

# Influence of breastfeeding on bone mineral metabolism after menopause

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## Summary

**Objective:** Lifestyle and gynecological history appear to influence bone mineral metabolism. There are conflicting data on the possible effects of breastfeeding on the subsequent development of densitometric osteoporosis or the development of fragility fractures. The objective of this study was to assess these effects.

**Material and methods:** Observational, cross-sectional, open study, carried out in 758 postmenopausal women who were classified into two groups, depending on whether they had breastfed their children or not. Data were collected on lifestyles, gynecological history and fragility fractures. They underwent a general analysis, with renal and hepatic function, lipids, ions, as well as biochemical markers of bone remodeling, parathyroid hormone (PTH) and vitamin D (25HCC). Bone mineral density (BMD) was determined in the lumbar spine and in the proximal extremity of the femur by dual X-ray absorptiometry (DXA). Likewise, a quantitative ultrasound (QUS) measurement was performed on the calcaneus of the dominant foot. The raw data, after being compared by groups, were adjusted by applying the propensity score matching method, making a more precise comparison of the variables studied.

**Results:** The results prior to the application of the propensity score were adjusted for age and body mass index (BMI), since in the baseline study there were significant differences in these variables between both groups (prevalence of hip fractures and kyphosis and in the following biochemical parameters: specifically uric acid, glucose, HDL-cholesterol, triglycerides and phosphorus). These differences disappeared after adjusting for the variables that were included in the model by the applied linear logistic regression.

After adjusting with the propensity score matching and with the finally obtained linear regression model, no influence of breastfeeding was obtained on bone mineral density, on the prevalence of densitometric osteoporosis or on the appearance of fragility fractures after menopause.

**Conclusion:** Breastfeeding is not associated with higher or lower bone mineral density values, the prevalence of densitometric osteoporosis, or the presence of fragility fractures.

**Key words:** breastfeeding, pregnancy, osteoporosis, fragility fractures, propensity score matching, bone density.



## INTRODUCTION

Osteoporosis is defined as a skeletal disease in which there is a decrease in bone strength that leads to an increased risk of fracture, usually due to mild trauma<sup>1</sup>. Although any fracture can be observed in clinical practice, with the exception of the skull bones, the most prevalent is the vertebral one and the most serious that of the proximal extremity of the femur<sup>2</sup>, given its significant morbidity and mortality<sup>3</sup>. Genetic, anthropometric, nutritional and lifestyle factors<sup>4-11</sup> influence the appearance of fragility fractures or osteoporotic fractures, but also gynecological and obstetric factors<sup>12</sup>. Among them, breastfeeding reportedly exerts an essential reproductive function in women and protects the mother from developing many diseases, such as cancer or diabetes<sup>11-14</sup>.

Its effect on bone mineral metabolism is less defined, however, and published results are often contradictory. Some of these studies indicate that prolonged breastfeeding could be associated with an increase in bone mineral density (BMD) and a lower prevalence of osteoporosis in postmenopausal women<sup>12-16</sup>, while others suggest precisely the opposite, that prolonged breastfeeding is a risk factor for the appearance of osteoporosis and fragility fractures<sup>17-21</sup>. Finally, reports have also been published that do not find any effect, neither beneficial nor harmful<sup>22-24</sup>.

Therefore, we have carried out the present study in a population of postmenopausal women to establish whether or not breastfeeding is associated with the subsequent appearance of densitometric osteoporosis and the presence of fragility fractures, with the particularity that the propensity score matching method was used. This provides a more precise comparison of the variables studied in the established groups, making them more homogeneous as we will describe in more detail in this paper.

## MATERIAL AND METHODS

A total of 758 women were included, who were studied in the Bone Metabolic Unit of the Insular University Hospital in the period between 2016-2020. They were informed of the objectives of the study and gave their informed consent. All completed a questionnaire, previously validated and used in other similar clinical studies on osteoporosis<sup>25,26</sup>. They also underwent a basic physical examination that included height and weight measurements to then calculate their body mass index (BMI). Subsequently, they were grouped into women who had breastfed (cases) and women who did not (controls).

