Osteoporosis: risk factors and diagnostic approach

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Chapter 2

The association of sex hormone levels with quantitative ultrasound, bone mineral density, bone turnover and osteoporotic fractures in older men and women.

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ABSTRACT

Objective Sex steroids play an important role in the maintenance of bone health. Association studies on sex steroids and fractures are not consistent. Our objective was to examine whether serum oestradiol (E2) and testosterone (T) are associated with quantitative ultrasound (QUS), bone mineral density (BMD), bone turnover markers and fracture incidence.

Design The Longitudinal Ageing Study Amsterdam (LASA), an ongoing cohort study including 623 men and 634 women, aged 65–88 years.

Measurements Serum levels of E2, T, SHBG, albumin, bone turnover markers serum osteocalcin (OC) and urinary deoxypyridinoline (DPD/Cr) were measured. QUS of the heel and BMD of the hip were assessed, and a 6-year fracture follow-up was performed.

Results Men in the lowest quartile (Q1) of bioavailable E2 (bioE2) had higher levels of bone turnover and lower BMD (B= –0.09, P< 0.01) and QUS than men in the highest quartile (Q4). This also applied to Q1 of bioT. Women in Q1 of bioE2 had higher levels of bone turnover and lower BMD (B= –0.07, P< 0.01) and QUS than women in Q4. In men and women, levels of bioE2 below the median were associated with an increased risk of osteoporotic fractures after all adjustments [hazard ratio (HR) 1.53, 95% confidence interval (CI) 1.02–2.29]. In men, univariate analysis revealed that low bioT was associated with an increased fracture risk (HR 1.91, 95% CI 1.03–3.56), but after adjustment for age, this association was no longer significant.

Conclusions Low levels of bioE2 and bioT were found to be associated with high bone turnover, low QUS and BMD and high risk of osteoporotic fractures in both men and women.

INTRODUCTION

Osteoporosis is one of a number of serious diseases facing an ageing population. Age-related bone loss is universal, affecting older men and women in all populations (1). The morbidity of osteoporosis is caused by fractures, leading to pain, decrease in physical and social functioning and loss of quality of life (2). Hence it is important to elucidate the predisposing factors that could be treated.

The causal relationship between postmenopausal osteoporosis and oestradiol (E2) deficiency has been established by densitometric studies showing that the accelerated bone loss induced by ovariectomy could be prevented by oestrogen therapy (3,4). In 1998, Riggs *et al* proposed a unitary model for the

pathophysiology of involutional osteoporosis. This model identified E2 deficiency as the major cause of both the early, accelerated and late, slow phases of bone loss in postmenopausal women, and as a contributing cause of bone loss in elderly men (5). Although the serum levels of both E2 and testosterone (T) tend to decline with age in men (6,7), E2 deficiency appears to be the dominant cause of bone loss, while T deficiency has additional effects (1).

E2 concentrations in postmenopausal women decrease to levels significantly lower than those in men of the same age (8), but as a similar role for E2 in skeletal metabolism in both sexes is indicated (9), a similar association between E2 and bone parameters in men and women might be expected. However, the results of various studies on this are not consistent. Some of the studies investigating the relationship between sex steroids and bone mineral density (BMD) in men have shown a positive associations between T and BMD (10), or between both T and E2 and BMD (11), some have found associations between E2 and BMD but not between T and BMD (12-15) and some found no relationship (16). Studies on sex steroids and osteoporotic fractures in both men and women have also shown different results (17-22).

The studies that investigate the relationship between sex steroids and bone health in both men and women are useful for comparing the effects of E2 and T on bone parameters measured in the same setting. In this study we investigated the relationship between the oestrogen and androgen status and various parameters of bone quality in older men and women, including quantitative ultrasound (QUS), BMD, bone turnover markers, bone loss and osteoporotic fractures, to provide a more complete picture of this relationship.

