Author/s:
Bjørnerem, Å;Ghasem-Zadeh, A;Wang, X;Bui, M;Walker, SP;Zebaze, R;Seeman, E

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<CATEG>ORIGINAL ARTICLE

BJØRNEREM ET AL.

BREASTFEEDING AND BONE MICROSTRUCTURE

Irreversible Deterioration of Cortical and Trabecular Microstructure Associated With Breastfeeding

Åshild Bjørnerem,[1,2] Ali Ghasem-Zadeh,[3] Xiaofang Wang,[3] Minh Bui,[4] Susan P Walker,[5] Roger Zebaze,[3] and Ego Seeman[3,6]

[1]Department of Clinical Medicine, UiT The Arctic University of Norway, Tromsø, Norway

[2]Department of Obstetrics and Gynecology, University Hospital of North-Norway, Tromsø, Norway

[3]Endocrine Centre and Department of Medicine, Austin Health, University of Melbourne, Melbourne, Australia

[4]Centre for Epidemiology and Biostatistics, School of Population and Global Health, University of Melbourne, Melbourne, Australia

[5]Mercy Hospital for Women, Department of Obstetrics and Gynecology, University of Melbourne, Melbourne, Australia

[6]Department of Health and Ageing, Australian Catholic University, Melbourne, Australia

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Address correspondence to: Åshild Bjørnerem, MD, Department of Clinical Medicine, Faculty of Health Sciences, UiT The Arctic University of Norway, 9037 Tromsø, Norway. E-mail: ashild.bjornerem@uit.no

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ABSTRACT

Estrogen deficiency associated with menopause is accompanied by an increase in the rate of bone remodeling and the appearance of a remodeling imbalance; each of the greater number of remodeling transactions deposits less bone than was resorbed, resulting in microstructural deterioration. The newly deposited bone is also less completely mineralized than the older bone resorbed. We examined whether breastfeeding, an estrogen-deficient state, compromises bone microstructure and matrix mineral density. Distal tibial and distal radial microarchitecture were quantified using high-resolution peripheral quantitative computed tomography in 58 women before, during, and after breastfeeding and in 48 controls during follow-up of 1 to 5 years. Five months of exclusive breastfeeding increased cortical porosity by 0.6% (95% confidence interval [CI] 0.3–0.9), reduced matrix mineralization density by 0.26% (95% CI 0.12–0.41) (both $p < 0.01$), reduced trabecular number by 0.22 per mm (95% CI 0.15–0.28), and increased trabecular separation by 0.07 mm (95% CI 0.05–0.08) (all $p < 0.001$). Relative to prebreastfeeding, at a median of 2.6 years (range 1 to 4.8) after cessation of breastfeeding, cortical porosity remained 0.58 SD (95% CI 0.48–0.68) higher, matrix mineralization density remained 1.28 SD (95% CI 1.07–1.49) lower, and trabeculae were 1.33 SD (95% CI 1.15–1.50) fewer and 1.06 SD (95% CI 0.91–1.22) more greatly separated (all $p < 0.001$). All deficits were greater than in controls. The results were similar at distal radius. Bone microstructure may be irreversibly deteriorated after cessation of breastfeeding at appendicular sites. Studies are needed to establish whether this

deterioration compromises bone strength and increases fracture risk later in life. © 2016 American Society for Bone and Mineral Research.

KEY WORDS: CORTICAL POROSITY; LACTATION; MATRIX MINERALIZATION; TRABECULAR BONE MICROARCHITECTURE</KWD>

Introduction

All factors influencing bone's structural strength express their effects through a final common cellular machinery of bone remodeling.[1,2] Bone remodeling is balanced during young adulthood. Equal volumes of old or damaged bone are removed and replaced by new bone upon the endocortical surface of the cortex maintaining cortical thickness, upon the surfaces of the intracortical canals maintaining their diameters, and upon trabecular surfaces maintaining their thickness, number, and connectivity.[3,4]