### Sample collection and laboratory techniques

Blood and urine samples were collected in the morning, between 8:00 and 9:00 am, after an overnight fast. Blood was collected in the appropriate specific tubes for each determination, with the least possible venous compression, and was centrifuged at 1,500 g for 10 minutes; the serum was separated into aliquots and stored within one hour from the extraction at -20°C until the biochemical analyzes were carried out, although most of them were carried out the same day as the extraction. Glucose, urea, creatinine, calcium, inorganic phosphorus, total proteins, total cholesterol and its fractions and triglycerides were measured using standardized and automated colorimetric techniques on an autoanalyzer (Kodak Ektachem Clinical Chemistry Slides).

Serum calcium was corrected according to total proteins by means of the following formula:

$$\text{Corrected calcium} = \frac{\text{previous calcium (mg/dl)}}{[0.55 + \text{total proteins (g/l)/16}]}$$

### Determination of ultrasound values in the calcaneus

Ultrasonographic parameters were estimated in the calcaneus of the dominant foot using a Sahara® Hologic® ultrasonographer (Bedford, Massachusetts, USA). This device measures both the broadband ultrasound attenuation (BUA) and the speed of sound (Speed of sound, SOS) in the region of interest of the calcaneus. The BUA and SOS values are combined into a single parameter called the quantitative ultrasound index (QUI), also known as the consistency or stiffness index, which is obtained by means of the formula:

$$\text{QUI} = 0.41 (\text{SOS}) + 0.41 (\text{BUA}) - 571$$

The T-score values were calculated from the values published as normal for the Spanish population<sup>27</sup>.

### Bone mineral density (BMD)

BMD was measured by dual radiological absorptiometry (DXA), both in the lumbar spine (L2-L4) and in the proximal extremity of the femur, with a Hologic Discovery® densitometer (Hologic Inc, Waltham, USA), whose accuracy is 0.75-0.16%. The measurements were made by the same operator, so there was no inter-observer variation. The T-score values were calculated from the values published as normal for the Canary Island population<sup>28</sup>.

### Diagnosis of osteoporosis and fragility fractures

Osteoporosis was considered to exist when a T-score equal to or less than -2.5 was obtained in any of the 3 anatomical locations where bone mineral density was determined: lumbar spine L2-L4, femoral neck or total hip.

The existence of a fragility fracture was diagnosed when they occurred without a trauma to justify it or when a maximum fall from the height of the woman in question. The fractures were confirmed by medical reports available in their medical history: emergency services, trauma, rehabilitation, or after analyzing x-rays.

### Ethics

Our study was carried out in accordance with the standards of the Declaration of Helsinki<sup>29</sup> and was approved by the Ethics Committee of the Insular University Hospital. All patients were informed of the objectives of the study and their informed consent was requested.

### Statistic analysis

#### Univariate analysis

Initially, we carried out an analysis of the numerical variables, studying whether or not they followed a normal distribution. Later we carried out a descriptive study. Categorical variables were summarized by percentages, and numerical variables by means and standard deviations if they followed normality, or as median and their interquartile range (percentiles = 25<sup>th</sup> - 75<sup>th</sup>) if they did not. To study the possible associations between categorical variables, the Chi-square test ( $\chi^2$ ) was used and the odds ratio (OR) was used as a measure of association, which was estimated using a 95% confidence interval (95% CI). In those cases in which there were cells with less than 5 cases, Fischer's exact test was applied.

**Table 1. Baseline characteristics of the women studied**

	Breastfeeding		P value
	Yes N = 457	No N = 301	
Age (years)	63.4 ± 11.7	57.3 ± 13.8	<0.001
BMI* (kg/m <sup>2</sup> )	27.8 ± 5.1	26.4 ± 6.1	<0.001
Tobacco use, n (%)			0.787
Yes	71 (15.5)	52 (17.3)	
No	305 (66.7)	199 (66.1)	
Ex-smoker	81 (17.7)	50 (16.6)	
Alcohol use, n (%)			0.582
Yes	205 (45.0)	126 (41.9)	
No	246 (53.9)	173 (57.5)	
Ex-drinker	5 (1.1)	2 (0.7)	
Physical activity, n (%)			0.897
Sedentary	303 (67.2)	205 (68.8)	
Light	123 (27.3)	77 (25.8)	
Moderate	25 (5.5)	16 (5.4)	
Diabetes			0.696
Insulin-dependent	9 (2.0)	6 (2.0)	
No insulin-dependent	53 (11.6)	29 (9.6)	
No diabetes	395 (86.4)	266 (88.4)	
Fractures, n (%)			
All fractures	157 (34.4)	84 (28.1)	0.071
Vertebral	45 (10.3)	26 (9.3)	0.665
Hip	20 (4.6)	4 (1.4)	<b>0.023</b>
Colles	36 (8.2)	20 (7.1)	0.606
Falls	167 (37.0)	93 (31.0)	0.089
Kyphosis	114 (25.5)	48 (16.0)	<b>0.002</b>
Current calcium intake (mg/día)	700 (600-850)	700 (537-850)	0.425