METHODS

Study population

Data for this study were collected within the Longitudinal Ageing Study Amsterdam (LASA), an ongoing interdisciplinary cohort study on predictors and consequences of changes in physical, cognitive, emotional and social functioning in the ageing population in the Netherlands (23). The sample represents the older Dutch population. The sample and data collection have been described in more detail elsewhere (24). In brief, a random sample of men and women, aged 55 years and over, stratified by age, sex and urbanization grade, was drawn from the population registers of 11 municipalities in three regions of the Netherlands.

The present study included respondents who participated in the medical assessment of the second cycle of LASA (1995–96), were born in or before 1930

(aged 65 and older as of 1 January 1996) and were living in Amsterdam, Zwolle and Oss and surrounding areas.
After undertaking a main interview and a medical interview at home (*n* = 1509), participants were invited to the VU University Medical Centre (VUMC) (respondents living in Amsterdam and surroundings) or a healthcare centre (respondents living in Zwolle and Oss and surroundings), where blood and urine samples were obtained in the morning after a light (calcium-low) breakfast. Blood was put on ice immediately, and processed within 60 min. Blood was centrifuged and stored at –20 º C until measurement in 1998/1999.
Serum levels of sex hormones, SHBG, albumin and bone markers were determined in 1285 persons. QUS measurements of the heel bone were assessed in all these participants. Subsequently, fracture data were collected for 6 years (from 1996 to 2002) in 1248 persons. BMD measurements of the total hip were obtained in a subsample including 493 participants in 1995/1996 and 313 participants in 1998/1999.
The following subjects were excluded from all analyses: one man and 11 women who used systemic oestrogens, one man and one woman who used testosterone, and 14 respondents who used bisphosphonates at baseline, leaving 1257 respondents (623 men and 634 women) to perform the analyses. In addition, 21 respondents who started using bisphosphonates during the follow-up were excluded from the analysis of fractures (leaving 1228 respondents) and bone loss (leaving 304 respondents).
All interviews were conducted by specially trained and intensively supervised interviewers (main interview) and nurses (medical interview), and were tape recorded in order to monitor the quality of the data. Informed consent was obtained from all participants and the study was approved by the Ethical Review Board of the VUMC.

Assessment of hormones
T concentrations were only measured in men by radioimmunoassay (RIA) (Coat-A-Count, DPC, Los Angeles, CA), with an interassay coefficient of variation (CV) of 11% at 2·6 nmol/l; E2 concentrations were measured by RIA (Diasorin Biomedica, Saluggia, Italy), with an interassay CV of 10% at 70 pmol/l. SHBG concentrations were measured by immunoradiometric assay (IMRA) (Orion Diagnostica, Espoo, Finland), with an interassay CV of 6% at 10 nmol/l. The detection limits were 1 nmol/l for T, 18 pmol/l for E2, and 6 nmol/l for SHBG. Levels of bioavailable T (bioT) and E2 (bioE2) were calculated using a law of mass action-based algorithm described previously by Vermeulen *et al.(*25) and Sodergard *et a.* (26) In this

algorithm the following constants were used: 3.14×10E8 for the binding of E2 to SHBG; 1.0×10E9 for the binding of T to SHBG; 4.21×10E4 for the binding of E2 to albumin; and 4.06×10E4 for the binding of T to albumin.

Assessment of bone markers

Serum levels of bone formation marker intact osteocalcin (OC) were measured using an IMRA (Biosource/Medgenix Diagnostics, Fleurus, Belgium). Urinary excretions of bone resorption marker deoxypyridinoline (DPD) were measured by competitive immunoassay (ACS 180, Bayer Diagnostics, Mijdrecht, the Netherlands). Values were corrected for creatinine (Cr) concentration in the same urine sample. Serum Cr level was measured using the Jaffe alkaline picrate reaction with a Hitachi 747 analyser, and was included as a marker for renal function.

