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Original Article

Reproductive, Menstrual and Menopausal Factors: Which Are Associated with Bone Mineral Density in Early Postmenopausal Women?

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Abstract. The associations between a number of reproductive and menopausal factors and bone mineral density (BMD) were studied in a sample of early postmenopausal women. The study included 580 women aged 45–61 years who completed a risk factor questionnaire containing sections on obstetric and menstrual history. BMD measurements were taken at the anteroposterior (AP) spine, greater trochanter, femoral neck, total radius and whole body, along with whole body bone mineral content (BMC). In analyses adjusting for key confounders, number of pregnancies was more strongly associated with increased BMD than number of live births at all sites ($p<0.05$ at femoral neck and total radius), and menstrual years was more strongly associated with increased BMD than years since menopause ($p<0.05$ at all sites). Hysterectomized women had a significantly higher adjusted mean BMD than non-hysterectomized women at all sites (AP spine: 0.999 g/cm^2 vs 0.941 g/cm^2, $p<0.001$), although there were no significant differences in BMD between hysterectomized women who had a bilateral oophorectomy and those whose ovaries were preserved. Negative associations between the duration of hot flushes and BMD were statistically significant ($p<0.05$) at the three non-hip sites. In multiple regression analyses containing all reproductive terms, duration of hormone replacement therapy (HRT) use, menstrual years and hysterectomy status were significantly associated with BMD at all five sites, whilst oral contraceptive use before the age of 23 years was significantly associated with increased BMD at all sites except the total radius. Breastfeeding duration, the duration of oral contraceptive use and premenopausal amenorrhea were found to have no association with BMD. Results for whole body BMC were consistent with those for the five BMD sites, across all the variables considered here. These findings confirm the importance of HRT use and duration of menses as predictors of BMD, whilst the results for hysterectomy status and early oral contraceptive use require further consideration.

Keywords: Bone density; Cross-sectional study; Menopause; Osteoporosis; Postmenopausal; Reproduction

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Introduction

It is well established that an early menopause is a risk factor for low bone mass [1], and that postmenopausal estrogen supplementation can increase bone mass [2]. Premenopausal amenorrhea [3] and the presence of menopausal symptoms [4,5] have also been suggested as risk factors for low bone mineral density (BMD), whereas oral contraceptive use has been linked with increased BMD [6,7]. The evidence regarding these latter factors, however, remains unclear. A hysterectomy is considered to be a risk factor for low BMD because it brings about an early menopause, although the effect of the hysterectomy itself, independent of years since menopause and use of hormone replacement therapy (HRT), is not clearly understood [8,9]. Finally, bone

mineral levels are known to change during pregnancy and lactation, but the long-term effects of these events on postmenopausal BMD are still uncertain [10,11].

The purpose of the analyses reported here was to investigate which reproductive and menopausal factors were associated with BMD in a cross-sectional study of early postmenopausal women. By examining a number of different reproductive variables together, their relative importance can be ascertained, as can the effect of each variable independent of all other relevant terms.

Subjects and Methods

The current study is a cross-sectional epidemiologic study assessing associations between BMD and potential risk factors. The study participants comprised two groups of women. The first group included postmenopausal women participating in the Early Postmenopausal Interventional Cohort (EPIC) study, whilst the second group comprised additional women who were ineligible or unwilling to take part in this study.

The EPIC study is a multicenter clinical trial, carried out in four study centers (Copenhagen, Denmark; Hawaii, USA; Nottingham, UK; and Portland, USA). The trial was established to test the efficacy of the bisphosphonate alendronate in preventing early postmenopausal bone loss over a 6-year period [12]. The subjects used for the present analysis came from the Nottingham center and were recruited between September 1992 and June 1993. Information supplied by the Nottinghamshire Family Health Services Authority was used to select a population-based sample of early postmenopausal women. First, 60 general practices were randomly selected from all those in the Nottingham Health district, and the senior partners from these were asked to give permission for their patients to be contacted. Then a stratified random sample of 150 women aged 45–59 years was selected from each participating practice, with the aim of obtaining approximately equal numbers of postmenopausal women in each of the following age groups: 45–49, 50–54 and 55–59 years. The lists of women we wished to contact were sent to the senior partner of each practice so as to avoid inappropriate contacts. The selected women were then sent information about the trial with an invitation to attend study information sessions, followed by a screening clinic for entry into the EPIC trial.

The inclusion criteria for the trial were that subjects were postmenopausal, in good general health and had a lumbar spine anatomy suitable for spine densitometry. Exclusion criteria included having taken HRT within the previous 3 months, evidence of any disease likely to have affected bone turnover, current use of medication which may affect bone metabolism, a history of allergy to bisphosphonates, gastrointestinal symptoms within the previous year and excessive body weight. Also, no more than 10% of trial subjects were permitted to have a spinal BMD below 0.8 g/cm^2. Full details of the recruitment procedure and exclusion criteria for the EPIC trial have been published elsewhere [13]. Once the trial had commenced the participating women were contacted by telephone to ask if they wished to take part in the present study.

A second group of women was recruited for the current study so as to achieve a final sample of women more typical of those in the general Nottingham population, and also to provide sufficient numbers of women who had experienced menopausal symptoms or taken HRT. This group comprised women who had been ineligible or unwilling to take part in the EPIC trial as well as non-responders. On this occasion, 20 women aged 45–59 years were selected at random from the 150 women originally selected for each practice. Women who were originally premenopausal, already participating in the EPIC trial or were previously excluded by the general practitioners were not re-contacted, whilst the remaining women were sent a letter asking if they wished to participate in the present study. For the present study these women were asked to have a single BMD scan and to complete a questionnaire. The only inclusion/exclusion criterion for this second group was that the subjects had to be postmenopausal. Those women previously excluded for other reasons such as the existence of medical conditions or through the use of HRT were now eligible since these exclusion criteria were predominantly for safety reasons and therefore were not necessary in the current study where no intervention was taking place. All women who were previously excluded from the EPIC trial due to low bone density were now eligible. All women in the study provided written informed consent and the study was approved by the Nottingham City Hospital Ethics Committee.