The data are expressed as means ± standard deviations, medians (IQR) and frequencies in number (%); \* BMI: body mass index.

To evaluate the association between a quantitative variable and a categorical variable, the Student's t-test or ANOVA (if there were more than 2 categories) was used for variables with normal distribution, or the non-parametric Mann-Whitney U test for the non-normal ones. In all cases, the significance level was considered at 5% ( $p < 0.05$ ).

#### Propensity score matching

To establish the association between breastfeeding and the presence of fragility fractures more precisely and to eliminate the influence of other variables, a similar non-lactating control (matching) was selected for each case of lactating women. This process was based on the method called propensity score matching, which in our case is defined by the conditional probability that breastfeeding is conditioned by those variables that could act as confounding factors. The propensity score was obtained

for each patient using logistic regression, in which the final variable was breastfeeding. The co-variables included in the model were selected using the complete enumeration algorithm and the Akaike information criteria (Akaike Information Criterion, AIC).

#### Matching

Subsequently, we performed an adjusted 1 to 1 analysis without replacement, based on the propensity score of each patient. The caliper or calibrator chosen was 0.7. After adjustment for the propensity score, the baseline characteristics were compared by McNemar's test for binary variables or with the t-test or Wilcoxon, as appropriate in each case, for continuous variables and paired data. The 13 variables selected by the program to be included in the matching were: age, BMI, falls, use of statins or thiazides, uric acid, total cholesterol, HDL-cholesterol, triglycerides, the presence of kyphosis and densitometric

**Table 2. Bone mineral density values obtained by densitometry (DXA) and ultrasound (QUS), values adjusted for age and BMI and prevalence of densitometric osteoporosis**

	Yes N = 457	No N = 301	P value
Densitometry (DXA)			
L2-L4 (g/cm <sup>2</sup> )	0.828 (0.7 ; 0.942)	0.842 (0.7 ; 0.980)	0.624
T-score	-2.0 (-2.8 ; -0.942)	1.9 (-3.0 ; -0.5)	
Femoral neck (g/cm <sup>2</sup> )	0.655 (0.6 ; 0.738)	0.673 (0.6 ; 0.768)	0.080
T-score	-1.6 (-2.3 ; -0.9)	-1.5 (-2.3 ; -0.6)	
Total hip (g/cm <sup>2</sup> )	0.784 (0.7 ; 0.881)	0.788 (0.7 ; 0.893)	0.923
T-score	-1.2 (-1.3 ; -1.1)	-1.2 (-1.3 ; -1.0)	
Ultrasounds(QUS)			
BUA (dB/mHz)	60.8 (58.9 ; 62.7)	60.9 (58.6 ; 63.2)	0.950
SOS (m/s)	1522 (1519 ; 1525)	1522 (1518 ; 1526)	0.963
QUI	78.1 (76.0 ; 80.1)	77.7 (75.2 ; 80.2)	0.824
Densitometric osteoporosis*, n (%)	205 (44.9%)	134 (44.5%)	0.927

Median (95% CI) adjusted for age and body mass index (BMI); \*: presence of a T-score lower than -2.5 in any of the 3 locations where bone mineral density (DXA) was determined, expressed in number (%).

values in L2-L4, femoral neck and total hip. Furthermore, we established the success of the propensity score adjustment by balancing the adjustment of the covariates in the two groups using the standardized differences. Those differences less than 10% supported the assumption of equilibrium between the two groups. The level of statistical significance was established at 5% (p<0.05). The data were analyzed using the R program, version 3.6.1 (R Development Core Team, 2019).