Assessment of ultrasound and BMD measurements

QUS measurements were measured at the calcaneus with the CUBA Clinical instrument (McCue Ultrasonics, Winchester, UK). The ultrasound system consisted of two transducers (emitting and receiving) faced with silicone rubber coupling pads. These were placed in direct contact on either side of the heel using a coupling gel. Broadband ultrasound attenuation (BUA, B/MHz) and speed of sound (SOS, m/s) were measured twice in both the right and left calcaneus. The feet were repositioned after the first measurement. Mean BUA and SOS were calculated from these four measurements. The CV, calculated in 20 healthy volunteers measured on five occasions consecutively within 1 h, was 3·4% for BUA and 1·3% for SOS (27,28). Dual X-ray absorptiometry (DXA) scans were obtained at the Department of Nuclear Medicine, using a Hologic QDR 2000 scanner (Hologic Inc., Waltham, MA). The right hip was scanned. BMD of the femoral neck and total hip was measured in g/cm^2.

Follow-up of fractures

Data on fractures that occurred between the second (1995/1996) and the third examination (1998/1999) were prospectively collected with a calendar. Respondents were asked to record falls and fractures and to mail the calendar to the institute every 3 months for 3 years. They were contacted by telephone if they were not able to complete the calendar, if no calendar was returned even after a reminder, or if the calendar was completed incorrectly. Proxies were contacted if participants were not able to respond. During the medical interviews and telephone interviews in 1998/1999 and 2001/2002, participants were asked

whether they had suffered a fracture in the past 3 years. If a participant died, their general practitioner or caregiver was contacted to supply information on whether a fracture had occurred since the last interview contact. The fracture was considered osteoporotic if it had occurred from a standing or sitting position; all accidental fractures, fractures of hands, fingers, feet, toes and skull were excluded. All reported osteoporotic fractures were used in the analyses, 90% of these were confirmed following verification with the general practitioner or the hospital.

Confounders

Potential confounders included sex, age, body mass index (BMI), use of oral corticosteroids, alcohol use, current smoking (yes/no), self-reported chronic diseases such as asthma or chronic obstructive pulmonary disease (COPD), osteoarthritis, rheumatoid arthritis or other joint disease, stroke and diabetes (29). BMI was calculated as weight (kg)/height squared (m^2). Body weight was measured without clothes and shoes using a calibrated balance beam scale. Height was measured using a stadiometer. Alcohol use, chronic diseases and use of oral corticosteroids were assessed by self-report during the main interview.

Statistical analysis

We used *t*-tests, Mann–Whitney tests and χ^2-tests to assess significant differences (P <0.05) in baseline variables in men (n = 623) and women (n = 634). Participants in the lowest quartile (Q1) of sex hormone levels were compared with those in the highest quartile (Q4, reference group), using Student's *t*-tests for continuous variables and the Mann–Whitney test for skewed continuous variables. The adjusted association between sex hormones and QUS measurements, BMD and bone markers was examined using multiple regression analysis. Linearity was examined with dummies by dividing sex hormone levels into sex-specific quartiles. These analyses were stratified by sex. The relationship between sex hormones and osteoporotic fractures was examined using a Cox proportional hazards regression model. The duration of follow-up was recorded for each participant from the date of blood sampling to the date of the first fracture, the date of death or the date of the last follow-up. There were 109 osteoporotic fractures (44 in men and 65 in women) reported in 6 years of follow-up from 1995/1996 to 2001/2002. To increase the power, the analyses on bioE2 and fractures were performed in men and women together, after arranging the respondents in sex-specific quartiles of bioE2. Hazard ratios (HRs) were reported with 95% confidence

intervals (CIs). Unadjusted analyses were performed first, and then potential confounders were added to the models.

RESULTS

The study sample (*n* = 1257) comprised 623 men and 634 women. Table 1 shows the baseline data and the serum concentration of sex hormones in the whole cohort and in a subsample of respondents in whom BMD measurements were made.

Table 2 shows differences between the lowest and highest quartiles of levels of bioT and bioE2 for age, BMI, sex hormones and the outcome measures in men and women. Men in Q1 of bioT scored significantly lower on both QUS measurements and higher on markers of bone formation (OC) and bone resorption (DPD/Cr) when compared with men in Q4 of bioT. Men in Q1 of bioT were also older, had lower levels of E2 and higher levels of SHBG than men in Q4 of bioT, but did not differ with respect to BMI. Men in Q1 of bioE2 had significantly lower BUA but not SOS, significantly lower BMD of total hip, and lower BMI when compared with men in Q4 of bioE2, but did not differ with respect to age.

