

# Gonadal sex steroid status and bone health in middle-aged and elderly European men

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## Abstract

**Summary** The influence of sex steroids on calcaneal quantitative ultrasound (QUS) parameters was assessed in a population sample of middle-aged and elderly European men. Higher free and total E<sub>2</sub> though not testosterone, were independently associated with higher QUS parameters.

**Introduction** The aim of this study was to investigate the association between QUS parameters and sex steroids in middle-aged and elderly European men.

**Methods** Three thousand one hundred forty-one men aged between 40 and 79 years were recruited from eight European centres for participation in a study of male ageing: the European Male Ageing Study. Subjects were

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invited by letter to attend for an interviewer-administered questionnaire, blood sample and QUS of the calcaneus (Hologic-SAHARA). Blood was assessed for sex steroids including oestradiol ( $E_2$ ), testosterone (T), free and bio-available  $E_2$  and T and sex hormone binding globulin (SHBG).

**Results** Serum total T was not associated with any of the QUS parameters. Free T and both free and total  $E_2$  were positively related to all QUS readings, while SHBG concentrations were negatively associated. These relationships were observed in both older and younger (<60 years) men. In a multivariate model, after adjustment for age, centre, height, weight, physical activity levels and smoking, free  $E_2$  and SHBG, though not free T, remained independently associated with the QUS parameters. After further adjustment for IGF-1, however, the association with SHBG became non-significant.

**Conclusion** Higher free and total  $E_2$  are associated with bone health not only among the elderly but also middle-aged European men.

**Keywords** Epidemiology · Oestradiol · Sex steroids · SHBG · Testosterone · Ultrasound

## Introduction

Osteoporosis is an important clinical and public health problem in men through its association with age-related fractures which account for substantial morbidity, economic cost and even mortality [1, 2]. The pathophysiology of bone loss in men is less well understood than in women.

There is increasing evidence for an effect of sex steroids, and in particular oestradiol ( $E_2$ ), on the maintenance of bone health not only in women but also in elderly men [3–9]. The effect of testosterone (T) on bone health is less well understood. Most studies have been undertaken in cohorts of elderly men (more than 60 years old) with few data in younger men. In a recent study of community-dwelling Australian older men (60 years and older), serum testosterone was independently associated with the risk of osteoporotic fracture [10], while in a European study serum testosterone had no effect on fracture risk [11]. The relative role of age-related changes in total vs free or bio-available fractions of serum sex steroids on bone health remains unclear [12]. Serum T and  $E_2$  are bound with high and low affinity to serum sex hormone binding globulin (SHBG) and albumin, respectively. Only fractions of circulating sex steroid are either free (non-bound to SHBG and albumin) or bio-available (non-bound to SHBG) [13]. Moreover, it is well established that the age-related decline of both free and bio-available fractions of sex steroids in men as well as a rise of SHBG starts as early as the fourth decade [14, 15]. Reductions in free and bio-available fractions of sex steroids are also more important than decreases of total concentrations. However, the relative contribution of total vs free or bio-available sex steroids in maintaining bone health in large samples of elderly and especially middle-aged men has received little attention. The question therefore remains whether these relatively early reductions of free and bio-available sex steroids as well as the rise of SHBG contribute to a decline of bone health in middle-aged men.

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The European Male Ageing Study (EMAS) is a population-based survey of ageing in both middle-aged and elderly European men. The aim of this study is to investigate the association between sex hormones, including T and E<sub>2</sub>, and the free and bio-available fractions of these hormones as well as SHBG with male bone health as estimated by quantitative ultrasound (QUS) of the calcaneus from 40 up to 80 years of age.

## Materials and methods

### Subjects

The subjects included in this analysis were recruited for participation in EMAS. Details concerning the study design and recruitment were described previously [16]. 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 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 years and over. Subjects were invited by letter to complete a postal questionnaire and attend for an interviewer-assisted questionnaire, which included questions about physical activity, and QUS of the heel. Subjects were recontacted usually within 4 weeks if they did not reply following a first letter.

