Endogenous sex hormones and bone mineral density among community-based postmenopausal women

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Summary: In order to describe the relationships between endogenous sex hormones and bone mineral density in healthy postmenopausal women, we carried out a cross-sectional study of 90 community-based women, all at least one year since their last menstrual period (mean 9.6 ± 4.9 years, range 1–22) and with a serum oestradiol level less than 100 pmol/l. None was currently using hormone replacement therapy. Serum oestradiol, testosterone, sex hormone binding globulin, dehydroepiandrosterone sulphate, and androstenedione were measured using standard techniques. Free oestradiol and testosterone indices were derived as the ratio of total hormone to sex hormone binding globulin, respectively. Total body, spine and hip bone mineral density (g/cm^2) were measured by dual energy X-ray absorptiometry.

Significant positive correlations were found between the free oestradiol and testosterone indices and bone mineral density at all sites. These relationships remained significant for the free oestradiol index after adjustment for age and body mass index. By stepwise multiple regression analysis, the free oestradiol index was an independent predictor of total body, spine and hip bone mineral density, accounting for 4–17% of the variance.

These findings suggest an independent positive relationship between endogenous free oestradiol and total body, spine and hip bone mass even in the late postmenopause.

Introduction

Osteoporotic fractures are a major cause of morbidity and mortality amongst postmenopausal and elderly women. Low bone mass is an important determinant of skeletal fragility and fracture risk.^{1,2} Several lines of evidence point to a pivotal role for sex steroids in the pathogenesis of low bone mass. Ovarian failure following menopause is associated with an accelerated rate of bone loss.^{3,4} Oestrogen replacement therapy prevents postmenopausal bone loss,⁵ increases bone mineral density in established osteoporosis,⁶ and is associated with a reduced fracture risk.^{7–9}

There is considerable inter-individual variation in the rates of postmenopausal bone loss and the responsible factors are not fully understood. It is possible that differences in the levels of endogenous sex hormones may be important. Case-control

studies comparing sex hormone levels among hip and vertebral fracture cases with controls have given conflicting results.^{10–13} However, vertebral bone mineral density has been positively correlated with endogenous sex hormone levels in pre- to early postmenopausal women,^{14–16} and similar findings have been reported at the radius for postmenopausal women.^{15,17,18}

There is a paucity of data relating endogenous sex hormones and bone mineral density in normal community-based postmenopausal women. Only one previous report has examined these relationships for hip bone mineral density,¹⁶ and never, to the best of our knowledge, for total body bone mineral density. In this paper we report the relationships between endogenous sex hormones and total body, spine and hip bone mineral density in a community-based sample of healthy postmenopausal women.

Materials and methods

Subjects

The subjects in this report were recruited between 1989 and 1990 into a pilot validation study compar-

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ing different dietary measurement methods. Community-dwelling women aged between 45 and 65 years, identified from two general practice registers in Cambridge, were recruited by letter. About 20% of those approached agreed to participate ($n = 169$) and they were requested to continue their usual dietary intakes during the study period. These women were unselected as to their health status. Subsequently, 141 of these women agreed to undergo bone mineral density measurement. Of these, 90 were postmenopausal as assessed by absence of menstrual bleeding for at least one year and a serum oestradiol level less than 100 pmol/l. These 90 subjects form the basis of this report. None was currently taking hormone replacement therapy, and all were ambulatory and enjoyed good general health. Subjects provided informed consent and the research protocol was approved by the hospital ethical committee. Weight and height were measured by trained observers and body mass index was calculated as weight in kg/(height in metres).²

Hormone assays

Non-fasting blood for the sex hormone assays was drawn between 0900 and 1500 hours and the serum removed and stored at -70°C until assayed. Total oestradiol was measured by radioimmunoassay using the DPC double antibody kit (Diagnostic Products Corporation, Los Angeles, CA 90045, USA). For analysis, we used measured values though this assay is less accurate in discriminating values below 20 pmol/l. For postmenopausal subjects the intra-assay coefficient of variation was up to 10%. Total testosterone was measured by radioimmunoassay after extraction of serum using diethyl ether. The ^{125}I -labelled testosterone derivative, the antibody and other reagents were provided by Dr M.J. Wheeler, St Thomas' Hospital, London SE1. The intra-assay coefficient of variation was 8–10%. Sex hormone binding globulin (SHBG) was measured by immuno-radiometric assay using the Farnos kit (Pharmacia Ltd, Milton Keynes, UK). The intra- and inter-assay coefficients of variation were less than 5% and 3.8–8.4%, respectively. A free testosterone index was calculated by dividing the total testosterone level by the sex hormone binding globulin level.¹⁹ We derived a free oestradiol index in a similar manner (total oestradiol/SHBG) to provide an estimate of the free biologically active hormone level. Androstenedione was measured by an ether extraction assay followed by standard radioimmunoassay using tritiated androstenedione as tracer. Intra- and inter-assay coefficients of variation were less than 5% and 3.6–9.0%, respectively. Dehydroepiandrosterone sulphate (DHEAS) was measured by a direct radioimmunoassay using iodinated DHEAS