With the appearance of estrogen deficiency at menopause, bone remodeling becomes unbalanced; each remodeling event resorbs more bone than it replaces, producing cortical thinning and porosity, perforation, and complete loss of trabeculae. Remodeling rate also accelerates; more remodeling sites appear upon the endocortical, intracortical, and trabecular surfaces, accelerating structural deterioration.[5] The rapid remodeling also reduces matrix mineralization density by replacing older, more mineralized bone with young, less completely mineralized bone.[4–6]

Breastfeeding is an estrogen-deficient state associated with bone loss.[6–9] Bone remodeling accelerates and may also become unbalanced. Studies in rodent and other animal models demonstrate that trabeculae perforate, their numbers decrease, and cortical porosity increases.[10–18] This structural deterioration has been reported to be reversible in some animal models.[7,17,18]

Bone loss during breastfeeding in humans is reported, but whether the microstructural deterioration is reversible after cessation of breastfeeding in human subjects is uncertain.[19–29] As trabecular bone loss, trabecular thinning, and perforation proceed rapidly after estrogen deficiency, the resulting bone fragility is attributed to deterioration in trabecular bone. However, ~80% of the skeleton is cortical; trabecular bone comprises only ~20% of the skeleton. The slower remodeling and loss of the fourfold larger cortical bone volume contributes more to total bone loss in absolute terms than rapid trabecular bone loss during aging.[30,31] Moreover, unbalanced intracortical remodeling is the main source of cortical bone loss and produces cortical porosity.[30] Increasing porosity of a compact structure like cortical bone reduces strength more than increasing porosity of an already porous structure like trabecular bone.[32] No study has been done examining the microstructural basis of bone loss or its reversibility during more than 1.5 years postpartum follow-up. We hypothesized that the rapid unbalanced remodeling associated with breastfeeding will produce bone loss, structural deterioration, and will reduce the completeness of matrix mineralization by replacing older bone with young bone. We also hypothesized that deficits in trabecular and cortical microstructure are likely partly irreversible because complete loss of trabeculae by perforation and formation of large pores may be incompletely restored after completion of breastfeeding and return of normal menstrual cyclicity.

Materials and Methods

We recruited 100 healthy pregnant women aged 20 to 42 years with a singleton pregnancy and term delivery at The Mercy Hospital for Women, Heidelberg, Melbourne, Australia, from 2008 to 2009. Initial assessment was at a mean of 14 days (range 3 to 25) post-delivery. The second

assessment occurred after a median of 5 months (range 2 to 6) in women who breastfed 6 to 10 times per day exclusively without formula or bottle feeding (Table 1).{TBL 1} To accurately capture changes during exclusive breastfeeding, each woman had the second assessment within 14 days of cessation of exclusive breastfeeding because the shift to intermittent breastfeeding is followed shortly by resumption of menses and possible slowing of remodeling as estrogen levels rise.[23] The third and final assessment occurred after a median total follow-up since delivery of 3.6 years (range 0.9 to 5.4). Total follow-up included a median of total breastfeeding for 13 months (range 3 to 31), exclusive breastfeeding for 5 months, intermittent breastfeeding (2 to 4 times daily combined with other nutrition for 7 months (range 0 to 25), and 2.6 years (range 1 to 4.8) follow-up after stopping breastfeeding. Resumption of menses occurred 7 months post-delivery (range 1 to 14) (which was shortly after cessation of exclusive breastfeeding), and follow-up after resumption of menses was 3 years (range 1.5 to 5.1).

We excluded 8 women who did not breastfeed, 6 who became pregnant, 10 who were lost to follow-up, as well as 18 women with movement artefacts during scanning of distal tibia and 24 women with movement artefact of distal radius. This left 58 breastfeeding women with three valid distal tibia scans and 52 women with three valid distal radial scans. We recruited a control group of 48 healthy non-pregnant non-lactating premenopausal women aged 18 to 44 years, followed for a median of 2.1 years (range 1.1 to 5.3) with two valid scans of distal tibia and 42 of them had two valid scans of distal radius. All participants gave written informed consent. The study was approved by the Austin and Mercy Human Research Ethics Committees.

