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Total duration of breastfeeding is associated with low bone mineral density in Iranian postmenopausal women

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ABSTRACT

Background: Although transient bone loss has been described during and immediately after pregnancy and lactation, the association between breastfeeding and bone mineral density (BMD) after menopause remains controversial.
Objective: To assess the association between breastfeeding and BMD in postmenopausal women in a population-based study.
Materials and Methods: We randomly selected 245 healthy, free-living postmenopausal women, ages 40 to 80, from the Tehran Lipid and Glucose Study. The duration of breastfeeding was recorded. BMD was measured at the lumbar spine and upper femur by dual X-ray absorptiometry (Lunar DPXMD 7164). Multiple-linear regression was used to determine the association between the total duration of breastfeeding and BMD at different sites.
Results: Means of age and years since menopause were 57.7 ± 7 and 9.4 ± 6.8 years, respectively. The mean number of parities was 5.1 ± 2.8. The median duration of breastfeeding was 48 months (25th to 75th percentiles: 24 and 108 months). Eleven percent of the women (n = 27) were osteoporotic in the femoral-neck region and 25.3% (n = 62) were osteoporotic in lumbar-spine sites. Breastfeeding duration was correlated inversely with femoral-neck BMD, Ward's triangle, and L2-L4. After adjusting for age, BMI, years since menopause, number of parities, 25-hydroxy vitamin D, and LnPTH in the multiple linear regression analyses, breastfeeding duration was inversely associated with BMD at the femoral neck (R2 = 0.31; P = 0.02), Ward's triangle (R2 = 0.32; P = 0.004), and L2-L4 (R2 = 0.18; P = 0.01), but not at other sites.
Conclusions: In healthy postmenopausal women, BMDs of the femoral neck, Ward's triangle, and the lumbar spine are inversely associated with the total duration of breastfeeding.

▸ *Implication for health policy/practice/research/medical education:*
It can help policymakers to consider breastfeeding as a potential risk factor for postmenopausal screening program.

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1. Background

Reproductive factors may decrease bone mass and, in turn, increase the risk of osteoporosis in women. Among these factors, pregnancy and breastfeeding have received considerable attention because of the major hormonal changes and the large transfer of calcium from

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the mother to fetus and infant that occurs during these periods, respectively (1). Breastfeeding is of particular interest due to the deleterious effects of hypoestrogenemia coupled with a loss of calcium in breast milk on maternal bone mass. Lactating women lose 250 to 400 mg of calcium daily through breast milk (2, 3). During breastfeeding, an increase in skeletal resorption and probably an increase in renal conservation of calcium occur (3). Contrary to pregnancy, breastfeeding is not associated with intestinal calcium hyperabsorption (4-6). A large number of short-term, prospective, controlled or noncontrolled studies suggest that breastfeeding is associated with a loss in bone mineral density (BMD) (1). It also has been suggested that there is a dose–response relationship between longer periods of breastfeeding and larger losses in BMD. However this reduction in BMD has been shown to be reversible 6-18 months after weaning (1); in other words, the decrease in BMD will be replaced a few months after the mother stops breastfeeding.

Despite the transient bone loss during breastfeeding, the long-term effects of these events on postmenopausal BMD are still a matter of controversy. Several studies have analyzed potential risk factors of osteoporosis; however, the results are somewhat inconsistent. These studies examined a wide range of population characteristics such as dietary and supplementary calcium intake, serum level of vitamin D, and the number of pregnancies (7-14). The findings of these studies also varied greatly, offering contrasting evidence on whether BMD increases or decreases later in life in women who have had multiple pregnancies and breastfed.

2. Objectives

Given the inconsistencies, in this study we examined the possible association between total duration of breastfeeding and BMD in healthy, , postmenopausal women who participated in a large, population-based study in Tehran, Iran, where vitamin D deficiency and low calcium intake are highly prevalent.