### 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). There was a question about prior fracture since the age of 25 years (response set = no/yes/don't know). The main study questionnaire included the physical activity scale for the elderly (PASE). This survey is designed to assess physical activity in epidemiologic studies of elderly persons. The PASE score combines information on leisure, household and occupational activity and is a continuous scale ranging from 0 to 1,100 [17]. There was a question about medications that subjects were taking. Height and weight were measured in a standardised fashion. Body weight was measured to the nearest 0.1 kg using an electronic scale (SECA, model no. 8801321009, SECA UK Ltd) and height to the nearest 1 mm using a stadiometer

(Leicester Height Measure, SECA UK Ltd). Each centre's electronic scales and stadiometers were calibrated on a monthly basis.

### Hormone measurements

A single fasting morning (before 1000 hours) 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 T and E<sub>2</sub> were carried out by gas chromatography mass spectrometry as described in Labrie et al. [18, 19]. The lower limit of T quantitation was 0.17 nmol/L and E<sub>2</sub> was 7.34 pmol/L. The coefficients of variation of T measurements were 2.9% within runs and 3.4% between runs and for E<sub>2</sub> were 3.5% within runs and 3.7% between runs. SHBG was measured by the Modular E170 platform electrochemiluminescence immunoassay (Roche Diagnostics, Mannheim, Germany) as previously described [20]. The free and bio-available (non-SHBG-bound) T and E<sub>2</sub> levels were derived from total hormone, SHBG and albumin concentrations using mass action equations and associations constants of Vermeulen et al. [21] and Van Pottelbergh et al. [22]. In addition, samples were transported in frozen state to a single laboratory for measurement of IGF-1 (University of Santiago de Compostela) by quimioluminescence. Within- and between-assay coefficients of variation (CVs) for IGF-1 were 7.4% and 2.9%, respectively. The detection limit of the assay was 20 ng/ml.

### Quantitative ultrasound of the heel

Quantitative ultrasound of the left heel was performed with the Sahara Clinical Sonometer (Hologic, Bedford, MA) using a standardised protocol. Each centre used the same machine model, and each was calibrated daily with the physical phantom provided by the manufacturer. Outputs included the rate of loss of ultrasonic intensity with frequency (broadband ultrasound attenuation [BUA] measured in dB/MHz using Fourier transformation of the recorded signal) and the velocity of ultrasound transmission through bone (speed of sound [SOS] measured in metres per second from the sound propagation time between the transducers). In addition, quantitative ultrasound index (QUI), a measure of stiffness, was derived from the BUA and SOS measures:  $QUI = 0.41(SOS) + 0.41(BUA) - 571$ . Short-term precision of the method was established on duplicate measurements performed in 20 randomly selected cohort members in Leuven. The in vivo CVs were 2.8% and 0.3% for BUA and SOS, respectively, and 2.3% for QUI. Repeat measurements (ten) were performed on a roving phantom at each of the eight centres. Standardised

CVs (SCVs) for within-machine variability ranged by centre: for SOS, from 1.0% to 5.6%, and BUA, from 0.7% to 2.7%. SCVs for between-machines variability were 4.8% for BUA and 9.7% for SOS [23].

## Analysis

Descriptive statistics were used to characterise the distribution of the heel QUS parameters (BUA, SOS and QUI) and sex hormone levels (after exclusion of 144 subjects who were taking therapies which may have influenced sex steroid levels). Linear regression was used to determine the association between each of the ultrasound parameters and the different sex steroid levels adjusting for age, height, weight, physical activity, smoking and centre with the results expressed as  $\beta$  coefficients and 95% confidence intervals (CI). The sex hormone variables were analysed as continuous data and categorised into quintiles, though for total T we also categorised individuals as either normal or hypogonadal (using two thresholds, 8 and 10 nmol/L) [24]. We examined initially the whole group and subsequently after stratification by age: less than and greater than 60 years. The associations were assessed visually using scatter plots, super-imposing linear lines and locally weighted scatter plot smooth (lowess) curves [25]. Statistical analysis was performed using STATA version 9.2 (<http://www.stata.com>).