High-resolution 3-dimensional peripheral quantitative computed tomography (HR-pQCT; XtremeCT, Scanco Medical AG, Brüttisellen, Switzerland) was used to obtain images at the

non-dominant distal tibia and distal radius.[33] A total of 110 slices were obtained at 22.5 and 9.5 mm from a reference line at the endplate of the distal tibia and radius, respectively. The 49 most proximal slices were chosen because the thicker cortex allows accurate assessment of porosity. Porosity within the total cortex and its compartments (compact cortex, outer and inner transitional zones), matrix mineralization density, trabecular number, thickness, separation, and bone volume/tissue volume (BV/TV) were quantified using StrAx1.0, a non-threshold-based method for quantifying microarchitecture in vivo.[34] The method automatically segments bone into its compartments (Straxcorp, Melbourne, Australia). The in vivo precision was 0.5 to 3.0%.[34] Daily quality control was carried out by scanning a phantom containing rods of hydroxyapatite (QRM, Moehrendorf, Germany).

StrAx1.0 analysis uses an algorithm that separates bone from background (soft tissue) and bone into its compact-appearing cortex, the fragmented cortex forming an outer and inner transitional zone and the trabecular compartment. From the segmented image, porosity is quantified as the proportion of voxels within the cortical compartment that contain void. Once deposited, osteoid is mineralized reaching ≥80% of full mineralization (1200 mgHA/cc) within days. Matrix mineralization is quantified using StrAx1.0 by determining the mean density of voxels with attenuation between 80% and 100% of fully mineralized bone. These voxels are unlikely to contain a pore because a pore results in voxel attenuation <80% of the maximum. So, variation attenuation within 80% and 100% of full mineralization reflects heterogeneity in mineralization. Voxels attenuating photons <80% are used to calculate porosity.

Areal bone mineral density (aBMD) at the spine and total hip was assessed using dual-energy X-ray absorptiometry (DXA, Lunar GE Prodigy, Madison, WI, USA) at baseline and after end of lactation and end of follow-up (first and third visits but not at the second visit).

Analysis of variance (ANOVA) was used to compare baseline features in women who breastfed and controls. Then *t* tests were used to determine whether the within-group mean change in absolute terms differed from zero (i) during exclusive breastfeeding (second minus first measurement), (ii) after end of exclusive breastfeeding (third minus second measurement), and (iii) across the total follow-up in breastfeeding group (third minus first measurement), and in controls (second minus first measurement). In addition, change in standardized terms relative to prebreastfeeding was calculated by dividing total change by baseline standard deviation (SD) for each trait. Annual rates of change in absolute terms were calculated for each period by dividing each of the changes by its respective follow-up. ANOVA was used to test whether the change in each trait in absolute terms in women who breastfed differed from the change in controls after adjustment for baseline age, parity, height, and weight. Analyses were performed using SAS Software package, v9.3 (SAS Institute Inc., Cary, NC, USA). Two-sided $p < 0.05$ was the level of significance chosen. The results with mean ± standard deviation (SD) are shown in the tables, whereas the results below show the mean values only to facilitate comprehension. The 95% confidence intervals (CI) are presented short in parentheses.

Results

The total follow-up of women who breastfed was a median of 3.6 years (range 0.9 to 5.4), which included exclusive breastfeeding for a median of 5 months (range 2 to 6), intermittent breastfeeding for a median of 7 months (range 0 to 25), and no breastfeeding at all for a median

of 2.6 years (range 1 to 4.8) (Table 1). Baseline age, height, weight, and tibial cortical porosity did not differ between women who breastfed and controls. However, trabecular number was higher (2.97 versus 2.63 per mm), trabeculae were thinner (0.187 versus 0.196) but less separated (1.24 versus 1.34 mm), and the degree of matrix mineralization was higher (66.1 versus 65.5%) compared with controls, respectively (all $p < 0.05$).

