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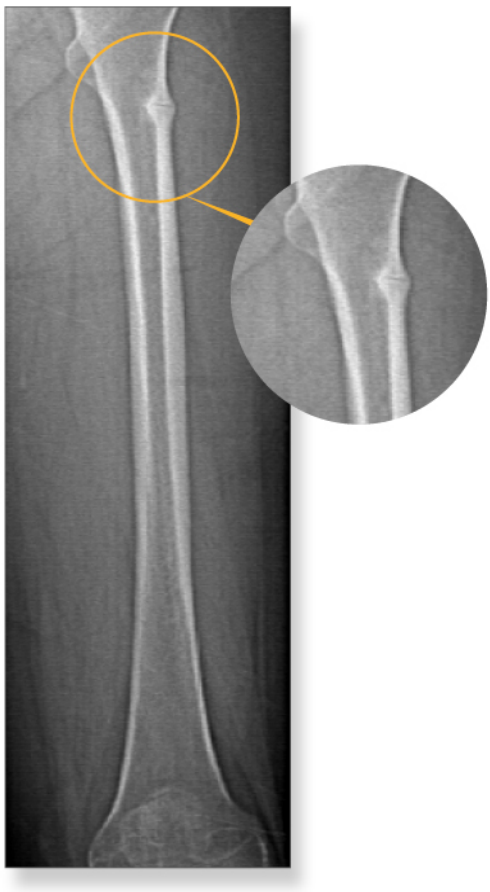

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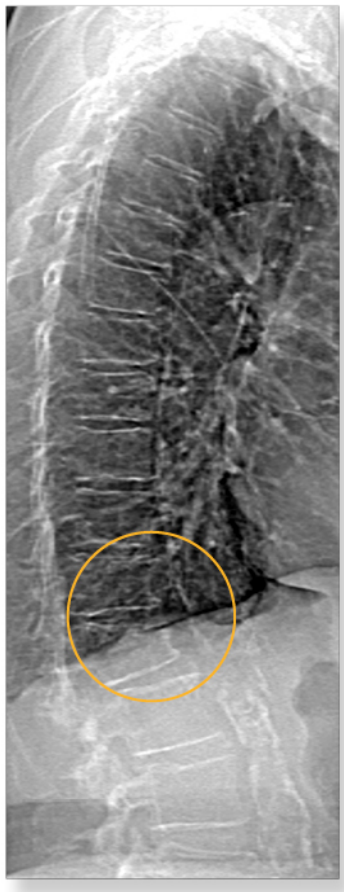

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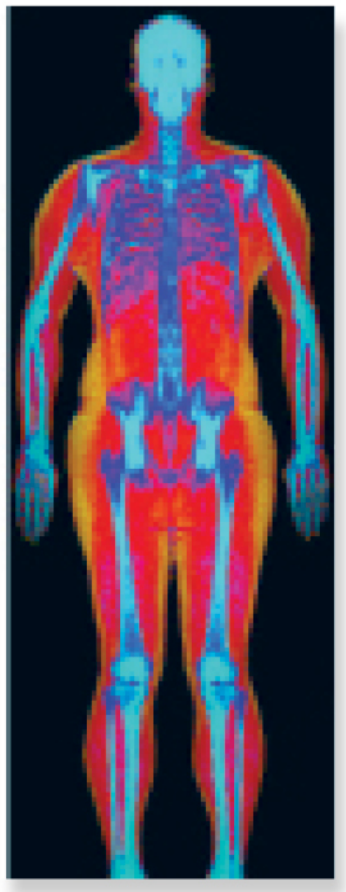

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COMMENTARY

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The Skeleton Is a Storehouse of Mineral That Is Plundered During Lactation and (Fully?) Replenished Afterwards