All subjects completed an interviewer-administered risk factor questionnaire, which contained sections on obstetric and menstrual history. Subjects were asked about pregnancies, breastfeeding, and oral contraceptive use, including the duration of use and the preparation used. Color photographs of various brands of oral contraceptive, which had been developed for a previous study of oral contraceptive use, were used to aid recall of preparations [14]. Menstrual history included age at menarche and menopause, and whether the woman's periods ended naturally, or if hysterectomized whether one or both ovaries were preserved. Any spells of premenopausal amenorrhea lasting for more than 3 months were recorded. Duration of menopausal symptoms was defined by the dates on which symptoms first started and finally stopped. Use of HRT was also ascertained, as were other potential risk factors for osteoporosis, including smoking history and fracture history in female relatives.

BMD was measured using dual-energy X-ray absorptiometry (Hologic 2000, Waltham, MA). Measurements were taken at two sites with a greater component of trabecular bone relative to the other sites (the anteroposterior (AP) spine and greater trochanter) and three sites with a greater component of cortical bone (the

femoral neck, radius/ulna (total radius) and whole body (75% cortical bone)). In addition, bone mineral content (BMC) was measured for the whole body. For subjects in the EPIC trial plus those initially excluded due to low BMD, their baseline scan prior to commencing therapy or exclusion respectively was used in the analyses reported here. For the other subjects a single scan was carried out around the same time that the questionnaire was administered. The coefficients of variation calculated for women in the EPIC trial are those which are used to assess the reproducibility of the local scanner. These subjects had their BMD measured twice within the space of 14 days, and the pairwise coefficients of variation were 1.48% for the AP spine, 1.77% for the femoral neck, 1.36% for the trochanter, 0.98% for the total radius and 0.90% for the whole body. Height and weight were measured at the time of the BMD scan.

Statistical analysis was carried out using the SAS package (SAS Institute, Cary, NC). Linear regression analyses were carried out to investigate associations between the reproductive terms and BMD after adjusting for selected confounders, and to determine which one of two or more related variables (e.g., number of pregnancies and number of live births) should be represented in the multiple regression analysis. The analyses of pregnancies, breastfeeding and oral contraceptive use were adjusted for age and weight, whereas the analyses of the menstrual and menopausal variables were controlled for age, years since menopause, duration of HRT use and whether or not the women had a hysterectomy. Multiple linear regression models were constructed for each of the five BMD sites and whole body BMC, adjusting the effect of each reproductive variable for all the other reproductive terms of interest, with additional adjustment for age, height, weight, duration of smoking, family history of fracture and a two-level factor indicating whether or not the subject was part of the main EPIC trial. The validity of the final regression models was checked in two ways. First, an analysis of residuals was carried out to check model assumptions, and identify any influential observations. Second, each term in the model was removed in turn, in order to check that fitting one term did not noticeably increase the standard errors of other terms in the model. A p-value of less than 0.05 (two-sided) was used to denote statistical significance, although associations reaching borderline significance ($0.05<p<0.10$) were also identified as being of potential interest. Analyses were carried out using all subjects with available data.

Results

Of the 60 general practices selected, 55 (91.7%) gave permission for their patients to be contacted. Of the 7564 women from these practices originally approached, 428 (5.6%) were randomized into the EPIC trial, and of these 349 (81.5%) agreed to participate in the cross-sectional study presented here. Six hundred

Table 1. Summary information for reproductive and menopausal variables (n = 580)

	Median (interquartile range)[a]	No. missing
No. of pregnancies	2 (1,3)	0
No. of live births	2 (1,3)	0
Ever breastfed – No. (%)	382 (65.9)	0
Breastfeeding duration (weeks)[b]	16 (4,44)	12
Ever used oral contraceptives – No. (%)	336 (57.9)	0
Duration of oral contraceptive use (months)[b]	47 (12,108)	11
Age at first oral contraceptive use	27 (23,31)	4
Age at menarche (years)	13 (12,14)	1
Age at menopause (years)	47 (43,50)	5
Years since menopause	6 (3,11)	5
Premenopausal amenorrhea – No. (%)	46 (8.0)	2
Hysterectomy – No. (%)	168 (29.7)	15
Hysterectomy only – No. (%)	95 (16.8)	
Unilateral oophorectomy – No. (%)	38 (6.7)	
Bilateral oophorectomy – No. (%)	35 (6.2)	
Ever used HRT – No. (%)	228 (39.3)	0
Duration of HRT use (months)[b]	21 (6,49)	8
Menopausal symptoms		
Any symptoms – No. (%)	508 (94.2)	41
Duration of longest symptom (months)[b]	48 (24,96)	116
Hot flushes – No. (%)	428 (79.6)	42
Duration of hot flushes (months)[b]	30 (12,60)	49
Night sweats – No. (%)	317 (59.1)	44
Duration of night sweats (months)[b]	24 (12,60)	51

[a] Unless stated otherwise.
[b] Median and interquartile range calculation only includes values greater than 0.

and two women who had not been included in the EPIC study were recontacted of whom 231 (38.4%) were eligible and agreed to take part. Of the remainder 156 (25.9%) were found to be ineligible and 215 (35.7%) either refused or did not respond. This gave a final sample size of 580 women. Of the 231 non-EPIC women, 106 were non-responders in the original recruitment, 59 refused to participate in the trial and 66 were ineligible, of whom 40 were excluded due to the use of HRT, 12 because of low BMD and 14 for a variety of other reasons including use of exclusion drugs (e.g., corticosteroids) and exclusion illnesses (e.g., gastrointestinal symptoms).