Women in Q1 of bioE2 scored significantly lower on both QUS measurements as well as BMD of total hip and femoral neck, and higher on both markers of bone turnover when compared with women in Q4 of bioE2. Women in Q1 of bioE2 had a lower BMI than women in Q4 of bioE2, but did not differ with respect to age.

Table 3 shows the associations between bioE2 and bone turnover markers in men and women, and the associations between bioT and bone turnover markers in men. After adjustment, men and women in Q1 of bioE2 and men in Q1 of bioT had higher levels of OC and DPD/Cr than those in Q4.

The associations between bioE2 and BUA, SOS and BMD of the total hip are shown in Table 4. Both men and women in Q1 and Q2 of bioE2 had lower BUA (in women, this also applied for SOS), and lower BMD of the total hip (in women, this also applied for BMD of the femoral neck, data not shown), staying significant after adjustments for all confounders, when compared to men and women in Q4. Men in Q1 of bioT had lower BUA, SOS and BMD of the total hip, also after all adjustments.

In Table 5, the inverse relationship between bioE2 and the 3-year difference in BMD of the femoral neck for women is shown. The same analysis performed for men and women with BMD of total hip, and for men with BMD of femoral neck, was not significant (data not shown).

Table 1. Baseline data and serum concentration of sex hormones in the main group and in the sub-sample with hip BMD.

Parameter	Men		Women	
	Total, N=623	BMD group, N=254	Total, N=634	BMD group, N=239
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Age, years	75.6 (6.6)	75.5 (6.4)	75.5 (6.6)	74.8 (6.3)
BMI, kg/m^2	26.1 (3.4)	26.0 (3.2)	27.6 (4.7)	27.8 (4.7)
Alcohol[a]:				
Non-drinkers, %	21.7	20.5	49.1	36.4
Moderate drinkers, %	69.2	69.3	41.2	49.0
Heavy drinkers, %	9.1	10.2	9.8	14.6
Corticosteroids use, current, %	3.5	2.0	4.4	5.4
Smoking, %	24.6	23.2	11.7	16.7
Asthma/COPD[b], %	16.9	16.9	12.0	13.0
Osteoarthritis/RA/Joint diseases[b], %	33.5	38.2	57.1	57.7
Stroke[b], %	8.5	6.7	6.2	5.9
Diabetes[b], %	6.3	5.9	9.1	8.4
Total T, nmol/l	15.5 (5.0)	15.4 (4.9)	-	-
BioT[d], nmol/l	6.3 (2.0)	6.0 (1.7)	-	-
Total E2, pmol/l	77.3 (25.0)	87.6 (24.3)	29.0 [22.8;36.3][c]	33.0 [26.2;39.8][c]
BioE2[e], pmol/l	55.2 (17.9)	61.3 (17.4)	18.2 [14.0; 24.2][c]	20.3 [15.3;27.3][c]
SHBG, nmol/l	41.2 [30.5;52.7][c]	42.0 [31.1;54.5][c]	48.4 [34.4; 65.3][c]	47.4 [34.1;63.7][c]
Albumin, g/l	42.0 (4.0)	39.0 (2.5)	42.0 (4.0)	38.8 (2.5)

[a] Alcohol: non-drinkers (0 glasses/week), moderate drinkers (1-21 glasses/week for men and 1-14 glasses/week for women), heavy drinkers (>21 glasses/week for men and >14 glasses/week for women).
[b] Self-reported.
[c] Given the skewed distribution, the median with interquartile range is shown.
[d] Calculated according to Vermeulen [25].
[e] Calculated according to Sodergard [26].

Table 2. Differences between lowest and highest quartiles of levels of bioT and bioE2 for age, BMI, sex hormones and outcome measures.