**RESULTS**

Table 1 of the women included in the study, grouped into women who had breastfed and women who had not. Those who had breastfed were older (63.4 ± 11.7 years versus 57.3 ± 13.8 years, p<0.001) and had a higher BMI, (27.8 ± 5.1 kg/m<sup>2</sup> versus 26.4 ± 6.1 kg/m<sup>2</sup>), were performed after adjusting for these two variables. The prevalence of hip fracture was higher among women who had breastfed significantly, a significance that subsequently disappeared when adjusting for age and BMI.

Table 2 shows the BMD values obtained in the lumbar spine (L2-L4) and in the proximal extremity of the femur with their corresponding T-scores. The ultrasound index values obtained in the calcaneus are also shown, specifically the ultrasound attenuation coefficient (BUA), the speed of sound (SOS) and the consistency index or stiffness (QUI). No statistically significant differences were observed in any of the values obtained between both groups studied. The prevalence of osteoporosis was similar between both groups: 44.9% in women who had breastfed and 44.5% in those who had not, (p=0.927).

Table 3 shows the biochemical values obtained in both groups studied before making the adjustment. Statistically significant differences (p<0.05) are observed in the serum values of uric acid, HDL-cholesterol, trigly-

cerides and phosphorus. All of these differences subsequently disappeared when propensity score matching was carried out.

Table 4 sets out the characteristics of the study patients after matching according to the propensity score of each of them. The variables selected by the program to carry out said matching are shown, which were a total of 13, including all those that had previously shown statistically significant differences in the crude comparisons. As a consequence of this matching, the sample size was substantially reduced to the point that the number of women was finally made up of 254 women in each group. As proof of the correctness of this pairing, it is observed that the standardized differences are less than 10%, which indicates the homogeneity of the variables between both groups.

Table 5 shows the data obtained by applying conditional logistic regression for the presence of fragility fractures. After matching, breastfeeding showed no association with fragility fractures.

**DISCUSSION**

Osteoporosis is a very prevalent disease in which fractures are its only clinical complication<sup>2,30</sup>. Various risk factors have been implicated in the etiopathogenesis of postmenopausal osteoporosis, related to lifestyle<sup>4-7,12</sup>, genetics<sup>8</sup> and even gynecological history<sup>12,14</sup>.

One of the etiopathogenic aspects on which there is no consensus is the effect that breastfeeding, which is carried out at a stage of life in which the woman is obviously younger, may have on the subsequent development of osteoporosis after menopause. Some studies suggest that the “negative calcium balance” that would occur during breastfeeding could generate a subsequent loss of bone mass that would manifest itself after menopause with an increased risk of developing densitometric osteoporosis and/or fragility fractures<sup>17-21</sup>.

**Table 3. Biochemical data of the patients included in the study, classified according to whether they had breastfed or not, adjusted for age and BMI**

	Breastfeeding		P value
	Yes N = 457	No N = 301	
Urea (mg/dL)	34 (28 - 41)	33 (27 - 40)	0.189
Creatinine (mg/dL)	0.8 (0.8 - 0.9)	0.8 (0.8 - 0.9)	0.460
Uric acid (mg/dL)	4.3 (3.7 - 5.2)	4.2 (3.6 - 5)	<b>0.028</b>
Glucose (mg/dL)	96 (89 - 105)	95 (88 - 103)	<b>0.054</b>
Total cholesterol (mg/dL)	213 (186 - 238)	212 (186 - 240)	0.843
HDL-cholesterol (mg/dL)	59 (50 - 68)	61 (51 - 72)	<b>0.040</b>
LDL-cholesterol (mg/dL)	128 (106 - 151)	130 (108 - 158)	0.615
Triglycerides (mg/dL)	110 (82 - 150)	98 (75 - 126)	<b>&lt;0.001</b>
Calcium (mg/dL)	9.9 (9.5 - 10.3)	9.8 (9.4 - 10.2)	0.092
phosphorus (mg/dL)	3.4 (3.1 - 3.8)	3.5 (3.1 - 3.9)	<b>0.029</b>
Total proteins (g/L)	7.1 (6.9 - 7.5)	7.1 (6.9 - 7.4)	0.924
25-hydroxycholecalciferol (ng/mL)	22.1 (16 - 30)	21.9 (16 - 31.1)	0.565
Parathyroid hormone (PTH) (pg/mL)	48 (36 - 75)	46 (35 - 70)	0.609
FATR* (UI/L)	82 (63 - 104)	79 (65 - 98)	0.694
Beta-crosslaps (ng/mL)	0.4 (0.2 - 0.61)	0.4 (0.2 - 0.61)	0.807
Osteocalcin (ng/mL)	20 (13 - 31)	19 (12 - 29)	0.319
Type I procollagen (P1NP) (ng/mL)	43 (31 - 60)	43 (27 - 59)	0.412