Information on the reproductive characteristics of the sample is provided in Table 1. Details of BMD values and more general characteristics of the sample can be found elsewhere [15]. Of the women in the study, 37 (6.4%) reported having had a previous low trauma fracture of the wrist or vertebrae after age 20 years. None of the women had suffered a previous hip fracture. When the two groups of study participants (EPIC trial participants and additional participants) were compared as regards reproductive history, the non-EPIC women were found to be further past the menopause on average (median 7.1 years vs 5.7 years; Mann–Whitney test, p=0.006), were more likely to have used HRT (58.4% vs 27.8%; chi-square test, p<0.001) and were more likely to have had a hysterectomy (42.9% vs 21.1%; chi-square test, p<0.001). There were no significant differences between the groups in terms of number of pregnancies, breastfeeding duration, use of oral contraceptives, duration of menopausal symptoms, age at menarche and history of amenorrhea.

Pregnancies and Live Births

There were statistically significant positive associations between number of pregnancies and BMD at the femoral neck and total radius after adjustment for age and weight, but not at the AP spine, greater trochanter and whole body or for whole body BMC (Table 2). The associations with bone mass were stronger for number of pregnancies than for number of live births at all five BMD sites and for whole body BMC (based on R^2 values). There was a reasonably strong positive association between age at first pregnancy and whole body BMC after adjustment for age, weight and number of pregnancies. Positive associations between this variable and BMD just reached statistical significance at the AP spine and whole body, but were not significant at the other sites. Women who had at least one pregnancy did not have a significantly higher BMD or BMC than never-pregnant women at any site after adjustment for age and weight, although an increased BMD in ever-pregnant women reached borderline significance ($0.05<p<0.10$) at the femoral neck and total radius (data not shown).

Breastfeeding

There were no significant associations between total duration of breastfeeding and BMD or BMC at any site after adjustment for age and weight (with positive associations at three BMD sites and negative associations at the other two and for whole body BMC), nor after further adjustment for the number of pregnancies.

Table 2. Association between BMD/BMC at five sites and number of pregnancies, number of live births and age at first pregnancy: regression analyses

	Estimate	SE	R^2 (%)	p
		No. of pregnancies[a]		
AP spine BMD	0.00281	0.00316	0.12	0.375
Greater trochanter BMD	0.00252	0.00227	0.18	0.267
Femoral neck BMD	0.00624	0.00245	0.87	0.011
Total radius BMD	0.00222	0.00109	0.60	0.041
Whole body BMD	0.00272	0.00202	0.27	0.181
Whole body BMC	0.00527	0.00611	0.08	0.389
		No. of live births[a]		
AP spine BMD	0.00088	0.00391	0.01	0.822
Greater trochanter BMD	0.00077	0.00281	0.01	0.784
Femoral neck BMD	0.00652	0.00302	0.62	0.032
Total radius BMD	0.00221	0.00134	0.39	0.100
Whole body BMD	0.00262	0.00251	0.16	0.297
Whole body BMC	0.00273	0.00755	0.01	0.718
		Age at first pregnancy[b]		
AP spine BMD	0.00304	0.00153	0.66	0.048
Greater trochanter BMD	0.00017	0.00113	0.00	0.877
Femoral neck BMD	0.00046	0.00122	0.02	0.704
Total radius BMD	0.00085	0.00053	0.41	0.114
Whole body BMD	0.00198	0.00099	0.66	0.046
Whole body BMC	0.00837	0.00292	1.03	0.004

[a] Adjusted for age and weight.
[b] Adjusted for age, weight and number of pregnancies.

Table 3. Adjusted[a] mean BMD and BMC values (and standard errors, SE) by age at first use of oral contraceptives

	Age at first contraceptive use (years)			p[b]	p[c]
	15–22 (n = 66)	23–27 (n = 128)	28–44 (n = 138)		
	Mean (SE)	Mean (SE)	Mean (SE)		
AP spine BMD	1.016 (0.018)	0.961 (0.012)	0.953 (0.013)	0.018	0.017
Greater trochanter BMD	0.722 (0.013)	0.656 (0.008)	0.673 (0.009)	<0.001	0.015
Femoral neck BMD	0.810 (0.015)	0.749 (0.009)	0.750 (0.011)	<0.001	0.005
Total radius BMD	0.534 (0.006)	0.525 (0.004)	0.521 (0.005)	0.274	0.127
Whole body BMD	1.083 (0.012)	1.042 (0.008)	1.037 (0.008)	0.005	0.007
Whole body BMC	2.175 (0.036)	2.101 (0.023)	2.095 (0.026)	0.163	0.126

[a] Adjusted for age, weight and duration of oral contraceptive use.
[b] From analysis of variance (2 degrees of freedom).
[c] Test for linear trend.

Oral Contraceptive Use

There were weak positive associations (age- and weight-adjusted) between total duration of oral contraceptive use and BMD which were of borderline significance at the femoral neck (p=0.06), but not significant at the other four BMD sites and for whole body BMC. When age at first use of oral contraceptives was grouped into three categories (so as to obtain a group of women who had first used oral contraceptives at a relatively young age (15–22 years), and two equal-sized comparison groups), the differences between the groups were statistically significant at all sites except the total radius and whole body BMC, and there were strong linear trends across the groups (Table 3). Women who first used oral contraceptives between ages 15 and 22 years had the highest adjusted mean BMD at these sites. Duration of oral contraceptive use prior to 1976 (when the estrogen content of preparations was lowered) was not associated with BMD at any site once age at first use was controlled for, neither was oral contraceptive use after 1976 associated with BMD.