	Men						Women		
Variables	BioT, Q1, 0.1–5.1 nmol/l	BioT, Q4, 7.7–12.9 nmol/l	N=623 P-value[a]	BioE2, Q1, 4.6–43.7 pmol/l	BioE2, Q4, 66.3–140.0 pmol/l	N=623 P-value[a]	BioE2, Q1, 2.9–13.9 pmol/l	BioE2, Q4, 24.2–73.0 pmol/l	N=634 P-value[a]
Age, years	79.2±6.2	72.3±5.5	<0.001	75.9±6.5	75.8±6.2	0.868	75.9±6.3	75.5±6.6	0.597
BMI, kg/m^2	25.9±3.8	26.2±3.1	0.458	24.9±3.7	27.1±3.0	<0.001	25.0±3.8	31.2±4.9	<0.001
Total T, nmol/l	11.3±5.0	19.4±4.3	<0.001	13.5±5.8	16.9±4.6	<0.001			
BioT, nmol/l	3.8±1.3	8.8±1.1	<0.001	5.2±2.3	7.2±1.8	<0.001			
Total E2, pmol/l	69.4±26.6	84.6±25.5	<0.001	50.2±12.3	107.5±19.3	<0.001	19.3 [9.0;22.3]	43.5 [36.7;54.5]	<0.001
BioE2, pmol/l	45.6±17.0	63.8±19.0	<0.001	34.6±8.1	78.5±12.8	<0.001	10.9 [6.1;12.5]	29.7 [27.3;37.4]	<0.001
SHBG, nmol/l	44.6 [34.5;63.5]	37.6 [28.7;47.3]	<0.001	43.4 [32.5;59.8]	38.6 [28.3;48.7]	0.002	69.5 [54.3;85.5]	34.1 [25.0;44.3]	<0.001
OC, nmol/l	2.1 [1.5;2.9]	1.7 [1.3;2.1]	<0.001	2.1 [1.6;2.8]	1.6 [1.2;2.2]	<0.001	2.6 [2.0;3.2]	1.9 [1.3;2.5]	<0.001
Urinary DPD/cr, nmol/mmol	5.2 [4.1;6.9]	4.3 [3.5;5.3]	<0.001	5.2 [4.1;6.7]	4.4 [3.5;5.3]	<0.001	6.1 [4.9;7.9]	5.5 [4.2;6.9]	0.003
BUA (mean), dB/MHz	76.1±20.3	82.9±16.9	0.002	77.6±20.2	85.8±16.6	<0.001	54.3±16.0	69.3±15.7	<0.001
SOS (mean), m/s	1616±47	1655±48	<0.001	1641±55	1638±46	0.676	1601±46	1617±46	0.002
BMD total hip [b], g/cm^2	0.87 [0.78;0.96]	0.90 [0.85;1.01]	0.075	0.86 [0.77;0.92]	0.95 [0.87;1.04]	<0.001	0.71±0.12	0.83±0.12	<0.001
BMD femoral neck [c], g/cm^2	0.70 [0.63;0.78]	0.73 [0.65;0.83]	0.108	0.71±0.13	0.77±0.13	0.104	0.61±0.10	0.70±0.11	<0.001

All differences are presented in mean ± standard deviation, or in median [interquartile range]. Q=quartile.

[a] Differences in mean values between highest and lowest quartile were examined with Student's t-test (mean±SD), or Mann-Whitney (median).

[b] The bone loss (BMD difference in total hip from 1996 to 1999) was measured in 156 men and 148 women, the differences between Q1 and Q4 were not significant.

[c] The bone loss (BMD difference in femoral neck from 1996 to 1999) was measured in 156 men and 148 women, with significant difference between Q1 and Q4 of levels of bioE2 in women, these results are presented in Table 5.

Table 3. Results of unadjusted and adjusted multiple regression analyses of levels of bioE2 with OC and Dpd/cr in men and women and of bioT with OC and Dpd/cr in men.