Exclusive breastfeeding

During a median of 5 months of exclusive breastfeeding, distal tibia cortical porosity increased by 0.6% (95% CI 0.3–0.9), whereas the degree of matrix mineralization decreased by 0.26% (0.12–0.41), trabecular number decreased by 0.22 per mm (0.15–0.28), thickness increased by 0.004 mm (0.003–0.005), separation increased by 0.07 mm (0.05–0.08), and BV/TV decreased by 0.29% (0.20–0.38) (all $p < 0.001$, Table 2). {TBL 2}

Intermittent breastfeeding, with other nutrition and resumption of menses

Despite the cessation of breastfeeding for a median of 2.6 years and regular menses for 3.0 years, distal tibia cortical porosity continued to increase by a further 1.8% (95% CI 1.4–2.1), whereas matrix mineralization continued to decrease by 0.97% (0.79–1.14), trabecular number decreased by a further 0.43 per mm (0.36–0.51), thickness increased by 0.014 mm (0.011–0.016), separation increased by 0.16 mm (0.13–0.18), and BV/TV decreased by 0.78% (0.66–0.90) (all $p < 0.001$).

After stopping breastfeeding

Residual deficits in microarchitecture and material composition were observed at a median of 2.6 years after complete cessation of any breastfeeding. Relative to measurements done before breastfeeding, cortical porosity remained 2.3% (95% CI 1.9–2.7) or 0.58 SD (48–0.68) higher,

and matrix mineralization density remained 1.23% (1.03–1.43) or 1.28 SD (1.07–1.49) lower (all $p < 0.001$) (Table 2, Fig. 1*A*).{FIG1} There were 0.65 per mm (0.56–0.74) or 1.33 SD (1.15–1.50) fewer trabeculae that were 0.017 mm (0.015–0.020) or 1.54 SD (1.33–1.74) thicker and 0.22 mm (0.19–0.26) or 1.06 SD (0.91–1.22) more separated. Trabecular BV/TV remained 1.07% (0.93–1.21) or 0.70 SD (0.61–0.79) lower (all $p < 0.001$). There was no overall gain in distal tibia total cross-sectional area (–0.005 mm² [–2.14, +2.13]).

There was a gain in aBMD of the spine and total hip between baseline and end of follow-up of 0.021 g/cm² (95% CI 0.004–0.038) or 0.16 SD (0.07–0.26), $p = 0.016$ and 0.010 g/cm² (–0.0001, 0.020) or 0.10 SD (0.03–0.18), $p = 0.052$, respectively. The weight loss of 2.2 kg (0.5–4.0) ($p = 0.014$) did not predict the changes in aBMD or microstructure at any site.

All trait changes were greater in women breastfeeding than in controls: Cortical porosity was higher, 2.3% (1.9–2.7) versus 0.8% (0.4–1.2); matrix mineralization density was lower, 1.23% (1.03–1.43) versus 0.29% (0.07–0.51); trabecular number was lower, 0.65 per mm (0.56–0.74) versus 0.29 per mm (0.20–0.39); trabecular thickness was greater, 0.017 mm (0.015–0.020) versus 0.008 mm (0.005–0.010); separation was greater, 0.22 mm (0.19–0.26) versus 0.10 mm (0.07–0.14); and BV/TV was lower, 1.07% (0.93–1.21) versus 0.45% (0.28–0.61) (all $p < 0.001$). Results were similar for distal radius (Tables 1 and 2). Annualized rates of changes in cortical porosity, matrix mineralization density, trabecular number, thickness, separation, and BV/TV were more rapid in breastfeeding women than in controls (all $p < 0.05$, Fig. 1*B*). All these comparisons were adjusted for age, height, weight, and parity.

Discussion

We report that women who exclusively breastfed had more rapid deterioration in microstructure than controls. Intracortical remodeling produced porosity and replaced older, more completely mineralized bone with younger, less completely mineralized bone. Remodeling upon trabecular surfaces removed them producing a reduction in trabecular number and greater separation between them. These morphological effects did not reverse despite cessation of lactation and the return of menses. Despite follow-up of a median of 3.6 years post-delivery, residual deficits remained relative to beginning of breastfeeding with higher cortical porosity, fewer trabeculae, and lower matrix mineralization.