3. Materials and Methods

3.1. Design and Subjects

The women studied in this cross-sectional study were selected from participants of the Tehran Lipid and Glucose Study [TLGS; (15)]. The TLGS is a population-based study, conducted to determine the prevalence of noncommunicable diseases among Tehran's urban population and to develop population-based measures to decrease the prevalence or prevent the rising trend of diabetes mellitus and dyslipidemia. A multistage, stratified, cluster random sampling technique was used to select 15,005 people ages 3 years and over from District 13 of Tehran (Latitude: 35° 4'), the capital of Iran. The district is located in the center of Tehran, and the age and sex distributions of its population are representative of the overall population of Tehran (15). The crude response rate was approximately 55.6%, and the respondents and nonrespondents did not differ significantly by age or gender. For this study, we extracted the codes of 245 postmenopausal women and invited them to take part in this survey. Menopause was defined according to the participants' self-reports; 6 months had to have passed since the date of the last menstrual period. Breastfeeding was defined as the months of the total duration of breastfeeding, according to the participants' reports. All the women studied were community dwellers, were between 40 and 80 years of age, were independent in their activities of daily living, and owned their homes , were independently mobile, and did not use any walking aids. Women were excluded if they used diuretics, corticosteroids, anticonvulsions, estrogens, androgens, alcohol consumption, consumption of supplemental vitamin D or calcium in the past 6 months, or history of any hepatic, renal, adrenal, or thyroid disorders or pancreatitis.

The study protocol was approved by the research ethics committee of the Research Institute for Endocrine Sciences at Shahid Beheshti University, Tehran, Iran.

3.2. Clinical and Laboratory Assessments

After receiving informed consent of the subjects, demographic data collection and anthropometric examinations were undertaken by a trained general physician. Weight was recorded using a Seca 707 weighing machine (range: 0.1-150 kg) with an accuracy of up to 100 gr. The machine was checked for precision after every 10 measurements. Height was measured without shoes using a tape stadiometer with a minimum measurement of 1 mm. Body mass index (BMI) was calculated by dividing weight (in kilograms) by height squared (in meters).

Blood samples were drawn between 8:00 AM and 9:00 AM into vacutainer tubes after 12–14 hours of overnight fasting in February and March; all samples were centrifuged within 35–40 min of collection (2,500 rpm, 30 min, and 4°C). Serum levels of 25(OH)D, parathyroid hormone (PTH), calcium, and phosphorus total were measured.

Serum 25(OH)D was measured using a radioimmunoassay (10) method (Gamma BCTDPD, IDS Ltd. Boldon, UK, CV < 8.9%); reference range for serum 25(OH)D was 23 to 113 nmol/L. Serum PTH measurement was done using an enzyme-linked immunosorbent assay (ELISA) method (Human iPTH, IDS Ltd. Boldon, UK, CV < 6.3%); the reference range for PTH was between 0.8 and 3.9 pmol/L. Serum calcium and phosphorus and total alkaline phosphatase levels were measured using colorimetric methods (Pars Azmon Co. Tehran, Iran).

3.3. Densitometry Assessment

BMD, expressed in grams per cm2, was measured by a trained operator at the lumbar spine and total hip with dual X-ray absorptiometry (DXA) using a Lunar DPXMD densitometer (Lunar 7164, GE, Madison, WI) according to the manufacturer's instructions. The instrument was calibrated weekly using appropriate phantoms. Precision error for BMD measurements was 1%–1.5% in the lum-

bar and 2%–3% in the femoral regions. The device normative data of the U.S. population for spine BMD and the Third National Health and Nutrition Examination Survey (NHANES III) for femur BMD were used as reference values. According to the World Health Organization classification system, t scores ≤ -2.5 indicate osteoporosis, and scores between -1 and -2.5 indicate osteopenia.

3.4. Statistical Methods

The Kolmogorov-Smirnov goodness-of-fit test was used to assess the normality of the distribution of the continuous data. All continuous data are expressed as mean ± SD or median and interquartiles 25%–75%, and all categorical variables are expressed as percentages. Logarithmic transformation was performed to normalize the distribution of serum PTH. Bivariate linear correlations were estimated by Pearson's correlation coefficients. Multiple linear regression analyses were performed in a stepwise method to determine the association between BMD as a dependent variable and age (years), BMI (kg/m2), years since menopause (years), LnPTH, 25(OH)D (nmol/L), and duration of breastfeeding as independent variables. Descriptive and multivariate statistics were performed with SPSS software (version 13.0; SPSS Inc. Chicago, Ill, USA). All statistical tests were two sided, and differences with probability values < 0.05 were considered statistically significant.

4. Results

In our sample of 245 postmenopausal women, the mean age and years since menopause were 57.5 ± 7 and 9.4 ± 6.8 years, respectively. Ninety-six participants (39.2%) were overweight and 106 (43.3%) were obese. The median serum vitamin D concentration was 54.4 nmol/l; the median level was below 50 nmol/l and 80 nmol/l in 46.1% and 78% of participants, respectively. Eight women (3.3%) were nulliparous. The median duration of breastfeeding was 48 months (25th to 75th percentile: 24 to 108 months). Sixty-four women (29.2%) were identified as having osteoporosis, 25 (11.2%) in the femoral neck and 55 (25.1%) in the lumbar spine; 99 (45.2%) were osteopenic; and 56 (22.9%) had normal BMD *(see Table 1)*.