Age at Menarche/Years since Menopause/Years of Menstruation

Age at menarche was negatively associated with BMD (i.e., the earlier the menarche the higher the BMD) after adjusting for current age and years since menopause, with associations reaching borderline significance at the AP spine and greater trochanter (Table 4). There was no association between age at menarche and whole body

Table 4. Association between BMD/BMC at five sites and age at menarche, years since menopause and years of menstruation: regression analyses

	Estimate	SE	R^2 (%)	p
		Age at menarche[a]		
AP spine BMD	−0.00707	0.00357	0.70	0.048
Greater trochanter BMD	−0.00457	0.00264	0.51	0.084
Femoral neck BMD	−0.00371	0.00293	0.27	0.206
Total radius BMD	−0.00019	0.00121	0.00	0.874
Whole body BMD	−0.00239	0.00230	0.18	0.300
Whole body BMC	0.00026	0.00803	0.00	0.974
		Years since menopause[b]		
AP spine BMD	−0.00429	0.00134	1.84	0.001
Greater trochanter BMD	−0.00245	0.00100	1.03	0.015
Femoral neck BMD	−0.00254	0.00111	0.89	0.023
Total radius BMD	−0.00148	0.00046	1.59	0.001
Whole body BMD	−0.00327	0.00086	2.33	<0.001
Whole body BMC	−0.00858	0.00305	1.35	0.005
		Years of menstruation[b]		
AP spine BMD	0.00490	0.00128	2.43	<0.001
Greater trochanter BMD	0.00278	0.00096	1.45	0.004
Femoral neck BMD	0.00266	0.00106	1.07	0.012
Total radius BMD	0.00131	0.00044	1.36	0.003
Whole body BMD	0.00322	0.00082	2.47	<0.001
Whole body BMC	0.00737	0.00293	1.08	0.012

[a] Adjusted for age and years since menopause.
[b] Adjusted for age, duration of HRT use and hysterectomy status.

BMC. After controlling for age, duration of HRT use and hysterectomy status, there were statistically significant associations between BMD and years of menstruation which were stronger (based on R^2 values) than for years since menopause at all sites except the total radius and whole body BMC. Furthermore, there was no association between age at menarche and BMD if years of menstruation was controlled for instead of years since menopause (data not shown).

Premenopausal Amenorrhea

Women who reported episodes of amenorrhea which persisted for at least 3 months did not have a significantly different BMD or whole body BMC when compared with the remainder of the sample, after adjustment for age, weight and years of menstruation.

Hysterectomy Status

After adjustment for age, years since menopause and duration of HRT use, hysterectomized women had a higher mean BMD than non-hysterectomized women, with highly significant differences at all five BMD sites and whole body BMC (Table 5). Hysterectomized women who also had a bilateral oophorectomy did not have a significantly different BMD or BMC compared with those who had one or both ovaries preserved, once HRT use was controlled for (data not shown). In analyses stratified by use/non-use of HRT and by age at menopause (<45/⩾45 years) there were significant differences between hysterectomized and non-hysterectomized women at the AP spine, total radius and whole body in all four subgroups (Table 5). At the femoral neck and greater trochanter, the higher BMD of hysterectomized women was only statistically significant among users of HRT, and in women having a menopause after

Table 5 Mean BMD/BMC of hysterectomised and non-hysterectomized women, with analyses stratified by HRT use and age at menopause: results of regression analyses[a]

	Hysterectomy[b]		Difference (SE)	*p*
	Yes	No		
All women (n=553)				
AP spine BMD	0.999	0.941	0.0578 (0.0157)	<0.001
Greater trochanter BMD	0.697	0.667	0.0305 (0.0118)	0.010
Femoral neck BMD	0.776	0.742	0.0343 (0.0130)	0.009
Total radius BMD	0.534	0.515	0.0193 (0.0053)	<0.001
Whole body BMD	1.073	1.029	0.0450 (0.0101)	<0.001
Whole body BMC	2.189	2.061	0.1285 (0.0359)	<0.001
Non-HRT users (n=332)				
AP spine BMD	0.973	0.926	0.0477 (0.0223)	0.034
Greater trochanter BMD	0.675	0.661	0.0135 (0.0165)	0.415
Femoral neck BMD	0.758	0.738	0.0195 (0.0185)	0.294
Total radius BMD	0.525	0.509	0.0163 (0.0077)	0.035
Whole body BMD	1.056	1.018	0.0383 (0.0143)	0.008
Whole body BMC	2.148	2.037	0.1110 (0.0508)	0.030
HRT users (n=221)				
AP spine BMD	1.027	0.967	0.0604 (0.0220)	0.007
Greater trochanter BMD	0.719	0.673	0.0460 (0.0170)	0.007
Femoral neck BMD	0.794	0.747	0.0475 (0.0182)	0.010
Total radius BMD	0.544	0.523	0.0209 (0.0072)	0.005
Whole body BMD	1.092	1.045	0.0468 (0.0141)	0.001
Whole body BMC	2.233	2.098	0.1349 (0.0509)	0.009
Menopause < 45 years (n=167)				
AP spine BMD	0.985	0.936	0.0493 (0.0244)	0.045
Greater trochanter BMD	0.689	0.670	0.0187 (0.0193)	0.335
Femoral neck BMD	0.765	0.753	0.0122 (0.0212)	0.564
Total radius BMD	0.533	0.515	0.0175 (0.0082)	0.036
Whole body BMD	1.064	1.017	0.0466 (0.0163)	0.005
Whole body BMC	2.145	2.050	0.0949 (0.0569)	0.097
Menopause ⩾ 45 years (n=386)				
AP spine BMD	1.001	0.946	0.0593 (0.0215)	0.006
Greater trochanter BMD	0.704	0.668	0.0364 (0.0157)	0.021
Femoral neck BMD	0.792	0.742	0.0505 (0.0174)	0.004
Total radius BMD	0.534	0.515	0.0188 (0.0073)	0.011
Whole body BMD	1.076	1.033	0.0433 (0.0136)	0.002
Whole body BMC	2.232	2.070	0.1612 (0.0486)	0.001

[a] Adjusted for age, years since menopause and duration of HRT use (except for non-HRT users).
[b] Values are mean BMD.