Women who exclusively breastfed lost bone rapidly

Deterioration in microarchitecture was observed relative to prelactation and was greater than in controls. As reported during menopause, trabecular bone was lost by a reduction in trabecular number producing an increase in trabecular separation, an effect that reduces trabecular strength more than trabecular thinning.[35] Trabecular bone loss occurs more rapidly than cortical bone loss, but the resulting deficits were similar (0.70 SD trabecular BV/TV versus 0.58 SD cortical porosity). However, increasing porosity of a compact structure like cortical bone reduces strength more than increasing porosity of an already porous structure like trabecular bone.[32] Loss of mineral occurs in three ways. First, each remodeling transaction deposits less new bone than was resorbed, so there is loss of mineral and matrix. Second, the smaller volume of newly deposited osteoid volume undergoes rapid primary mineralization reaching ~80% of its peak mineralization density within days to weeks, but secondary mineralization, the enlargement of crystals deposited during primary mineralization, takes months if not years to complete.[36] A

third possible mechanism is peri-osteocytic osteolysis, focal resorption of mineral from perilacunar matrix as documented during lactation in rodents.[37]

Deterioration in microstructure and matrix mineralization density persisted despite cessation of lactation and return of menses

Bone loss slowed, but did not cease, after the end of exclusive lactation. Nor did deficits in bone microstructure or matrix mineralization density revert to prebreastfeeding values during follow-up. Estrogen deficiency associated with menopause or lactation increases the rate of bone remodeling.[23] More remodeling events upon the surfaces of intracortical canals, endocortical surface, and trabeculae create cavities and microstructural deterioration.

If the rapidity of remodeling was the only abnormality associated with lactation and estrogen deficiency, the microstructural deficits produced would be expected to be fully reversed with restoration of estrogen production and slowing of remodeling because all the cavities excavated eventually refill completely.

However, estrogen deficiency is also accompanied by the appearance of a remodeling imbalance—less bone is deposited than was resorbed during lactation. The negative bone balance is small, about –5%; more than 95% of a volume of bone resorbed by the osteoclasts is replaced by osteoblasts of each remodeling unit. The rapidity of remodeling drives structural deterioration. Perforated trabeculae result in permanent loss of trabeculae. Preferential loss of thin trabeculae reduces trabecular number but increases the *mean* thickness of trabeculae; we do not suggest lactation is accompanied by bone formation upon trabeculae making them thicker. High intracortical remodeling produces large coalescent pores, which may also fail to refill after

lactation. Secondary mineralization takes many months to reach completion when remodeling slows down after return of menses.[7,18–29]

The occurrence of cortical porosity is well documented by histomorphometry in lactating beagle dogs but reversibility of porosity in this animal model was not reported.[10] Studies in rodent species variously report reversibility, partial, or no reversibility after weaning.[13–18] Studies in rodents also demonstrate reduced mineralization of bone matrix.[12,18] Although trabecular bone is remodeled in rodents, rodents continue to grow and do not remodel cortical bone, or do so to a limited degree late in life, so this species may be less suitable as a model for examining cortical (Haversian) remodeling and porosity in human subjects.

Contrary to changes in appendicular microarchitecture,[29] some investigators report either partial or complete reversibility of BMD at the spine or hip after breastfeeding, and some suggest even higher spine BMD after breastfeeding.[7,19–28] The reasons for the reversibility at central skeleton are not clear.

The only other study examining bone microarchitecture of the appendicular skeleton also reported incomplete recovery in bone microarchitecture.[29] However, because 80% of porosity is less than 100 microns in diameter, threshold-based segmentation performed in this study underestimates cortical porosity because voxels containing pores also contain matrix and are disregarded using this approach. Threshold-based segmentation also overestimates trabecular density because intracortical remodeling producing porosity results in cortical fragments, which are measured as “trabecular bone,” overestimating recovery of trabecular bone.