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ABSTRACT

During lactation, mammals resorb mineral from the maternal skeleton to provide calcium to milk. Rodents lose 25% to 35% of skeletal ash weight, ash calcium content, and bone mineral content as measured by dual-energy X-ray absorptiometry (DXA), and have compromised material properties of bone as assessed by crushing vertebrae and 3-point bend tests of femora or tibias. The strength, stiffness, and toughness of vertebrae, femora, and tibias are reduced by as much as 60%. The effects of lactation are not uniform throughout the skeleton, but instead resorption is much more marked in the trabecular-rich spine than in the appendicular skeleton or whole body. Women who breastfeed exclusively lose an average of 210 mg calcium in milk each day, whereas nursing of twins or triplets can double and triple the output of calcium. Clinical data are also consistent with skeletal calcium being released during lactation to provide much of the calcium needed for milk production. Lumbar spine bone mineral density (BMD), as assessed by DXA, declines by a mean of 5% to 10% among numerous studies during 3 to 6 months of exclusive lactation, whereas largely cortical sites (hip, forearm, whole body) show half that loss or no significant changes. Micro-CT of rodents and high-resolution peripheral quantitative computed tomography (HR-pQCT) imaging of women confirm that lactation causes microarchitectural deterioration of bone. These skeletal losses occur through two pathways: upregulated osteoclast-mediated bone resorption and osteocytic osteolysis, in which osteocytes remove mineral from their lacunae and pericanalicular spaces. After weaning, the skeleton is fully restored to its prior mineral content and strength in both animal models and humans, despite persistent microarchitectural changes observed in high-resolution imaging. Osteoblasts upregulate to lay down new osteoid, while osteocytes remineralize their surroundings. The factors that stimulate this post-weaning skeletal recovery remain unclear. In most studies, a history of lactation does not increase the risk, but may protect against, low BMD and fragility fractures. © 2017 American Society for Bone and Mineral Research.

KEY WORDS: LACTATION; BREAST; NEONATE; CALCIUM; PARATHYROID HORMONE-RELATED PROTEIN; ESTRADIOL; MICRO-CT; DXA; OSTEOCLASTS; OSTEOCYTIC OSTEOLYSIS; BONE RESORPTION

Skeletal Demineralization During Lactation

During lactation, mammals resorb mineral from the maternal skeleton to provide calcium to milk (the first reference comprehensively cites more than 1000 articles from the primary literature).[1] Rodents face a particularly high demand for calcium delivery, given their large (8 to 12) litter sizes and short (3-week) duration of lactation. Although intestinal calcium absorption is upregulated during lactation in rats and mice, the maternal skeleton undergoes marked resorption to support milk production.[1] The extensive evidence of skeletal mineral losses includes 25% to 35% decreases in skeletal ash weight and ash calcium content; 25% to 35% reductions in bone mineral content (BMC) or density (BMD) by dual-energy X-ray absorptiometry (DXA); metabolic balance studies confirming a marked negative calcium balance; marked increases in bone resorption compared with bone formation markers; and compromised material properties of bone as assessed by crushing vertebrae and 3-point bend tests of femora or tibias.[1] The strength, stiffness, and toughness of vertebrae, femora, and tibias are reduced by as much as 60% while ductility is increased.[1]

The effects of lactation are not uniform throughout the skeleton. Ash weight, ash mineral content, and BMC or BMD decline more markedly in trabecular-rich sites (spine, proximal femora, and proximal tibias) than in purely cortical sites of the appendicular skeleton and whole body.[1] When vertebrae and femora from the same animals have been assessed, lactation induced a 64% decrease in maximal sustainable vertebral load compared with only a 26% reduction in femoral bending load.[2] A calcium-restricted diet or larger litter sizes cause greater losses of skeletal mineral content and further reductions in bone strength, whereas a calcium-enriched diet and smaller litters

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have the opposite effects.[1] Milk production creates an obligatory calcium loss, such that maternal tetany and sudden death can be provoked with a calcium-restricted diet, a bisphosphonate or osteoprotegerin/denosumab to block skeletal resorption, and especially a combination of these interventions.[1] Most lactation data come from mice, rats, and sheep. African green monkeys, which are the closest model to humans, lose 20% of lumbar spine BMD during 20 weeks of lactation.[3]

Women who breastfeed exclusively (ie, all infant nutrition comes from milk) lose an average of 210 mg calcium in expressed milk each day.[1] Nursing of twins or triplets can double and triple the output of calcium, respectively, whereas outputs of 500 to 1200 mg per day have been documented in wet nurses.[1] Clinical data are consistent with skeletal calcium being released during lactation to provide much of the calcium needed for milk production. Unlike rodents, intestinal calcium absorption remains at the prepregnant rate in breastfeeding women.[1] Metabolic studies have shown a significant negative calcium balance even when calcium supplements are consumed.[1] Biochemical markers of bone resorption are disproportionately increased over bone formation markers.[1] Radioisotopes that were deposited in the skeleton years earlier are released to enter breast milk.[4]