as tracer. Intra- and inter-assay coefficients of variation were 3.0–4.8% and 6.8–8.8%, respectively.

Bone mineral density measurements

Total and regional bone mineral density (BMD) were measured by dual energy X-ray absorptiometry (DEXA) using the Lunar DPX (Lunar Radiation Corp, Madison, WI, USA, software 3.1).²⁰ Bone mineral density results are expressed as areal density in g/cm^2 . Precision for total body bone mineral density (28 paired measurements in normal subjects) was 0.9%. Spine (L2–L4) and hip precision (24 paired measurements in normal subjects) were 1.2% and 2.2–3.7%, respectively; neck 2.5%, greater trochanter 2.2% and Ward's triangle 3.7%.²¹

Statistical analyses

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS-X) using the mainframe computer at the University of Cambridge. Pearson and partial correlation coefficients were computed and two-sided statistical tests were used throughout. Stepwise multiple linear regression analysis was used to determine the independent effects of the individual sex hormones.

Results

The baseline demographic, hormonal and bone density measurements for the 90 postmenopausal subjects are given in Table I. The sex hormone measurements were in the range expected for postmenopausal women.

Table II gives the Pearson correlation coefficients for the baseline study characteristics. Age was negatively correlated with DHEAS ($r = -0.34$, $P < 0.01$) and L2–L4 bone mineral density ($r = -0.23$, $P < 0.05$). Body mass index was positively correlated with total oestradiol and the free oestradiol index ($r = 0.57$, $P < 0.001$ and $r = 0.62$, $P < 0.001$, respectively), total testosterone and the free testosterone index ($r = 0.23$, $P < 0.05$ and $r = 0.51$, $P < 0.001$, respectively) and negatively with SHBG ($r = -0.29$, $P < 0.01$). Body mass index was also positively correlated with bone mineral density at all sites. All the regional bone density measurements as well as total body bone mineral density were highly intercorrelated. As expected the sex hormones were also highly correlated with each other.

Statistically significant positive correlations were seen between total oestradiol and the free oestradiol index and bone mineral density at each of the sites measured (r ranging from 0.26–0.43 and

Table I Distribution of variables in 90 postmenopausal women

	Mean (<i>s.d.</i>)	Range	
Age (years)	58.4 (3.8)	49–65	
Height (cm)	163.8 (66)	147.0–177.0	
Weight (kg)	67.6 (11.8)	47.6–118.8	
BMI (kg/m ²)	25.2 (4.3)	18.0–45.5	
Years postmenopause	9.6 (4.9)	1–22	
Oestradiol (pmol/l)	13.1 (13.7)	1–68	<i>n</i> = 90
Free oestradiol index*	0.46 (0.43)	0.1–2.1	<i>n</i> = 84
Testosterone (nmol/l)	1.68 (0.65)	0.4–3.6	<i>n</i> = 90
Free testosterone index*	0.031 (0.02)	0.006–0.097	<i>n</i> = 84
DHEAS (μmol/l)	1.54 (1.07)	0.22–4.8	<i>n</i> = 84
SHBG (nmol/l)	63.2 (25.8)	28–153	<i>n</i> = 84
Androstenedione (nmol/l)	6.04 (2.2)	1.0–12.5	<i>n</i> = 79
Total body BMD	1.078 (0.09)	0.926–1.322	<i>n</i> = 88
L2–L4 BMD	1.081 (0.17)	0.770–1.643	<i>n</i> = 90
Neck BMD	0.871 (0.11)	0.648–1.224	<i>n</i> = 90
Trochanter BMD	0.769 (0.11)	0.523–1.018	<i>n</i> = 90
Ward's triangle BMD	0.753 (0.14)	0.488–1.222	<i>n</i> = 90

BMI = body mass index; DHEAS = dehydroepiandrosterone sulphate; SHBG = sex hormone binding globulin; BMD = bone mineral density (g/cm²). *Calculated as total hormone/SHBG.¹⁹

0.32–0.49, respectively). The free testosterone index was significantly correlated also with bone mineral density at each site (*r* ranging from 0.23–0.37). Less consistent and weaker correlations were seen for DHEAS and SHBG (inverse), and no correlation was found between androstenedione levels and bone mineral density. The correlation coefficients were no longer significant after controlling for age and body mass index by partial correlation analysis except for the free oestradiol index (total body BMD *r* = 0.21, *P* = 0.058; L2–L4 BMD *r* = 0.25, *P* = 0.025; neck BMD *r* = 0.15, *P* = 0.17; trochanter BMD *r* = 0.21, *P* = 0.055; and ward BMD *r* = 0.22, *P* = 0.045).