	Men		Women	
Variables	OC (N=623)	Dpd/cr (N=604)	OC (N=634)	Dpd/cr (N=615)
	B	B	B	B
Unadjusted				
BioE2				
Q1	0.5***	0.9***	0.7***	0.7*
Q2	0.3*	0.6*	0.5***	0.6*
Q3	0.2	0.3	0.3*	0.1
Q4	reference	reference	reference	reference
Adjusted [a]				
BioE2				
Q1	0.4**	0.9***	0.6***	0.9**
Q2	0.2	0.7**	0.4**	0.8**
Q3	0.1	0.3	0.3*	0.2
Q4	reference	reference	reference	reference
Unadjusted				
BioT				
Q1	0.7***	1.2***		
Q2	0.2*	0.5		
Q3	0.0	0.2		
Q4	reference	reference		
Adjusted[a]				
BioT				
Q1	0.6***	0.8**		
Q2	0.2	0.4		
Q3	0.0	0.1		
Q4	reference	reference		

* p <0.05; ** p <0.01; *** p <0.001; Q=quartile.

[a] Adjusted for age, BMI, serum creatinine, corticosteroid use, alcohol use, smoking, and chronic diseases.

Table 4. Results of unadjusted and adjusted multiple regression analyses of levels of bioE2 with BUA, SOS and BMD of total hip in men and women and of bioT with BUA, SOS and BMD of total hip in men.

	Men			Women		
Variables	BUA (N=612)	SOS (N=612)	BMD total hip (N=254)	BUA (N=630)	SOS (N=630)	BMD total hip [b] (N=239)
	B	B	B	B	B	B
Unadjusted						
BioE2						
Q1	-7.7***	2.7	-0.13***	-14.8***	-15.7**	-0.13***
Q2	-7.7***	-6.2	-0.08**	-11.7***	-17.6**	-0.06**
Q3	-3.4	-3.6	-0.02	-4.9*	0.1	-0.05*
Q4	reference	reference	reference	reference	reference	reference
Adjusted [a]						
BioE2						
Q1	-6.1**	2.4	-0.09**	-8.8***	-12.0*	-0.07**
Q2	-6.9**	-6.4	0.05	-7.3***	-15.4**	-0.02
Q3	-3.0	-4.7	0.01	-2.5	2.4	-0.03
Q4	reference	reference	reference	reference	reference	reference
Unadjusted						
BioT						
Q1	-6.9**	-38.6***	-0.06*			
Q2	-1.6	-19.4***	-0.04			
Q3	0.2	-14.8**	0.02			
Q4	reference	reference	reference			
Adjusted [a]						
BioT						
Q1	-6.8**	-38.5***	-0.06*			
Q2	-2.0	-19.5***	-0.06			
Q3	-0.3	-14.7**	0.04			
Q4	reference	reference	reference			

* p <0.05; ** p <0.01; *** p <0.001; Q=quartile.

[a] Adjusted for age, BMI, corticosteroid use, alcohol use, smoking, and chronic diseases

[b] Similar results were found for BMD femoral neck (results not shown).

Table 5. Results of unadjusted and adjusted multiple regression analyses of levels of bioE2 with BMD of the femoral neck, and bone loss in femoral neck in women.

Variables	BMD of femoral neck, g/cm^2, N=148		
	BMD in 1996	BMD in 1999	Bone loss from 1996 to 1999
	B	B	B
Unadjusted			
BioE2			
Q1	-0.08***	-0.12***	- 0.03***
Q2	-0.06*	-0.08**	- 0.02*
Q3	-0.05**	-0.05*	- 0.01
Q4	reference	reference	reference
Adjusted[a]			
BioE2			
Q1	-0.03	-0.06*	0.03**
Q2	-0.04	-0.05*	0.02*
Q3	-0.04	-0.05	0.01
Q4	reference	reference	reference

p <0.05; ** p <0.01; *** p <0.001; Q=quartile.
[a] Adjusted for age, BMI, corticosteroid use, alcohol use, smoking, and chronic diseases.

Fig.1. Risk of osteoporotic fractures in men and women after dichotomization of respondents according to the level of bioE2.