## Results

### Subjects

Three thousand one hundred forty-one men (mean age 59.7 years) were included in the analysis. Of these, 1,618 were less than 60 years, and 1,523 were 60 years or older. Mean height was 173.7 cm and weight 83.5 kg; see Table 1. Mean PASE score was 198.1 (SD=91.4). Of the men, 21% reported that they currently smoke, whilst 57% reported consuming alcohol at least 1 day per week. Twenty-six per cent reported a previous fracture since the age of 25. Mean BUA was 80.2 dB/MHz (SD=18.7) and SOS 1550.9 m/s (SD=33.7). QUI was 97.9 (SD=21.5). The QUS parameters were all associated with prior fracture: after adjustment for age and centre, compared to those who did not report a previous fracture, those who did had a lower BUA ( $\beta$  coefficient=-4.797 db/Mhz,  $p<0.001$ ), SOS ( $\beta$  coefficient=-9.491 m/s,  $p<0.001$ ) and QUI ( $\beta$  coefficient=-5.860,  $p<0.001$ ).

The mean values for the sex hormones are shown in Table 2. Mean T level was 16.6 nmol/L, free T 292.2 pmol/L, and bio-available T was 7.1 nmol/L. Mean  $E_2$  level was 74.0 pmol/L, free  $E_2$  1.3 pmol/L, bio-available  $E_2$  51.2 pmol/L,

**Table 1** Subject characteristics

Variable	N=3,141
Mean (SD)	
Age at interview (years)	59.7 (10.9)
Height (cm)	173.7 (7.3)
Weight (kg)	83.5 (13.8)
Body mass index (kg/m <sup>2</sup> )	27.6 (4.0)
PASE score (0–1,100)	198.1 (91.4)
Broadband ultrasound attenuation (dB/MHz)	80.2 (18.7)
Speed of sound (m/s)	1,550.9 (33.7)
Quantitative ultrasound index	97.9 (21.5)
%	
Currently smoke (yes vs no)	21.2
Alcohol consumption $\geq$ 1 day/week	56.7
Previous fracture since age 25 (yes vs no)	25.9

SHBG 42.7 nmol/L and IGF-1 133.2 ng/mL. Using a cut-off of serum total T concentration of 8 and 10 nmol/L, respectively, 4.1% and 11.6% of men respectively had evidence of T deficiency.

### Influence of sex hormones on ultrasound parameters

After adjustment for age, centre, height, weight and physical activity as determined by PASE score and current smoking, there were significant positive associations between the QUS parameters BUA, SOS and QUI and serum gonadal steroid concentrations including both free, total and bio-available  $E_2$  and free and bio-available T; see Table 3. No association with total T was present, though the QUS parameters were significantly lower in hypogonadal

**Table 2** Sex hormone descriptives

Variable	N=3,141
Mean (SD)	
Testosterone (nmol/L)	16.6 (5.9)
Free testosterone (pmol/L)	292.2 (87.2)
Bio-available testosterone (nmol/L)	7.1 (2.2)
Oestradiol (pmol/L)	74.0 (25.0)
Free oestradiol (pmol/L)	1.3 (0.4)
Bio-available oestradiol (pmol/L)	51.2 (17.3)
SHBG (nmol/L)	42.7 (19.6)
IGF-1 (ng/mL)	133.2 (43.4)
N (%)	
Testosterone level <8 nmol/L	128 (4.1)
Testosterone level <10 nmol/L	361 (11.6)