The effects are species specific. Studies in mice and rats report reversibility, but these animals *model* cortical bone, they do not *remodel* cortical bone. They continue to grow throughout life,

and this may explain why the loss of bone is reversible. Humans remodel their cortical bone, and high remodeling of trabecular bone can cause loss of trabecular plates and loss of connectivity. We suggest that estrogen deficiency associated with lactation is, like menopause, accompanied by two changes, one being the reversible remodeling deficit produced by the rapid increase in bone remodeling accompanying the onset of lactation and the delay and slowness of the refilling phase of each remodeling transaction. There is an increase in the number of excavated cavities, and, concurrently, refilling of fewer cavities dug *before* delivery occurs. This produces a perturbation in *surface* level remodeling, the net effect being a rapid decrease in BMD.[38]

All of this would be fully reversible at the end of lactation in humans if it were not for the second abnormality: the appearance of a negative basic multicellular unit (BMU) balance. Estrogen deficiency alters the life span of the executive cells of bone. Osteoclasts live longer; osteoblasts die sooner. This produces an irreversible loss of bone and permanent structural deterioration because refilling of an excavated site is incomplete. Trabeculae are about 100 to 200 microns; a resorption cavity may be this deep causing perforation, which is why menopause causes loss in number of trabeculae, not thinning. Trabeculae are irreversibly lost. We suggest that this may be occurring during lactation. It is more modest because lactation is only 6 to 9 months, so remodeling returns.

There is another change of interest: osteocytic osteolysis, so there is loss of mineral. After lactation, remineralization occurs rapidly, but this is primary mineralization. Secondary mineralization is slow, which may take months if not years. Hence, we suggest and report persisting deficits in the mean degree of mineralization of matrix. This is likely to become fully reversed, but it takes time.

This study has several limitations. The sample size was modest and follow-up may have been insufficient to detect reversal of structural changes and restoration of matrix mineralization density produced by high remodeling rate. Measurements were not available before pregnancy so that the observations may be confounded by the effects of pregnancy, if any, on bone morphology and the subsequent changes produced at completion of pregnancy. The ideal control, pregnant women who did not breastfeed their offspring, could not be recruited to address this design limitation.

In summary, breastfeeding is associated with deterioration of cortical and trabecular bone microstructure and a reduction in completeness of secondary matrix mineralization at the appendicular skeleton. The bone loss was irreversible during 2.6 years of follow-up after the end of lactation and 3 years after resumption of regular menses. The relevance of these deficits in terms of compromising bone strength and increasing fracture risk remain to be established. Epidemiological studies report that lactation is neutral or protective against fracture; no long-term deleterious effects on fracture risk are reported.[7,39,40] Further studies are needed to establish whether the deterioration in microarchitecture of the appendicular skeleton contributes to fracture risk later in life.

Disclosures

ÅB has received lectures fees from Amgen and Eli Lilly. AG-Z is remunerated by StraxCorp as senior image analyst and is one of the inventors of the StrAx1.0 algorithm. RZ has received grant and/or research support from Amgen, Merck Sharp & Dohme, Servier, Warner-Chilcott, AKP, Genzyme, Sanofi, GSK, and Pfizer. He is a shareholder in StraxCorp, is remunerated by StraxCorp as president of R&D, and is one of the inventors of the StrAx1.0 algorithm. RZ is

also a director on the board of StraxCorp. ES has received research support and has lectured at national and international meeting symposia funded by Amgen, Allergan, Asahi, Genzyme, and Merck Sharp & Dohme. He is a director of the board and shareholder in StraxCorp, is remunerated by StraxCorp as chief medical officer, and is one of the inventors of the StrAx1.0 algorithm. All authors state that they have no other conflicts of interest.

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Authors' roles: Study design and conduct: all authors. Data collection: ÅB, AGZ, and XW. Responsibility for StrAx analysis: RZ. Statistical analyses: ÅB and MB. Drafting manuscript: all authors. Data interpretation and approving final version of manuscript: all authors.