\* FATR: tartrate-resistant acid phosphatase.

In fact, during lactation, the mother supplies the fetus with around 300 mg of calcium daily, the source of which is mainly bone, which produces a loss of between 5-10% of maternal bone mass<sup>31</sup>, being enough for 3-6 months lactation for this loss to occur<sup>32</sup>. However, when studying and trying to establish the gynecological and/or obstetric factors that can influence bone mineral metabolism, some authors assess only the presence or absence of pregnancies<sup>16</sup>, others study the number of pregnancies<sup>21</sup> with no shortage of who analyzes the age at which the first pregnancy occurs<sup>20</sup>. On the other hand, other authors suggest that the organism adapts to this situation, since it is transitory. With several compensatory homeostatic mechanisms, it restores balance in bone mineral metabolism. Other authors suggest that when breastfeeding lasts up to one year, it would be correct to inform the mother of the need for her to acquire nutritional and physical activity habits that facilitate this recovery<sup>33,34</sup>.

There are also notable differences in the method to be used to assess the effect of breastfeeding on bone mineral metabolism. Some studies analyze changes in BMD<sup>16,35</sup>, while others consider the risk of developing fragility fractures<sup>12,15,36</sup>, especially hip fractures<sup>18,37</sup>. Interestingly, we have not found studies in the literature that analyze the effect of breastfeeding on a very signi-

ficant aspect of the skeleton, which is bone quality, to such an important extent that some authors consider that it contributes more to fracture risk than the amount measured by BMD<sup>38</sup>.

Some studies have been carried out in order to know what are the changes in bone mineral metabolism in women at the time they are breastfeeding. Thus, Carneiro et al. suggested the hypothesis that in these women there is an uncoupling between osteoblasts and osteoclasts that leads to a rapid loss of bone mass<sup>39</sup>.

In a review carried out by Sower on the effect of pregnancy and lactation on bone mineral metabolism, a wide variability is collected in the results obtained in the different publications, which is considered to be largely due to the heterogeneity of the methodology used in these studies<sup>40</sup>.

A total of 758 women were included in our study, of whom 301 (39.7%) had not breastfed and 457 (60.3%) had. All of them were postmenopausal and in the analysis of their clinical characteristics in the baseline evaluation, we found the existence of statistically significant differences in age and BMI, which is why the densitometric values and the analytical parameters collected in Tables 2 and 3 are compared after adjusting for these two variables.

**Table 4. Characteristics of the study women after propensity-score matching**

	Breastfeeding		p value	% of standardized difference*
	Yes N = 254	No N = 254		
Age (years)	60.4 ± 11.0	60.1 ± 11.1	0.712	-2.9676
BMI (kg/m <sup>2</sup> )	27.2 ± 5.7	27.0 ± 6.2	0.665	-3.4992
Falls	80 (31.5)	82 (32.3)	0.923	1.6807
Statins	79 (31.1)	86 (33.9)	0.550	5.8122
Thiazides	26 (10.2)	23 (9.1)	0.775	-4.1077
Uric acid (mg/dL)	4.4 ± 1.2	4.4 ± 1.3	0.567	-4.8984
Total cholesterol (mg/dL)	214.7 ± 39.1	216.7 ± 45.9	0.615	4.2572
HDL-cholesterol (mg/dL)	62.4 ± 15.0	62.1 ± 16.1	0.845	-1.6184
Triglycerides (mg/dL)	4.6 ± 0.4	4.6 ± 0.4	0.711	2.8626
L2-L4 (g/cm <sup>2</sup> )	0.8 ± 0.2	0.8 ± 0.2	0.972	0.2958
T-score	-1.9 ± 1.5	-1.8 ± 1.7		
Femoral neck (g/cm <sup>2</sup> )	0.7 ± 0.1	0.7 ± 0.1	0.258	9.1552
T-score	1.5 ± 1.1	-1.4 ± 1.2		
Total hip (g/cm <sup>2</sup> )	0.8 ± 0.1	0.8 ± 0.2	0.880	1.2448
T-score	-1.3 ± 1.4	-1.2 ± 1.6		
Kyphosis	53 (20.9)	46 (18.1)	0.470	-7.1422
Fragility fractures n (%)	75 (29.5)	74 (29.1)	1	-0.8647
Densitometric osteoporosis** n (%)	115 (44.1)	117 (44.8)	0.933	-1.540