**Table 3** Association between sex hormones, SHBG and QUS parameters

	BUA (dB/Mhz) β coefficient <sup>1</sup> (95% CI)	SOS (m/s) β coefficient <sup>a</sup> (95% CI)	QUI β coefficient <sup>a</sup> (95% CI)
Total testosterone (per 10 nmol/L)	-0.030 (-1.267, 1.207)	1.348 (-0.886, 3.581)	0.705 (-0.715, 2.125)
Free testosterone (per 10 pmol/L)	0.161 (0.073, 0.250)*	0.343 (0.184, 0.503)*	0.215 (0.114, 0.317)*
Bio-available testosterone (nmol/L)	0.597 (0.238, 0.956)*	1.433 (0.785, 2.081)*	0.841 (0.428, 1.254)*
Total oestradiol (per 10 pmol/L)	0.545 (0.269, 0.820)*	1.300 (0.804, 1.796)*	0.788 (0.472, 1.103)*
Free oestradiol (per 10 pmol/L)	44.022 (28.057, 59.987)*	89.144 (60.321, 117.967)*	55.025 (36.676, 73.375)*
Bio-available oestradiol (per 10 pmol/L)	1.101 (0.701, 1.501)*	2.348 (1.626, 3.070)*	1.390 (0.930, 1.850)*
SHBG (per 10 nmol/L)	-0.792 (-1.173, -0.411)*	-1.006 (-1.696, -0.317)*	-0.640 (-1.075, -0.205)*
Total testosterone (nmol/L)			
≥10	Referent	Referent	Referent
8–10	-0.342 (-2.853, 2.168)	-2.363 (-6.894, 2.168)	-1.624 (-4.523, 1.274)
<8	-4.798 (-8.194, -1.403)*	-10.728 (-16.856, -4.601)*	-7.593 (-11.498, -3.689)*
Total testosterone (nmol/L)			
≥8	Referent	Referent	Referent
<8	-4.752 (-8.130, -1.374)*	-10.408 (-16.504, -4.312)*	-7.375 (-11.261, -3.490)*
Total testosterone (nmol/L)			
≥10	Referent	Referent	Referent
<10	-1.848 (-3.962, 0.266)	-5.190 (-9.005, -1.375)*	-3.653 (-6.090, -1.216)*

\* $p < 0.05$ <sup>a</sup> Adjusted for age, centre, height, weight, PASE score and current smoking

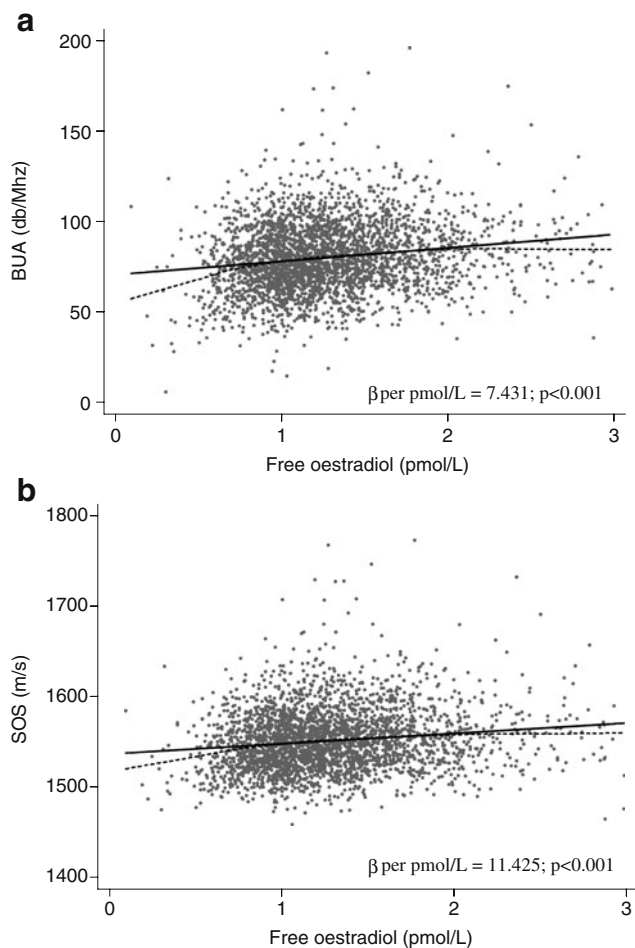
men compared to eugonadal men using either the 8 or 10 nmol/L threshold. In contrast with the gonadal sex steroid concentrations, SHBG levels were negatively associated with all three QUS parameters. After stratification by age, broadly similar associations were observed above and below the age of 60 years for total, free and bio E<sub>2</sub> levels, though stronger associations were observed with free and bio T levels among the older men (data not shown). Figure 1 shows the relationship between free E<sub>2</sub> levels and BUA and SOS. Using a locally weighted scatterplot smoothed line, the main effect was increased BUA and SOS with increased values of E<sub>2</sub> with no evidence of a threshold effect. In a multivariate model including free T, free E<sub>2</sub> and SHBG as continuous variables, after adjustment for age, centre, height, weight, physical activity (as assessed by PASE) and current smoking, both free E<sub>2</sub> and SHBG remained significantly associated with all three QUS parameters; see Table 4. Overall, the multivariate model explained 10–12% of the overall variation of QUS measures. After further adjustment for IGF-1, the association with SHBG became non-significant (data not shown). In a multivariate model using the same variables with the sex steroids and SHBG categorised into quintiles (see also Table 4), higher free E<sub>2</sub> levels were associated with higher QUS parameters. For SHBG, the association appeared non-linear; compared to the lowest quintile, those in the second and third quintiles had higher QUS parameters, while those in the highest