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Fig. 1. (*A*) Residual deficits or overall changes (95% confidence intervals) in distal tibia microarchitecture expressed as standard deviation (SD) units relative to beginning breastfeeding for cortical porosity, matrix mineralization density, trabecular number, thickness, separation, and bone volume/tissue volume (BV/TV) (calculated as the difference between first and third measurement, divided by the baseline SD). (*B*) Annualized mean (95% confidence intervals) rate of change in women breastfeeding for distal tibia microarchitecture during exclusive and intermittent breastfeeding and overall (exclusive, intermittent, and after cessation of breastfeeding) and in controls for cortical porosity (%), matrix mineralization density (%), trabecular number (1/mm), thickness (mm × 100), separation (mm), and BV/TV (%) (calculated as the difference between first and second, second and third, and first and third measurement of each trait, divided by its respective follow-up).

Table 1. Baseline Features, Duration of Lactation, and Follow-up in 58 Women Who Breastfed and 48 Controls

	Breastfed	Controls
Age (years)	33.5 (24.8–42.8)	33.4 (18.1–44.6)
Height (cm)	163.7 (146–178)	164.0 (150–184)
Weight (kg)	72.7 (48–102)	67.6 (45–117)
Parity (range)	1.9 (1–4)	0.8 (0–3)[c]
White ethnicity, *n* (%)	50 (86.2)	39 (81.3)
Distal tibia	*n* = 58	*n* = 48
Total cortical porosity (%)	56.9 ± 4.0	57.8 ± 4.1
Compact cortex porosity (%)	38.4 ± 3.7	38.0 ± 3.7
Outer transitional zone porosity (%)	39.6 ± 2.7	39.4 ± 3.1

Inner transitional zone porosity (%)	83.5 ± 2.5	84.5 ± 2.7
Matrix mineralization density (%)	66.1 ± 1.0	65.5 ± 1.3[b]
Trabecular number (1/mm)	2.97 ± 0.49	2.63 ± 0.55[c]
Trabecular thickness (mm)	0.187 ± 0.011	0.196 ± 0.014[c]
Trabecular separation (mm)	1.24 ± 0.21	1.34 ± 0.27[a]
Trabecular bone volume/tissue volume (%)	4.67 ± 1.53	4.13 ± 1.53
Distal radius	*n* = 52	*n* = 43
Total cortical porosity (%)	51.8 ± 5.0	51.7 ± 6.4
Compact cortex porosity (%)	34.7 ± 3.5	35.1 ± 4.7
Outer transitional zone porosity (%)	38.2 ± 2.5	38.8 ± 3.8
Inner transitional zone porosity (%)	84.4 ± 3.0	84.9 ± 2.8
Matrix mineralization density (%)	66.3 ± 1.3	66.1 ± 1.7
Trabecular number (1/mm)	2.40 ± 0.46	2.25 ± 0.47
Trabecular thickness (mm)	0.187 ± 0.011	0.194 ± 0.013[b]
Trabecular separation (mm)	1.35 ± 0.33	1.39 ± 0.34
Trabecular bone volume/tissue volume (%)	3.29 ± 1.77	2.97 ± 1.58
Duration of exclusive lactation (months)[*]	5.0 (2–6)	
Duration of total (any) lactation (months)[*]	13.0 (3–31)	
Resumption of menses (months postpartum)[*]	7.1 (1–14)	
Follow-up after resumption of menses (years)[*]	3.0 (1.5–5.1)	
Follow-up after end of any lactation (years)[*]	2.6 (1–4.8)	
Total follow-up (years)[*]	3.6 (0.9–5.4)	2.1 (1.1–5.3)[c]

Mean (range), mean ± standard deviation, or [*]median (range).
[a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.001$ differences between groups.

Table 2. Mean (95% Confidence Intervals [CI]) Changes in Distal Tibial and Distal Radial Microarchitecture Associated With Breastfeeding and in Controls