There was a significant negative correlation between duration of breastfeeding and femoral-neck osteoporosis (r = -0.2, p = 0.01), Ward's triangle (r = -0.25, p <0.01), and L2-L4 BMD (r = -0.18, p = 0.03) *Table 2* The number of

Table 1. Characteristics of the study population.

Variable	Mean ± SD
General characteristics	
Age, years	57.5 ± 6.9
Body weight, Kg	69.6 ± 12.1
BMI, kg/m^2	29.6 ± 4.9
Years since menopause, years	9.4 ± 6.8
Parity, n	5.1 ± 2.8
Duration of breastfeeding, month	68.3 ± 57.1
Biochemical characteristics	
Serum 25(OH) D, nmol/L	73 ± 62.3
Serum PTH, Pmol/L	27.2 ± 17.1
Serum calcium, mmol/L	2.3 ± 0.2
Serum phosphorus, mmol/L	1.1 ± 0.2
Densitometric characteristics	
Femoral neck, g/cm^2	0.838 ± 0.130
Ward's triangle, g/cm^2	0.684 ± 0. 155
Trochanter, g/cm^2	0.723 ± 0.116
Total hip, g/cm^2	0.900 ± 0.136
Lumbar spine, L2-L4, g/cm^2	1.019 ± 0.163

Table 2. Pearson's correlation coefficients between age, body mass index, years since menopause, duration of breastfeeding, number of births, 25-hydroxy vitamin D (25(OH)D), and LnPTH and densitometric characteristics.

	Body mass index	Years since menopause	Duration of breast-feeding	Number of parities	25(OH)D	LnPTH	Femoral neck BMD	Ward's triangle BMD	L2-L4 BMD
Age	- 0.09	0.68 [a]	0.29 [a]	0.2 [a]	0.02	- 0.09	- 0.3 [a]	- 0.31 [a]	- 0.13
Body Mass Index	-	- 0.01	0.06	0.06	0.08	0.06	0.3 [a]	0.32 [a]	0.22 [a]
Years since menopause	-	-	0.17 [b]	0.1	- 0.01	- 0.08	- 0.24 [a]	- 0.22 [a]	- 0.12
Duration of breast-feeding	-	-	-	0.25 [a]	- 0.05	0.16	- 0.2 [b]	- 0.25 [a]	- 0.18 [b]
Number of parities	-	-	-	-	- 0.09	0.12	- 0.08	- 0.11	- 0.05
25(OH)D	-	-	-	-	-	- 0.25[a]	0.02	0.04	0.02
LnPTH	-	-	-	-	-	-	- 0.07	- 0.06	0.01
Femoral neck BMD	-	-	-	-	-	-	-	0.93[a]	0.72 [a]
Ward's triangle BMD	-	-	-	-	-	-	-	-	0.75 [a]

[a] $P < 0.01$, [b] $P < 0.05$

Table 3. Linear stepwise regression analyses of duration of breastfeeding and densitometric characteristics in 245 postmenopausal women

Variable	Regression coefficient	SE (β)	Standardized regression coefficient	*P* Value
Dependent Variable: BMD total hip				
Age (years)	- 0.006	0.002	-0.319	0.00
Body mass index (kg/m^2)	0.008	0.002	0.293	0.00
Duration of breastfeeding (month)	-0.103			0.21
Years since menopause (year)	0.144			0.19
Number of parities				
LnPTH (Pmol/L)	-0.03			0.6
25(OH) D (nmol/L)	0.003			0.97
R^2 = 0.215	-0.102			0.2
Dependent Variable: BMD femoral neck				
Age (years)	- 0.007	0.001	- 0.393	0.00
Body mass index (kg/m^2)	0.007	0.002	0.252	0.00
Duration of breastfeeding (month)	0.000	0.000	- 0.181	0.02
Years since menopause (year)	- 0.141			0.18
Number of parities	- 0.061			0.43
LnPTH (Pmol/L)	- 0.028			0.71
25 (OH) D (nmol/L)	- 0.036			0.99
R2 = 0.309				
Dependent Variable: BMD trochanter				
Age (years)	-0.16			0.14
Body mass index (kg/m^2)	0.008	0.002	0.347	0.00
Duration of breastfeeding (month)	-0.14			0.07
Years since menopause (year)	-0.005	0.001	-0.295	0.00
Number of parities	-0.08			0.31
LnPTH	-0.01			0.89
25 (OH) D (nmol/L)	-0.079			0.31
R^2 = 0.23				
Dependent Variable: BMD L2-L4				
Age (years)	- 0.071			0.55
Body mass index (kg/m2)	0.007	0.003	0.214	0.01
Duration of breastfeeding (month)	- 0.001	0.000	- 0.230	0.01
Years since menopause (year)	- 0.005	0.002	- 0.231	0.01
Number of parities	- 0.105			0.22
LnPTH	0.121			0.15
25 (OH) D (nmol/L)	- 0.027			0.75
R^2 = 0.175				

parities was not associated with BMD at any site.