Table 6. Reproductive and menopausal predictors of BMD: results from multiple linear regression analyses[a] (n = 527)

	Trabecular bone sites				Cortical bone sites					
	AP spine		Greater trochanter		Femoral neck		Total radius		Whole body	
	Estimate[b] (SE)	p	Estimate[b] (SE)	p	Estimate[b] (SE)	p	Estimate[b] (SE)	p	Estimate[b] (SE)	p
Duration of OC use (months × 12)	0.0004 (0.0012)	NS	0.0005 (0.0009)	NS	0.0002 (0.0009)	NS	0.0003 (0.0004)	NS	0.0004 (0.0008)	NS
OC use before age 23 (yes vs no)	0.0504 (0.0198)	0.011	0.0564 (0.0142)	<0.001	0.0618 (0.0152)	<0.001	0.0058 (0.0070)	NS	0.0379 (0.0124)	0.002
Years of menstruation	0.0040 (0.0013)	0.002	0.0029 (0.0009)	0.001	0.0026 (0.0010)	0.009	0.0011 (0.0004)	0.015	0.0030 (0.0008)	<0.001
Hysterectomy status (yes vs no)	0.0492 (0.0151)	0.001	0.0248 (0.0109)	0.023	0.0254 (0.0116)	0.029	0.0170 (0.0053)	0.001	0.0417 (0.0095)	<0.001
Duration of HRT use (months × 12)	0.0097 (0.0024)	<0.001	0.0064 (0.0017)	<0.001	0.0054 (0.0018)	0.003	0.0041 (0.0008)	<0.001	0.0071 (0.0015)	<0.001
Duration of hot flushes[c] (months × 12)	−0.0037 (0.0016)	0.020	0.0001 (0.0011)	NS	−0.0023 (0.0012)	0.065	−0.0015 (0.0006)	0.009	−0.0024 (0.0010)	0.016
Amenorrhea (yes vs no)	0.0008 (0.0208)	NS	0.0120 (0.0150)	NS	−0.0043 (0.0160)	NS	0.0052 (0.0073)	NS	0.0035 (0.0130)	NS
No. of pregnancies	0.0020 (0.0034)	NS	0.0018 (0.0025)	NS	0.0052 (0.0026)	0.048	0.0021 (0.0012)	0.079	0.0022 (0.0021)	NS
Breastfeeding duration (weeks × 10)	−0.0011 (0.0017)	NS	−0.0006 (0.0012)	NS	−0.0007 (0.0013)	NS	−0.0002 (0.0006)	NS	−0.0001 (0.0011)	NS
R^2 (all reproductive terms)	7.8%		6.4%		6.5%		7.9%		10.3%	
R^2 (total including confounders)	23.7%		27.8%		33.6%		27.4%		31.1%	

OC, oral contraceptive.
NS: P>0.10.
[a] Each variable is adjusted for all the other variables in the table and age, height, weight, family history of fracture, study factor (EPIC vs non-EPIC) and smoking duration.
[b] The parameter estimate indicates the estimated change in BMD per unit increase in the explanatory variable. For dichotomous variables this is an estimate of the difference in BMD between those with and without the particular risk factor.
[c] Values which were previously missing for this variable were imputed using Buck's method [16].

age 45 years, whereas for whole body BMC the association was not significant among women who had a menopause before the age of 45 years. Hysterectomized women given HRT had higher BMD at all sites than those not given HRT. When women were grouped according to whether their hysterectomy was due to fibroids, heavy or irregular periods or for some other reason, a further subgroup analysis found that BMD was raised for all three groups compared with non-hysterectomized women.

Menopausal Symptoms

The total number of menopausal symptoms reported and duration of night sweats were not associated with BMD or BMC after controlling for age, years since menopause, duration of HRT use and hysterectomy status. The duration of hot flushes was, however, negatively associated with bone mass, with associations reaching statistical significance ($p<0.05$) for whole body BMC and for three BMD sites, the exceptions being the femoral neck ($p = 0.06$) and the greater trochanter ($p = 0.65$). There were also negative associations between the duration of the menopausal symptom which persisted longest and BMD/BMC; these were statistically significant ($p<0.05$) for the total radius and whole body BMD and for whole body BMC, of borderline significance ($0.05<p<0.10$) for BMD at the AP spine and greater trochanter, but non-significant for femoral neck BMD ($p = 0.21$). These negative associations were observed both in users of HRT and in women who had never used HRT.

Multiple Regression Analyses

Coefficients and significance levels of all the terms selected for the multiple regression analyses are presented in Table 6. The results for whole body BMC (not presented) were similar to those for whole body BMD in all cases. Years of menstruation, hysterectomy status and duration of HRT use were found to have strong independent associations with BMD at all sites. For years of menstruation and duration of HRT use the associations with BMD are reasonably linear (Figs. 1, 2). Oral contraceptive use before the age of 23 years was also found to be significantly associated with higher BMD at four sites, although there was no association at the total radius. Of the remaining variables, the duration of hot flushes remained significantly associated with BMD at the AP spine, total radius and whole body after multivariate adjustment. The associations between the number of pregnancies and BMD were reduced, but were of borderline significance at the femoral neck and the total radius. Breastfeeding duration, the duration of oral contraceptive use and a history of amenorrhea were not associated with BMD at any site. The reproductive