To determine the independent effects of the sex hormones, they were entered into a multiple stepwise linear regression model along with age, years postmenopause and body mass index. Total oestradiol, total testosterone and sex hormone binding globulin were subsequently dropped because the free oestradiol and free testosterone indices (which were considered more biologically meaningful) were derived from these variables and also their removal did not alter the regression coefficients of the variables in the final models. It was also possible to drop age, free testosterone index, dehydroepiandrosterone sulphate, and androstenedione without altering the regression coefficients, leaving the free oestradiol index, years postmenopause and body mass index in the final models. The regression coefficients (Beta) and their standard errors are given in Table III. An independent,

though modest effect, was seen for the free oestradiol index on total body, spine (L2–L4), femoral neck and Ward's triangle bone mineral density. For trochanter bone mineral density, this effect just failed to reach statistical significance (*P* = 0.054). The proportion of the variance explained by the free oestradiol index varied from 4 to 17%. The relationship between the free oestradiol index and bone density was partially confounded by body mass index as its regression coefficients were reduced by 10–40% by entering free oestradiol index and body mass index alone into the models. Despite these reductions, however, the free oestradiol index remained statistically significant at each bone site except for the femoral neck (*P* = 0.12).

Discussion

Among community-based women who were on average 9.6 years postmenopause, we have shown a positive relationship between an index of free oestradiol levels and spine, hip and total body bone mineral density. While these women were volunteers who agreed to participate in dietary assessment surveys, they were unselected as regards health status, though all were ambulatory and enjoyed good general health. Although we would advocate some caution in generalizing our results to community dwelling postmenopausal women at large in view of our relatively low recruitment rate, we believe that our findings have implications for

Table II Pearson correlation coefficients

	BMI	Yrs pm	E2	FreeE2	T	FreeT	DHEAS
Age	0.17	0.59 [‡]	-0.09	-0.11	-0.09	-0.19	-0.34 [†]
BMI		0.07	0.57 [‡]	0.62 [‡]	0.23 [*]	0.51 [‡]	0.14
Yrs pm			-0.03	-0.04	-0.06	-0.09	-0.06
E2				0.90 [‡]	0.50 [‡]	0.68 [‡]	0.42 [‡]
Free E2					0.35 [†]	0.82 [‡]	0.39 [†]
T						0.63 [‡]	0.40 [‡]
Free T							0.44 [‡]
DHEAS							
SHBG							
A							
TBody							
L2-L4							
Neck							
Troc.							
Ward							
	SHBG	A	TBody	L2-L4	Neck	Troc	Ward
Age	0.16	-0.18	-0.15	-0.23 [*]	-0.03	-0.02	-0.13
BMI	-0.29 [†]	0.07	0.49 [‡]	0.29 [†]	0.31 [†]	0.44 [‡]	0.33 [†]
Yrs pm	0.06	-0.09	-0.26 [*]	-0.30 [†]	-0.09	-0.03	-0.13
E2	-0.28 [†]	0.41 [‡]	0.43 [‡]	0.30 [†]	0.26 [*]	0.32 [†]	0.37 [‡]
Free E2	-0.57 [‡]	0.33 [†]	0.49 [‡]	0.41 [‡]	0.32 [†]	0.44 [‡]	0.40 [‡]
T	0.11	0.52 [‡]	0.15	0.06	0.12	0.09	0.16
Free T	-0.60 [‡]	0.46 [‡]	0.37 [‡]	0.33 [†]	0.23 [*]	0.35 [‡]	0.29 [†]
DHEAS	-0.20	0.60 [‡]	0.18	0.23 [*]	0.24 [*]	0.18	0.23 [*]
SHBG		-0.04	-0.22 [*]	-0.28 [*]	-0.11	-0.28 [†]	-0.13
A			0.14	0.14	0.14	0.03	0.11
TBody				0.83 [†]	0.77 [‡]	0.80 [‡]	0.80 [‡]
L2-L4					0.70 [‡]	0.74 [‡]	0.73 [‡]
Neck						0.80 [‡]	0.91 [‡]
Troc.							0.78 [‡]