In the multiple linear stepwise regression analyses, duration of breastfeeding was independently associated with BMD at the femoral neck (Beta = -0.18, *P* = 0.02), Ward's triangle (Beta = -0.22, *P* = 0.00), and L2-L4 (Beta = -0.23, *P* = 0.01) but not at other sites after adjusting for age, BMI, years since menopause, number of births, 25-hydroxy vitamin D, and LnPTH *(see Table 3)*. Age and BMI were significantly associated with BMD of the femoral sites; all these variables, to some extent, explained 29% of the total variance of BMD of the femoral sites. In the model, which was developed for BMD of the lumbar spine, only BMI and years since menopause were associated with BMD. This model explained 17% of total variance of BMD of the lumbar spine *(see Table 3)*.

5. Discussion

In this cross-sectional population-based study of healthy postmenopausal women living in Tehran, BMD was negatively associated with total duration of breastfeeding after controlling for other relevant confounders. Total duration of breastfeeding was found to be the second- and third-most important predictors of lumbar-spine and femoral-neck BMD, respectively, in a multiple linear stepwise regression analysis model (see Table 3).

The low postmenopausal BMD may be a result of the impaired postweaning BMD recovery. We submit that variables such as vitamin D status, calcium intake, and nutrition, particularly in closely spaced pregnancies accompanying breastfeeding, may influence the recovery of postweaning BMD. The impaired postweaning BMD followed by inadequate calcium and vitamin D intake in subsequent years may result in low BMD in postmenopausal period. We do not have any data regarding vitamin D, calcium intake, and nutrition status of this population in the postweaning period. However, the prevalence of vitamin D deficiency and low calcium intake has been rather high in Iranian women in the 2000s (16-18). In our study, the vitamin D level was below 50 nmol/l and 80 nmol/l in 46% and 78% of women, respectively. Although we did not have data on the calcium intake of the participants in our study, in the TLGS population, women over the age of 40 had a mean calcium intake of approximately 713 + 20 mg/day (17, 18). Moreover, in this population, BMD recovery occurred during the period of the 8-year war in Iran, which was accompanied by a restricted supply of nutrients nationwide. In a single blood sample, about 20 years after the war, vitamin D deficiency and low calcium intake were rampant, explaining the worsening conditions faced in the postweaning periods at the time.

Some previous studies, with findings similar to ours, were conducted at the borderline nutritional setting. Chowdhury et al., for instance, reported a negative association between BMD and total duration of breastfeeding amenorrhea in 400 marginally nourished Bangladeshi women (7). In a study of 1,486 postmenopausal Turkish women, Dursun et al. found a negative association between total duration of breastfeeding and BMD (9). Dursun et al. did not record the intake of calcium or the plasma level of vitamin D, but other studies have indicated that the prevalence of vitamin D deficiency is rather high in Turkish women (19, 20). On the other hand, some studies that were conducted in different settings reported no association between the two (2, 12). In 2,080 postmenopausal women in Germany, Hadji et al. did not find any association between BMD and breastfeeding (12). Moreover, in 456 premenopausal and 713 postmenopausal Japanese women ages 40 to 69, Kojima et al. reported no significant correlations between total breastfeeding period and BMD (21).

Our study was a population-based study of apparently healthy urban postmenopausal women; however, it has some noteworthy limitations. One limitation, as in most related studies, is that our study used a cross-sectional design, which does not allow for causative inferences. The second limitation is that we used a single blood sample to measure serum 25(OH)D, PTH, and other biochemical variables. This method has been used in other similar investigations with similar results. Intra-individual measurements of these variables can have considerable variability as a result of diurnal and episodic fluctuations. The third limitation was the impact of recall bias resulting from relying on the participants' memory of the number of births and duration of breastfeeding up to 20–30 years following their breastfeeding periods. Finally, we were unable to capture data about some relevant variables, such as participants' level of physical activity.

In conclusion, our data showed the possible role of long-term breastfeeding as a risk factor for postmenopausal osteoporosis, which should be taken into consideration by countries with a high prevalence of vitamin D deficiency or low calcium intake.

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Conflict of Interest

None declared.

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