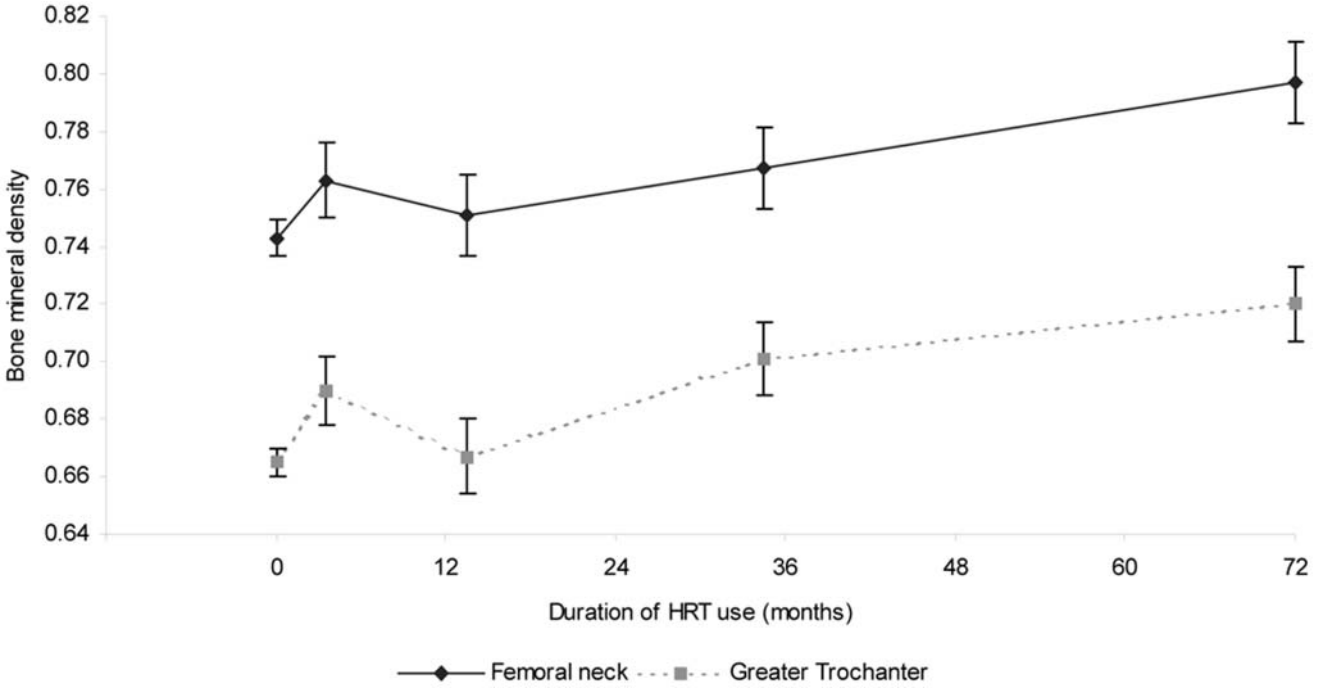

Fig. 1. Adjusted mean BMD (± 1 SE) by duration of HRT use (hip sites). The first level (0) represents the group of women who had never used HRT. The remaining women were categorized into quartiles on the basis of their duration of use and the adjusted mean BMD was plotted against the midpoint of each group. The mean BMD was adjusted for age, height, weight, family history of fracture, study factor (EPIC vs non-EPIC), smoking duration, number of pregnancies, breastfeeding duration, duration of oral contraceptive use, oral contraceptive use before age 23 years, amenorrhea >3 months, hysterectomy status, years of menstruation and duration of menopausal symptoms.