quintile had lower measurements. On formal testing, there was a significant negative (linear) trend with BUA only, though this disappeared after adjustment for IGF-1 (data not shown). Results were the same when either total or bio-available E<sub>2</sub> were used instead of free E<sub>2</sub> (data not shown).

## Discussion

In this study, we found no association between any of the QUS parameters and total T. There were significant associations between the QUS parameters, E<sub>2</sub>, free T and SHBG, and these were observed in men both above and below 60 years of age. As expected, the results were similar for free and bio-available sex steroid concentrations. In a multivariate analysis including free E<sub>2</sub>, SHBG and free T, the association with free T was no longer significant.

EMAS is the first study to explore the relationship between gonadal steroid status and bone health as measured by QUS of the calcaneus in a large group of both middle-aged and elderly men. This type of data is important as mechanisms related to bone loss in old age may differ from those related to maintenance of peak bone mass in middle-aged men. In ageing men, reductions in free and bio-available fractions of sex steroids which may negatively impact on bone health are already apparent from the fourth decade on. Our data are consistent with previous studies in older men showing that bone health is more strongly



The solid lines represent the continuous relationship, the dashed lines represent locally weighted scatterplot smoothing (LOWESS).  $\beta$  is the coefficient from linear regression.

**Fig. 1** Association between free oestradiol and **a** BUA and **b** SOS. Linear vs non-parametric lowess plots. Free oestradiol <3 pmol/L

associated with (total and free)  $E_2$  than with T. In fact, total T was not significantly related to any of the bone parameters in this cohort, irrespective of age. However, compared to those with normal levels of T, those with hypogonadism as based on a threshold of 8 or 10 nmol/L [24, 26] had lower BUA, SOS and QUI. This is consistent with recent findings in the MrOS study in which femoral bone mineral density ( $BMD_a$ ) (assessed by dual-energy X-ray absorptiometry [DXA]) was reduced only in men with T levels <7 nmol/L [27].

In agreement with previous studies [28–31], consistent positive associations between  $E_2$  and all QUS parameters were found both in middle-aged and in elderly men. Our findings add further evidence to support the view that  $E_2$  is the major determinant of bone health in elderly men. In keeping with some [22] but not all studies [28, 32–34], we

found a positive association across the entire range of  $E_2$  concentrations with no evidence of a threshold effect.