**P* < 0.05; [†]*P* < 0.01; [‡]*P* < 0.001. BMI = body mass index; Yrs pm = years postmenopause; E2 = total oestradiol; Free E2 = free oestradiol index; Free T = free testosterone index; DHEAS = dehydroepiandrosterone sulphate; SHBG = sex hormone binding globulin; A = androstenedione; TBody = total body mineral density; L2-L4 = L2-L4 bone mineral density; Neck = neck bone mineral density; Troc = trochanter bone mineral density; Ward = Ward's triangle bone mineral density. Bone mineral density is given in g/cm².

the mechanisms of bone loss among normal healthy women in the late postmenopause. Studies examining the relationships between endogenous oestrogens and bone mass in postmenopausal women have given conflicting results. A number of studies have reported positive effects of endogenous oestradiol on spinal bone mineral density in pre- to early postmenopausal women,^{14,15} though this finding has not been universal.¹⁶ Both cross-sectional¹⁷ and longitudinal studies,¹⁸ have demonstrated a positive relationship between oestrogen levels and radial bone mineral content. In contrast, a number of small studies among selected older women more than 5 years postmenopause failed to show any oestrogen effect on bone mass.²²⁻²⁴ We believe that our study is the first to

show a positive relationship between endogenous free oestradiol levels and total body and hip bone mineral density among women in the late postmenopause. The biological plausibility of the relationships observed, and their consistency, supports the existence of a genuine relationship. Of interest, recent reports have indicated that hormone replacement therapy can retard bone loss or even increase bone mineral density in elderly women.^{25,26} Although the relationships between the free oestradiol index and bone mass were reduced after adjusting for body mass index, an independent effect was still apparent at all sites except for the femoral neck. This partial confounding may be due to the aromatization of adrenal androgens to oestrone in adipose and skeletal tissue, which

Table III Relationship of variables with bone mineral density by stepwise multiple regression analysis

	Beta (g/cm ²)	SE	P	R ²
1. Total body BMD				
BMI	0.007	0.003	0.009	0.24
Yrs pm	-0.005	0.002	0.009	0.08
Free E2 Index	0.055	0.026	0.04	0.04
2. L2-L4 BMD				
Free E2 Index	0.154	0.041	0.0004	0.17
Yrs pm	-0.009	0.004	0.01	0.08
3. Neck BMD				
Free E2 Index	0.085	0.031	0.007	0.10
4. Troc BMD				
BMI	0.011	0.003	0.0002	0.20
5. Ward BMD				
Free E2 Index	0.135	0.038	0.0007	0.16

BMD = bone mineral density (g/cm²); SE = standard error; BMI = body mass index (kg/m²); Yrs pm = years postmenopausal; Free E2 Index = total oestradiol/sex hormone binding globulin; TBody BMD = total body BMD; Troc BMD = trochanter BMD; Ward BMD = Ward's triangle BMD.

subsequently is converted to oestradiol.²⁷ The observed pan-skeletal effect of endogenous free oestradiol on bone mass is consistent with data showing that oestrogen replacement therapy prevents bone loss at all skeletal sites.²⁸

We found a positive relationship between free rather than total oestradiol levels and bone mineral density. These findings are consistent with the widely held view that it is the unbound hormone which is biologically active. Additional supportive data were reported by Van Hemert *et al.* who found that sex hormone binding globulin was a powerful

predictor of metacarpal bone loss among 746 postmenopausal women, an effect which was attributed to its modulating effect on free hormone levels.²⁹

The limitations of our study in addition to the modest sample size and cross-sectional design should be acknowledged. Only one non-fasting serum sample was used to characterize each woman and measurement errors for the hormone assays were likely relatively large. We used a relatively crude estimate of free oestradiol levels and the oestradiol assay was not ideal for measuring the low levels expected in postmenopausal women. However, these limitations would be expected to reduce the strength of the relationships observed rather than to produce spurious ones.

In summary, our results show a positive relationship between endogenous free oestradiol and total body, spine and hip bone mineral density in postmenopausal women. These relationships were modest, a one standard deviation increase in free oestradiol index being associated with a 6.4–7.6% increase in bone mineral density. Nevertheless, these data suggest that even at low oestradiol values and within the limited range, that endogenous free oestradiol appears to have a positive influence on bone mass even in the late postmenopause.

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