	Breastfeeding			Controls
	Exclusive 2nd minus 1st measurement	Intermittent 3rd minus 2nd measurement	Overall 3rd minus 1st measurement	2nd minus 1st measurement
Distal tibia	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)
Total cortical porosity (%)	0.6 (0.3, 0.9)[c]	1.8 (1.4, 2.1)[c]	2.3 (1.9, 2.7)[c]	0.8 (0.4, 1.2)[c,f]
Compact cortex porosity (%)	0.3 (0.01, 0.6)[a]	0.8 (0.5, 1.2)[c]	1.2 (0.7, 1.6)[c]	0.3 (–0.1, 0.7)[f]
Outer transitional zone porosity (%)	0.2 (0.004, 0.5)[a]	1.2 (0.9, 1.5)[c]	1.4 (1.1, 1.8)[c]	0.3 (–0.1, 0.7)[f]
Inner transitional zone porosity (%)	0.4 (0.2, 0.5)[c]	0.9 (0.7, 1.1)[c]	1.2 (1.0, 1.5)[c]	0.4 (0.2, 0.6)[c,f]
Matrix mineralization density (%)	–0.26 (–0.41, –0.12)[c]	–0.97 (–1.14, –0.79)[c]	–1.23 (–1.43, –1.03)[c]	–0.29 (–0.51, –0.07)[a,f]
Trabecular number (1/mm)	–0.22 (–0.28, –0.15)[c]	–0.43 (–0.51, –0.36)[c]	–0.65 (–0.74, –0.56)[c]	–0.29 (–0.39, –0.20)[c,f]
Trabecular thickness (mm)	0.004 (0.003, 0.005)[c]	0.014 (0.011, 0.016)[c]	0.017 (0.015, 0.020)[c]	0.008 (0.005,0.010)[c,f]
Trabecular separation (mm)	0.07 (0.05, 0.08)[c]	0.16 (0.13, 0.18)[c]	0.22 (0.19, 0.26)[c]	0.10 (0.07, 0.14)[c,f]
Trabecular bone volume/tissue volume (%)	–0.29 (–0.38, –0.20)[c]	–0.78 (–0.90, –0.66)[c]	–1.07 (–1.21, –0.93)[c]	–0.45 (–0.61, –0.28)[c,f]
Distal radius				
Total cortical porosity (%)	0.6 (0.2, 1.0)[b]	1.6 (1.3, 2.0)[c]	2.2 (1.8, 2.6)[c]	0.8 (0.2, 1.4)[b,f]
Compact cortex porosity (%)	0.3 (–0.1, 0.6)	1.4 (1.1, 1.7)[c]	1.7 (1.3, 2.0)[c]	0.4 (–0.1, 1.0)[f]
Outer transitional zone porosity (%)	0.04 (–0.2, 0.3)	1.2 (0.9, 1.4)[c]	1.2 (0.9, 1.5)[c]	0.3 (–0.2, 0.8)[f]
Inner transitional zone porosity (%)	0.02 (–0.1, 0.1)	0.4 (0.3, 0.6)[c]	0.5 (0.3, 0.6)[c]	0.1 (–0.1, 0.3)[f]
Matrix mineralization density (%)	–0.06 (–0.25, 0.13)	–0.86 (–1.02, –0.69)[c]	–0.92 (–1.13, –0.71)[c]	–0.28 (–0.56, 0.01)[f]
Trabecular number (1/mm)	–0.04 (–0.12, 0.04)	–0.35 (–0.42, –0.27)[c]	–0.39 (–0.46, –0.31)[c]	–0.18 (–0.28, –0.08)[c,e]
Trabecular thickness (mm)	0.002 (–0.0002, 0.004)	0.011 (0.008, 0.013)[c]	0.013 (0.010, 0.015)[c]	0.008 (0.005, 0.011)[c]
Trabecular separation (mm)	0.03 (0.001, 0.05)[a]	0.10 (0.07, 0.13)[c]	0.12 (0.09, 0.15)[c]	0.07 (0.03, 0.10)[c,e]
Trabecular bone volume/tissue volume (%)	–0.08 (–0.14, –0.02)[b]	–0.52 (–0.63, –0.41)[c]	–0.61 (–0.72, –0.49)[c]	–0.22 (–0.33, –0.12)[c,f]

Changes in absolute terms during exclusive and intermittent breastfeeding, overall (exclusive, intermittent, and after cessation of breastfeeding), and in controls.
[a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.001$ for within-group changes. [d]$p < 0.05$, [e]$p < 0.01$, [f]$p < 0.001$ comparing overall changes in women who breastfed versus controls.