The fact that SHBG binds but also inactivates gonadal steroids is an important determinant of the bioavailability for tissue action and metabolism. Not surprisingly, and in accordance with most [33, 35–37] but not all earlier observations [11, 28], serum SHBG was inversely related to bone QUS parameters in the men in our study. It is well established that serum SHBG starts to rise in men as early as the fourth or fifth decade [14, 15]. The resulting age-related decrease of bioavailability of free sex steroids may explain the negative correlation of sex hormone binding globulin with bone density. An alternative explanation is that SHBG may have a direct negative effect of bone, independently of sex steroids. Interestingly, in line with our findings in men, SHBG has been reported to be associated with hip fracture risk, both in older men and postmenopausal women, independently of serum total and bioavailable  $E_2$  and T concentrations [9, 38].

That sex steroids are a determinant of bone density not only in elderly (>60 years) but also middle-aged men is a novel observation. Most previous studies have been small in scale or undertaken only in elderly men. In keeping with our finding, similar associations have been observed between sex steroid levels and bone density (at the radius but not at spine and hip) in a small cross-sectional sample of middle-aged (and elderly) men [39]. This suggests that hormonal mechanisms to maintain bone mass act from middle age onwards.

In our study, serum concentrations of sex steroids accounted only for a small proportion of the age-related decrease in QUS parameters. This underlines the fact that age-related bone loss in men (at least as determined by QUS) is due to a complex interplay of hormonal changes and other contributing factors.

The main strength of our study is that it is based on large-scale, population-based data and on the use of standardised methods to assess bone health and sex hormone status. There are a number of limitations to consider in interpreting the results. The study was cross-sectional, and given this design, it is not possible to determine the temporal nature of the observed associations for which prospective data are needed. The overall response rate for participation was 45%. It is possible that those invited but who did not take part may differ with respect to levels of sex hormones than those who took part, and therefore the data concerning absolute levels of these parameters need to be interpreted with caution. Any factors influencing participation, however, are unlikely to have influenced the association between the sex hormones and QUS parameters which are based on an internal comparison of those who participated. Our results are based on assessment of the calcaneus, a predominantly (95%)