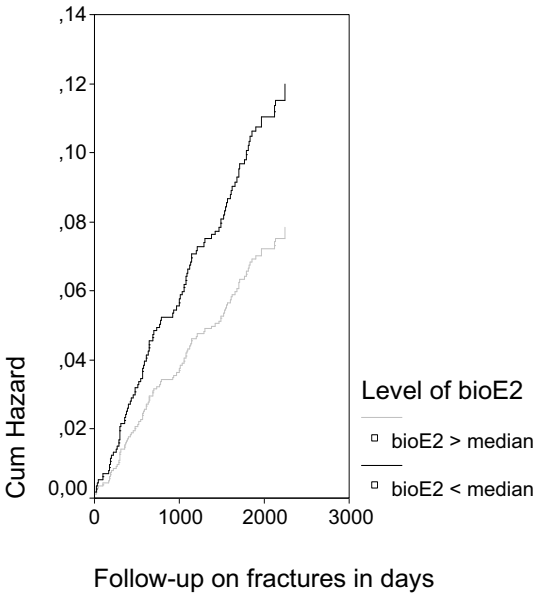

Table 6. Results of unadjusted and adjusted Cox regression analyses of levels of bioE2 with osteoporotic fractures in men and women, analyzed together after sex-specific dichotomization, and of levels of bioT with osteoporotic fractures in men.

Variables	Men and Women, Osteoporotic fractures N=109[a]	Men only, Osteoporotic fractures N=44
	BioE2, Quartiles pooled	BioT, Quartiles pooled
	HR	HR
Unadjusted		
Q1+Q2	1.56 (1.06-2.28)*	1.92 (1.04-3.55)*
Q3+Q4	reference	reference
Adjusted[b]		
Q1+Q2	1.52 (1.02-2.27)*	1.91 (1.03-3.56)*
Q3+Q4	reference	reference
Adjusted[c]		
Q1+Q2	1.53 (1.02-2.29)*	1.54 (0.81-2.93)
Q3+Q4	reference	reference

Q=quartile. * p<0.05

[a] When we repeated the same analysis in the subjects with known level of mobility and exercise (1114 respondents with 89 fractures), the association between bioE2 and fractures became stronger, without adjustment for mobility and exercise (HR=2.1; 95% CI 1.35 –3.35) or with it (HR=2.1; 95%CI 1.35-3.37). This was due to a sub-sample effect. In this sub-sample, the association between bioE2 and fractures was also significant, when men and women were analysed separately (for men, HR=2.20; 95%CI 1.08-4.50, for women, HR=2.39; 95%CI 1.28-4.48 after all adjustments).

[b] Adjusted as [c], but without adjustment for age.

[c] Adjusted for sex, age, BMI, corticosteroids use, alcohol use, current smoking and chronic diseases.

In men and women together, levels of bioE2 below the median were associated with an increased risk of osteoporotic fractures (HR 1.53, 95% CI 1.02–2.29) (Fig. 1). In men, low bioT was associated with an increased fracture risk in univariate analysis, and this stayed significant after adjustment for almost all confounders. However, after adjustment for age, this association was no longer significant (Table 6).

Men who suffered a fracture (*n* = 44) were significantly older than those without a fracture (*n*= 572) (77.9 and 75.3 years old, respectively), but did not differ in BMI, proportion on corticosteroids, alcohol use, smoking, or one of a number of chronic diseases.

Women who suffered a fracture (*n*= 65) did not differ in age, BMI, proportion on corticosteroids, alcohol use, smoking, or one of a number of chronic diseases as compared with women without a fracture.
All of the analyses were also performed with total serum levels of T and E2. These results were similar but are not shown because the associations of bioT and bioE2 with all bone parameters were stronger.