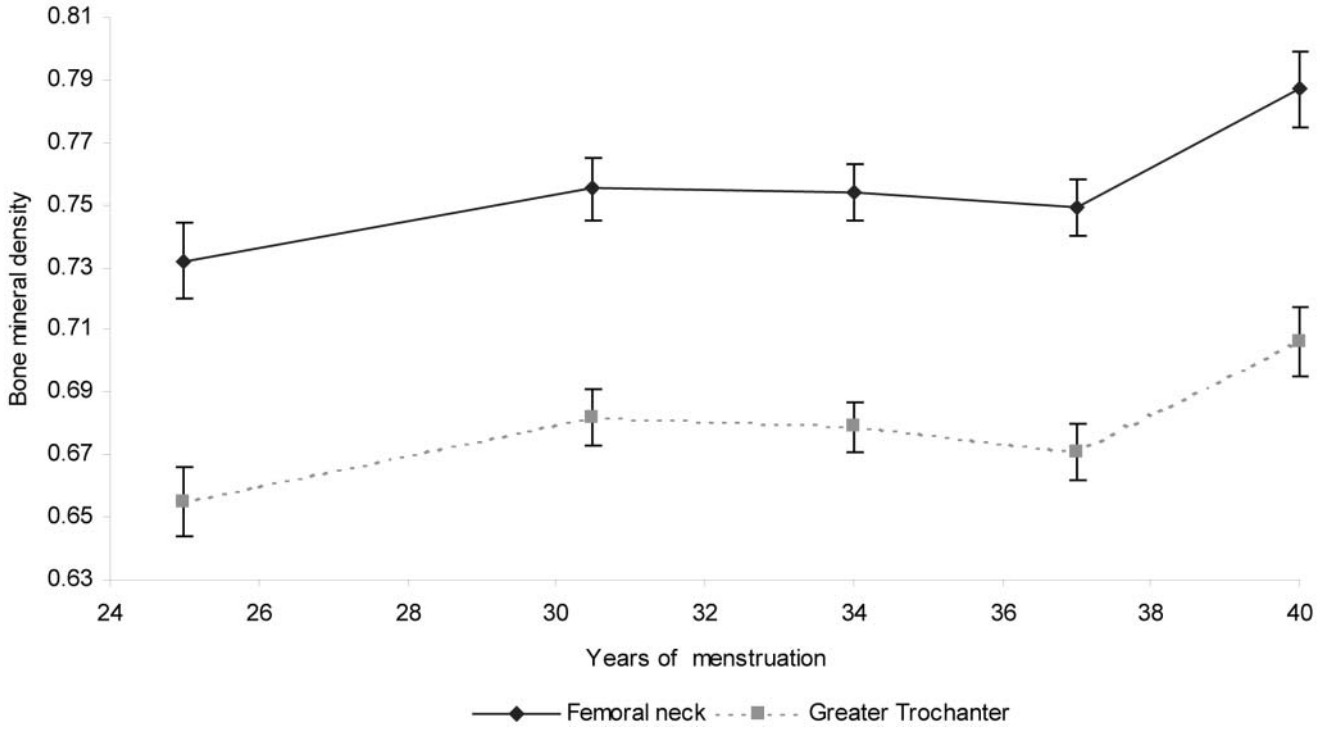

Fig. 2. Adjusted mean BMD (± 1 SE) by years of menstruation (hip sites). Women were categorized into quintiles based on the number of years of menstruation and the adjusted mean BMD was plotted against the midpoint of each group. The mean BMD was adjusted for age, height, weight, family history of fracture, study factor (EPIC vs non-EPIC), smoking duration, number of pregnancies, breastfeeding duration, duration of oral contraceptive use, oral contraceptive use before age 23 years, amenorrhea >3 months, hysterectomy status, duration of HRT use and duration of menopausal symptoms.

variables examined here explained between 6% and 10% of the total variation in BMD (Table 6), and 6% of the total variation in whole body BMC.

Discussion

This study has confirmed the importance of HRT use and years of menstruation as predictors of BMD. In addition, we found that a previous hysterectomy and early use of oral contraceptives were strongly associated with increased BMD. Smaller, but statistically significant inverse associations were found between the duration of hot flushes around the time of the menopause and BMD. There was some evidence of a small positive effect of number of pregnancies on BMD but no evidence of an association with breastfeeding in these early postmenopausal women. This study also investigated the effect of these variables on whole body BMC, and found results to be consistent with those for BMD across all the reproductive and menopausal terms considered here.

While the protective effects of HRT use are well known [2], our study was important in that it confirms previous studies which have found that the number of menstrual years is a predictor of increased BMD [17–19]. In particular, our results support those of Kritz-Silverstein and Barrett-Connor [18], who found that the number of reproductive years accounted for a greater proportion of the total variation in BMD at every site studied than did age at menopause (equivalent to our findings for years since menopause). Our results suggest that the effects of age at menarche and age at menopause may be adequately summarized by a single measure of menstrual years.

We found some evidence that the duration of hot flushes around the time of the menopause was associated with reduced BMD, although the duration of night sweats did not appear to influence BMD. Two other studies have also suggested that menopausal symptoms may be a marker for low BMD [4,5]. One of these [4] found this association to exist only amongst women who were not taking HRT; our study, in contrast, found that the relationship between menopausal symptoms and BMD was not dependent on use of HRT.

A number of short-term prospective studies have monitored changes in BMD during pregnancy and lactation. Two recent reviews conclude that whilst bone loss occurs during lactation, and to a lesser extent during pregnancy, this loss of bone is restored shortly after the return to normal menses [10,11]. This is likely to explain why retrospective studies have failed to find a consistent effect of pregnancies (or live births) and lactation on future BMD [17,20–22]. Our results do provide very limited support for a possible positive link between number of pregnancies and BMD, in that there were borderline associations at two sites of cortical bone

(femoral neck and total radius). This might be explained by the observation that parathyroid hormone (PTH) levels decline during pregnancy, and this decline may be protective in that excess levels of PTH in primary hyperparathyroidism have been linked with loss of cortical bone [23].

The evidence as to whether oral contraceptive use is associated with increased BMD remains inconclusive [6,7,24]. Our analysis did not find any association between the duration of oral contraceptive use and BMD, although women who used oral contraceptives at a relatively young age had a much higher BMD than the remainder of the sample. The magnitude of this result is surprising but is unlikely to be due to selection bias, as the participants did not know their BMD at the time of recruitment into the study. The rationale for this could be that oral contraceptives expose women to higher doses of hormones than those produced endogenously early in reproductive life, when menstruation is irregular. Another explanation is that there may be other characteristics associated with early pill use which cause increases in bone mass, but which were not taken into account here. To our knowledge no other study has specifically looked at the relationship between age at first use of oral contraceptives and BMD; however, Recker et al. [25] did find a greater gain in bone mass in users of oral contraceptives compared with non-users among a sample of women aged 18–26 years. Our finding could not be explained by the fact that the early contraceptive users were users of this medication before 1976, after which there was a move towards lower doses of both estrogen and progestogen in oral contraceptive pills.

The present study found that hysterectomized women had a higher BMD than non-hysterectomized women, irrespective of whether their ovaries had been removed or conserved, and this association was independent of HRT use. Two previous studies have also found BMD to be higher in hysterectomized women [8,22]. In contrast, two other studies found that a hysterectomy was associated with reduced BMD [9,26], whilst a further two found no association between these variables [27,28]. The reason for our finding as regards hysterectomy status is unclear. One explanation may be due to the timing of HRT use, in that if hysterectomized women took HRT immediately after a surgical menopause there would be continuous exposure to estrogen at a time of rapid early postmenopausal bone loss which would be expected to be beneficial. This would not explain why the association is also present in women who have never used HRT. Our results also indicate that the association was not affected by the reason for having the hysterectomy.

A limitation of the present study is that it involved the recall of past behavior and events. Recalling periods of amenorrhea may be particularly difficult, as may the duration of oral contraceptive use much of which took place more than 20 years ago. The large number of missing values for the menopausal symptoms data suggests that this information may also have been inaccurately recalled. Misclassification or poor recall would tend to underestimate any true effect of the reproductive measures on BMD. As only 12 women knew their BMD at the time of interview, recall of past reproductive behavior would not have been biased by knowledge of a low BMD.

