip (per S.D.)	**−0.104 (−0.171, −0.037)***	**−0.135 (−0.212, −0.057)***	**−0.121 (−0.192, −0.051)***	**−0.157 (−0.240, −0.074)***
Lumbar spine (per S.D.)	**−0.086 (−0.158, −0.015)***	**−0.171 (−0.253, −0.089)***	**−0.094 (−0.169, −0.019)***	**−0.195 (−0.283, −0.107)***

*$P<0.05$.

is negatively related to bone density (49–51) and positively related to fracture risk (52). We recently reported a similar negative association with bone QUS parameters, even after adjusting for potential confounders, in our cohort (53). Similar findings have also been documented in ageing women (54). However, the mechanism of this association remains unclear. Part of the association is likely to reflect the fact that SHBG is the principal determinant of bioavailability of free sex steroids. However, even after adjusting for free E_2 and free testosterone, SHBG remained strongly associated with bone turnover, suggesting that SHBG may potentially have a direct negative effect on bone, independent of sex steroids. Alternatively, calculated concentrations of circulating free sex steroid may not accurately reflect local bioavailability of these hormones at the target tissue level (55–57). Calculation of free sex steroid concentrations assumes that the binding affinity between both sex steroids and SHBG is constant in the entire population and does not vary with age while, in reality, it may be affected by genetic variants or isoforms of SHBG, age and levels of other SHBG-binding steroids (54, 58–61).

Previously, PTH contributed significantly to the bone turnover rate in some (62) but not all studies (2). Importantly, in this cohort, PTH was a significant determinant of bone turnover in multivariable models independently of other lifestyle and hormonal factors. This confirms that age-related secondary hyperparathyroidism is a significant determinant of age-related BMD decrease in men (62, 63). In line with the fact that IGF1 is known to act directly on bone cells and to stimulate bone remodelling, bone turnover marker levels were also found to be positively associated with IGF1 levels in this study. Although similar observations have been made in adolescents (64, 65), data in a general male population are very limited (2, 8).

In our population-based sample, both QUS parameters and DXA-assessed BMD_a were inversely related to bone turnover rate, independent of age, weight, height, lifestyle factors and key hormones regulating bone turnover. These findings are concordant with previous studies (1, 3, 14–16) and confirm the importance of bone remodelling for bone health in men, although it should be noted that the proportion of the variance in bone parameters explained by our multivariable models was $<26\%$. They show that the rate of bone turnover is a significant determinant of bone density, even in weight-bearing sites where bone metabolism is under a strong influence of the mechanical effect of body weight. While the associations between bone turnover and QUS tended to be slightly weaker than with DXA, findings were consistent across different skeletal sites and across different assessment methods, supporting the concept that QUS measurements in calcaneal bone should be primarily regarded as an indicator of bone density (66). In line with this concept, QUS parameters and DXA-assessed BMD_a have been shown to be equally predictive for incident fractures, in both sexes (67–69).

The main strength of the current analysis is that it used well-established methods to assess the impact of a wide variety of potential determinants of bone remodelling and skeletal integrity in a large, community-based population of middle-aged and elderly men. Our finding that, even after adjustment for lifestyle and hormonal variables known to regulate male bone metabolism, bone markers remained significantly associated with bone health provide strong evidence that other factors influence bone remodelling and determine skeletal integrity. What constitutes this residual variance in bone turnover rate will require more research. Future studies should clarify the extent to which other determinants (e.g. genetic background, nutritional status and underlying comorbidities) contribute to skeletal maintenance in ageing men.

Our study also has limitations. Our findings are based on data in middle-aged and older European men and may not apply to other groups of men. Also, the participants were home-dwelling volunteers recruited in selected centres and may not be representative of the general population. A small number of men ($n=249$; 7%) were excluded from the analysis due to incomplete data and it is possible that these men were different in terms of health status from those that were included. However, there was no difference in age, smoking status, alcohol consumption or hormone levels between those included and those excluded providing some reassurance against this. Bone density and turnover were assessed by established methods; however, bone mass was estimated only at weight-bearing sites and bone turnover analysed only by two biochemical markers. Bone density as assessed by DXA was performed in only a subset of the subjects. The free fractions of testosterone and E_2 were calculated rather than measured and may not reflect the absolute values. Whilst a wide variety of potential determinants of bone health were assessed in EMAS, it is possible that unmeasured factors explain the observed results. Analysis of the influence of bone remodelling on osteoporotic fracture was not possible due to lack of prospective fracture data at this time. Finally, in a cross-sectional study, associations can be demonstrated but it is not possible to determine cause-and-effect relationships or to disentangle the temporal nature of the observed associations.

In conclusion, E_2, SHBG, IGF1 and PTH contribute significantly and independently to bone turnover in middle-aged and older European men. Higher rates of bone remodelling are negatively associated with male bone health independently of age, lifestyle factors and hormonal exposure.

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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