DISCUSSION

In this study, we found that low endogenous serum levels of bioE2 and bioT were associated with low QUS measurements, low total hip BMD, high levels of bone turnover markers, and high risk of osteoporotic fractures in both men and women.
Our findings that low levels of E2 and T were associated with high bone turnover are consistent with other studies (30-32).
The positive relationship observed in our study between bioE2 and BMD is also found in other studies. Both in men and women, the level of bioE2 has been related to BMD (9,12–15,30,33). We also found an inverse association between bioE2 and bone loss in the femoral neck in women, but the bone loss in the total hip was not significant. In men, we observed a positive relationship between bioT and BMD, and this is also consistent with other studies (9,10,33,34). We found a positive relationship between sex steroids and ultrasound parameters. This is important because low BUA values have been identified as a strong predictor for hip fractures and any fracture (35).
A trend in the direction of a negative association between the serum levels of bioE2 and fractures was observed, when men and women were analysed separately (data not shown). This trend was borderline significant as the incidence of fractures in this healthy population was low. When we performed this analysis on men and women together after sex-specific dichotomization of respondents according to the levels of bioE2, a significant increased risk of osteoporotic fracture was found in men and women with the lower (below the median) levels of serum bioE2. This association remained significant after adjustments for confounding variables. The results of other studies on the association between sex hormones and fractures are not consistent. In the Rancho Bernardo study, low levels of E2 were associated with vertebral fractures in older men but not in women (18). In another study, an association between lower serum E2 (< 15.5 pmol/l) and vertebral fractures was found, independently of BMD (21). In a large study where women who subsequently had hip fractures and women who

subsequently had vertebral fractures were compared with control women from the same cohort (20), the women with undetectable serum E2 concentrations (< 18 pmol/l) had a risk ratio of 2.5 for subsequent hip fracture and subsequent vertebral fracture, as compared with the women with detectable serum E2 concentrations. Some studies, however, have reported no difference in sex steroid levels between postmenopausal women with or without a fracture (19,36). In the men in our study, low bioT was associated with an increased fracture risk in univariate analysis (HR 1.91, 95% CI 1.03–3.56), but after adjustment for age, this association was no longer significant. This might be explained by the small sample size, because the power analysis performed revealed that to obtain statistically significant results on bioT and osteoporotic fractures in men with adjustments for confounders, the sample size had to be 2–2.5 times larger than it was in our study. One study showed a significant association between low serum T and increased fracture risk in men (37). A recent study on E2, T and the risk for hip fractures in elderly men showed that men with a lower E2 level had an increased risk of suffering a hip fracture, while no significant higher risk for men with low T was observed (17). However, the risk for a hip fracture increased in men with both low E2 and T in that study, supporting the role for T in the risk of fracture. Free T has also been associated with prevalent osteoporosis-related fractures (34).

Our findings support the importance of endogenous sex steroids for bone health in older persons. This might have clinical implications. Since the Women's Health Initiative Study, the discussion about risks and benefits of hormone replacement therapy (HRT) for treatment of osteoporosis in women is ongoing (38,39). The International Consensus Group on HRT considers HRT as a first-line option for the primary prevention of osteoporosis-related fractures in postmenopausal women with increased risk, even if asymptomatic (40). The aim of hormone therapy for osteoporosis is to prevent fractures. Our data show that even a small decrease in serum bioE2 (from the highest to the lowest quartile in our study) is associated with a higher risk of osteoporotic fracture. It would be interesting to investigate which dose of oestrogens is sufficient to shift the endogenous serum level of bioE2 from the lowest to the highest quartile in a given population and whether such an intervention could prevent fractures. When the low-dose preparations are used to prevent osteoporosis, bone preservation seemed to be less efficacious compared with traditional doses, but fracture data are not yet available for the low-dose preparations (41). In addition, side-effects of very low doses of oestrogen are uncertain. In men, the use of T or selective oestrogen receptor modulators (SERMs) still needs to be justified.

The strengths of our study lie in its prospective cohort of men and women, in a representative group of the Dutch population, its mixed cross-sectional and longitudinal design, and the combination of bone turnover, bone density and fractures as outcome measures. A limitation is the relatively small number of fractures, which did not allow us to show a significant relationship between bioT and osteoporotic fractures.

In summary, low levels of bioE2 and bioT were associated with low QUS measurements, low BMD of total hip, high level of bone turnover markers and increased risk of osteoporotic fracture in both men and women. Our study shows consistent results on all bone health parameters that we examined, and therefore provides a complete picture of the associations between sex steroids and bone health in an elderly population.

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