Data are expressed as mean ± standard deviation and frequencies: n (%); the calibrator (caliper) selected was 0.5; \*: note that all standardized differences were less than or equal to 10%; \*\*: presence of a T-score lower than -2.5 in any of the 3 locations where bone mineral density (DXA) was determined.

**Table 5. Conditional logistic regression for the presence of fragility fractures. After matching, breastfeeding showed no association with fragility fractures**

	Breastfeeding		p value*	OR (95% CI)**
	Yes N = 254	No N = 254		
<b>Fragility fractures</b>			0.002	-
No, n (%)	179 (70.5)	180 (70.9)		1 (Reference)
Yes, n (%)	75 (29.5)	74 (29.1)		1.018 (0.704 – 1.447)

\*: likelihood ratio test; \*\*: conditional logistic regression; OR: odds ratio.

The distribution of lifestyles, such as tobacco use, physical activity in leisure time and the prevalence of diabetes, showed similar prevalence figures, without obtaining statistically significant differences. In a study by Yan et al. in Chinese women, they found that the differences observed in BMD in postmenopausal women who had breastfed and those who had not, were due to age, BMI and the number of pregnancies and not to the fact of having or not breastfed<sup>21</sup>. Given the known effect of age and BMI on BMD<sup>9</sup> in our study, we decided to adjust for these variables.

Women in both groups, lactating and non-lactating, showed similar BMD values in both the lumbar spine and the proximal end of the femur. Some studies have described that women who breastfeed have lower BMD values than those who do not<sup>20,24,32</sup>, but there are other authors who find the opposite: a protective effect with higher BMD values and a lower risk of densitometric osteoporosis<sup>18,31</sup>. A study carried out in Korea in more than one million women<sup>41</sup> found that the parameters that were independently associated with an increased risk of

fracture were the presence of late menarche, early menopause and, therefore, a shorter reproductive period, but not breastfeeding, a finding that concurs with our results.

In the literature consulted, we did not find studies that linked breastfeeding with bone quality assessed by ultrasound in postmenopausal women, and we only found one study carried out in premenopausal women that reported a beneficial effect<sup>42</sup>.

In our study, no statistically significant differences were observed in the ultrasound indices, so we can accept that breastfeeding has no effect, either positive or negative, on bone quality estimated by these measurements. We consider the statistically significant differences that we have found in the biochemical data to be clinically irrelevant<sup>43</sup>, as they are within the range of normality established by the laboratory and do not have a clinical impact.

By applying the statistical technique of the propensity score matching method, we achieved a better fit of the

women to homogenize both groups. The variables established by the program to be included in the adjustment are shown in table 4 and it can be seen that the standardized difference percentage ranges between -7.1422 and 9.1552. This indicates a very good fit, which has been established by consensus as less than 10%. Although as a consequence of this adjustment, the number of women studied decreased to 254 in each group, thanks to it we were able to establish more precisely, by applying conditional logistic regression, that breastfeeding has no effect on the presence of fragility fractures after menopause.

In conclusion, our study suggests that breastfeeding has no positive or negative effect on bone mineral metabolism after menopause, according to the biochemical results obtained (with markers of bone remodeling, vitamin D and PTH) and the densitometric (with DXA and QUS). Finally, the propensity score matching method allowed us to confirm that it did not influence the prevalence of fragility fractures after menopause either.



**Conflict of interests:** The authors declare no conflict of interest.

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