A further issue of importance is that this study assessed the impact of the reproductive characteristics on BMD at five different skeletal sites as well as BMC for the whole body. The disadvantage of using more than one outcome is that it raises the problem of multiple significance testing. Rather than using multiple comparison procedures which require the different outcome measures to be non-correlated, our interpretation of results took account of the findings at all five sites, whilst the different reproductive factors chosen for investigation were all derived from pre-planned hypotheses.

Despite the extensive literature on reproductive factors and BMD, many issues remain unresolved. In particular, it is uncertain whether nulliparity and primigravidity are risk factors for low BMD after the menopause, or whether oral contraceptive use at a young age has an effect on peak bone mass. The reason why some epidemiologic studies are finding that hysterectomized women have an increased BMD is also unclear and warrants detailed investigation. The role that the presence and duration of menopausal symptoms have in terms of being able to predict BMD and rates of bone loss has to date received little attention, and is another important area for future research.

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References

1. Gallagher JC, Goldgar D, Moy A. Total bone calcium in normal women: effect of age and menopausal status. J Bone Miner Res 1987;2:491–6.
2. Eastell R. Treatment of postmenopausal osteoporosis. N Engl J Med 1998;338:736–46.
3. Drinkwater B, Brummer B, Chesnut C. Menstrual history as a determinant of current bone density in young athletes. JAMA 1990;263:545–8.
4. Naessen T, Persson I, Ljunghall S, Bergstrom R. Women with climacteric symptoms: a target group for prevention of rapid bone loss and osteoporosis. Osteoporos Int 1992;2:225–31.
5. Salamone LM, Gregg E, Wolf RL, et al. Are menopausal symptoms associated with bone density and changes in bone density in premenopausal women? Maturitas 1998;29:179–87.
6. Corson SL. Oral contraceptives for the prevention of osteoporosis. J Reprod Med 1993;38:1015–20.
7. Tuppurainen M, Kroger H, Saarikoski S, Honkanen R, Alhava E. The effect of previous oral contraceptive use on bone mineral density in perimenopausal women. Osteoporos Int 1994;4:93–8.
8. Johansson C, Mellestrom D, Milsom I. Reproductive factors as predictors of bone density and fractures in women at the age of 70. Maturitas 1993;17:39–50.
9. Watson NR, Studd JWW, Garnett T, Savvas M, Milligan P. Bone

loss after hysterectomy with ovarian conservation. Obstet Gynecol 1995;86:72–7.
10. Sowers M. Pregnancy and lactation as risk factors for subsequent bone loss and osteoporosis. J Bone Miner Res 1996;11:1052–60.
11. Eisman J. Relevance of pregnancy and lactation to osteoporosis? (commentary). Lancet 1998;352:104.
12. Hosking D, Chilvers CED, Christiansen C, et al. Prevention of bone loss with alendronate in postmenopausal women under 60 years of age. N Engl J Med 1998;338:485–92.
13. Coupland CAC, Cliffe SJ, Lyons AR, Tolley K, Hosking DJ, Chilvers CED. Costs of recruiting women for an osteoporosis prevention trial. J Epidemiol Biostat 1997;2:179–83.
14. UK National Case-Control Study Group. Oral contraceptive use and breast cancer risk in young women. Lancet 1989;I:973–82.
15. Grainge MJ, Coupland CAC, Cliffe SJ, Chilvers CED, Hosking DJ. Association between a family history of fractures and bone mineral density in early postmenopausal women. Bone 1999;24:507–12.
16. Little RJA, Rubin DB. Statistical analysis with missing data. New York: Wiley, 1987.
17. Fox KM, Magaziner J, Sherwin R, et al. Reproductive correlates of bone mass in older women. J Bone Miner Res 1993;8:901–8.
18. Kritz-Silverstein D, Barrett-Connor E. Early menopause, number of reproductive years and bone mineral density in postmenopausal women. Am J Public Health 1993;83:983–8.
19. Osei-Hyiamin D, Satoshi T, Ueji M, Hideto T, Kano K. Timing of menopause, reproductive years, and bone mineral density: a cross-sectional study of postmenopausal Japanese women. Am J Epidemiol 1998;148:1055–61.
20. Murphy S, Khaw K-T, May H, Compston JE. Parity and bone mineral density in middle-aged women. Osteoporos Int 1994;4:162–6.
21. Kritz-Silverstein D, Barrett-Connor E, Hollenbach KA. Pregnancy and lactation as determinants of bone mineral density in postmenopausal women. Am J Epidemiol 1992;136:1052–9.
22. Tuppurainen M, Kroger H, Saarikoski S, Honkanen R, Alhava E. The effect of gynecological risk factors on lumbar and femoral bone mineral density in peri- and postmenopausal women. Maturitas 1995;21:137–45.
23. Bilezikian JP, Silverberg SJ, Shane E, Parisien M, Dempster DW. Characterisation and evaluation of asymptomatic primary hyperparathyroidism. J Bone Miner Res 1991;6:(Suppl):S85–9.
24. Murphy S, Khaw K-T, Compston JE. Lack of relationship between hip and spine bone mineral density and oral contraceptive use. Eur J Clin Invest 1993;23:108–11.
25. Recker RR, Davies KM, Hinders SM, Heaney RP, Stegman MR, Kimmel DB. Bone gain in young adult women. JAMA 1992;268: 2403–8.
26. Purdie DW, Steel SA, Howey S. Hysterectomy, oophorectomy and BMD in a UK urban population. In: Current research in osteoporosis and bone mineral measurement V. London: British Institute of Radiology, 1998:6.
27. Carranza-Lira S, Murillo-Uribe A, Tejo NM, Santos-Gonzalez J. Changes in symptomatology, hormones, lipids, and bone density after hysterectomy. Int J Fertil Women Med 1997;42:43–7.
28. Larcos G. Hysterectomy with ovarian conservation: effect on bone mineral density. Aust N Z J Obstet Gynecol 1998;38:452–4.

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