**Table 4** Association between free testosterone, free oestradiol and sex hormone binding globulin and QUS parameters

	BUA (dB/Mhz)		SOS (m/s)		QUI	
	$\beta$ coefficient (95% CI)	$P_{\text{trend}}$	$\beta$ coefficient (95% CI)	$P_{\text{trend}}$	$\beta$ coefficient (95% CI)	$P_{\text{trend}}$
Free testosterone (per 10 pmol/L)	0.050 (−0.057, 0.157) <sup>a</sup>		0.110 (−0.083, 0.303) <sup>a</sup>		0.073 (−0.050, 0.196) <sup>a</sup>	
Free oestradiol (per 10 pmol/L)	37.407 (18.152, 56.662) <sup>a,*</sup>		76.184 (41.375, 110.994) <sup>a,*</sup>		46.447 (24.325, 68.569) <sup>a,*</sup>	
SHBG (per 10 nmol/L)	−0.752 (−1.137, −0.367) <sup>a,*</sup>		−0.910 (−1.606, −0.213) <sup>a,*</sup>		−0.581 (−1.020, −0.141) <sup>a,*</sup>	
Free testosterone quintiles (pmol/L)						
<21	Referent <sup>b</sup>		Referent <sup>b</sup>		Referent <sup>b</sup>	
221–263	−0.037 (−2.157, 2.084) <sup>b</sup>		0.528 (−3.301, 4.358) <sup>b</sup>		0.195 (−2.250, 2.641) <sup>b</sup>	
263–307	1.006 (−1.232, 3.244) <sup>b</sup>		1.799 (−2.243, 5.841) <sup>b</sup>		1.114 (−1.470, 3.698) <sup>b</sup>	
307–359	0.542 (−1.838, 2.922) <sup>b</sup>		1.404 (−2.895, 5.703) <sup>b</sup>		0.766 (−1.978, 3.510) <sup>b</sup>	
≥359	0.192 (−2.545, 2.928) <sup>b</sup>	0.509 <sup>b</sup>	0.957 (−3.986, 5.900) <sup>b</sup>	0.394 <sup>b</sup>	0.731 (−2.425, 3.887) <sup>b</sup>	0.391 <sup>b</sup>
Free oestradiol quintiles (pmol/L)						
<0.9	Referent <sup>b</sup>		Referent <sup>b</sup>		Referent <sup>b</sup>	
0.9–1.1	1.503 (−0.616, 3.622) <sup>b</sup>		3.390 (−0.438, 7.218) <sup>b</sup>		1.685 (−0.761, 4.131) <sup>b</sup>	
1.1–1.3	3.367 (1.177, 5.557) <sup>b,*</sup>		7.047 (3.091, 11.002) <sup>b,*</sup>		3.957 (1.432, 6.481) <sup>b,*</sup>	
1.3–1.6	3.074 (0.787, 5.362) <sup>b,*</sup>		6.714 (2.582, 10.846) <sup>b,*</sup>		3.764 (1.128, 6.400) <sup>b,*</sup>	
≥1.6	5.659 (3.153, 8.165) <sup>b,*</sup>	<0.001 <sup>b</sup>	11.189 (6.662, 15.715) <sup>b,*</sup>	<0.001 <sup>b</sup>	6.563 (3.678, 9.447) <sup>b,*</sup>	<0.001 <sup>b</sup>
SHBG quintiles (nmol/L)						
<27	Referent <sup>b</sup>		Referent <sup>b</sup>		Referent <sup>b</sup>	
27–35	1.715 (−0.369, 3.799) <sup>b</sup>		3.296 (−0.468, 7.060) <sup>b</sup>		1.657 (−0.748, 4.062) <sup>b</sup>	
35–43	1.084 (−1.079, 3.246) <sup>b</sup>		3.240 (−0.666, 7.146) <sup>b</sup>		1.563 (−0.931, 4.058) <sup>b</sup>	
43–56	−0.040 (−2.269, 2.189) <sup>b</sup>		0.355 (−3.671, 4.381) <sup>b</sup>		0.386 (−2.185, 2.958) <sup>b</sup>	
≥56	−2.145 (−4.510, 0.220) <sup>b</sup>	0.020 <sup>b</sup>	−2.397 (−6.669, 1.875) <sup>b</sup>	0.091 <sup>b</sup>	−1.631 (−4.352, 1.091) <sup>b</sup>	0.124 <sup>b</sup>

\* $p < 0.05$ <sup>a</sup> Multivariable model including free T, free E<sub>2</sub> and SHBG as continuous variables; age, centre, height, weight, PASE score and current smoking<sup>b</sup> Multivariable model including free T, free E<sub>2</sub> and SHBG categorised into quintiles; age, centre, height, weight, PASE score and current smoking

trabecular bone which may be more sensitive to variation in sex hormone levels than cortical bone. The results may therefore be difficult to extrapolate to other skeletal sites. Unlike DXA there are no published methods for cross calibrating between QUS scanners, and the results reported are the data obtained at each centre (though we included an adjustment for centre in the analysis). Any errors related to measurement are, however, likely to be non-directional and would tend, if anything, to reduce the risk of finding significant biological associations. The study was based on assessment of middle-aged and elderly European men, and extrapolation beyond this group should be undertaken with caution. In addition, our findings are based on QUS of the calcaneus as a measure of bone architecture rather than DXA. Prospective studies have, however, confirmed the value of QUS for predicting fracture risk in both sexes. In ageing men, QUS measurements predict the risk of hip and

any non-spine fracture and almost as well as hip BMD<sub>a</sub> measurements [40].

In conclusion, higher free and total E<sub>2</sub> are associated with bone health not only among the elderly but also middle-aged European men. The overall effect on male bone health of these hormonal variations, however, is modest.

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