Lumbar spine BMD, as assessed by DXA, declines by a mean of 5% to 10% among numerous studies during 3 to 6 months of exclusive lactation, whereas largely cortical sites (hip, forearm, whole body) show less than half that loss or no significant changes.[1] This is consistent with the animal data in that the trabecular-rich spine is more greatly affected than the appendicular skeleton. As noted, the range of a 5% to 10% decline in lumbar spine BMD indicates mean changes among available studies; within each study, a few lactating women showed no change in BMD, the majority lost BMD, but some women lost as much as 20% from the lumbar spine.[1] Adolescents lost a mean 10% to 15% of trabecular BMD in several studies.[1] Greater milk output predicts a greater loss of bone mineral density.[5] The rate of decline in BMD ranges from 1% to 3% *per month*, which is quite marked given that a loss of >1% *per year* is considered rapid after menopause. Cohort studies and randomized interventions have demonstrated that high calcium intakes do not reduce the amount of skeletal mineral content lost during lactation.[1] Although there understandably have been no objective tests of bone strength in women, lactation increases the risk of vertebral compression fractures but has not been associated with appendicular fractures.[6]

By What Mechanism(s) Does the Skeleton Lose Mineral Content During Lactation?

The first clearly defined mechanism is increased osteoclast-mediated bone resorption. Histomorphometric studies in rodents and primates have revealed increased osteoclast numbers and eroded trabecular and endocortical surfaces.[1] Micro-computed tomography (micro-CT) imaging has confirmed resorption of trabecular and endocortical surfaces in the central vertebrae, conversion of trabeculae from plate-like to rod-like structures, 30% to 50% reductions in trabecular bone volumes, 20% decreases in trabecular thickness, trabecular perforations, reduced tissue mineralization, and decreased whole bone stiffness.[1] Trabecular bone within the proximal ends of tibias and femora show similar losses, whereas the cortical compartment of the long bones show modest decreases in cortical thickness, cross-sectional area, tissue mineral density, and whole bone stiffness, and increases in cortical porosity and endosteal perimeter.[1] The greater surface area of trabecular bone enables osteoclasts to resorb it much more rapidly than cortical bone. These microstructural changes can be prevented with bisphosphonates or osteoprotegerin/denosumab and blunted with high-dose estradiol.[1]

There have been no histomorphometric studies from lactating women, but the marked increase in bone resorption markers suggests increased osteoclast numbers and activity.[1] High-resolution peripheral quantitative computed tomography (HR-pQCT) has been used in only two clinical studies of breastfeeding women, including Bjornerem and colleagues in this issue of *JBMR*.[7] HR-pQCT can only assess peripheral sites such as the radius and distal tibia, which lose much less mineral content than the lumbar spine based on DXA. In the first study, Brembeck examined 81 lactating women who had experienced a 4% decrease in BMD of the lumbar spine and femoral neck after a mean 8 months of exclusive lactation and compared them to 21 healthy controls.[8] Within the distal tibia, they found decreases of 0.5% in cortical volumetric BMD (vBMD), 2.5% in cortical thickness, and 2.5% in trabecular thickness. The effects were greater in those who lactated longer, whereas no changes were found in women who lactated <4 months.[8] In the new study by Bjornerem and colleagues,[7] 58 women breastfed exclusively for 5 months and were compared with 48 controls. The extent of BMD loss in the spine or hip by DXA was not determined. Five months of exclusive lactation increased distal tibial cortical porosity by 0.6%, reduced matrix mineralization density by 0.26%, reduced trabecular number by 0.22 per mm, and increased trabecular separation by 0.07 mm. Changes in the radius were similar.[7] Overall, the HR-pQCT findings from these two studies suggest comparatively modest effects of lactation to resorb skeletal microarchitecture of the distal tibia. (For comparison, postmenopausal women had a 4% to 8% increase in cortical porosity of the distal tibia over 2 years in a clinical trial[9] and 300% higher cortical porosity when compared with premenopausal women in a cross-sectional study.[10]) However, it should be kept in mind that this technology is not examining the vertebrae or femoral neck, where comparatively greater (5% to 10%) losses appear to occur as assessed by DXA; the distal tibia is not specifically examined by DXA.

A second more recently defined mechanism by which the skeleton loses mineral content is osteocytic osteolysis, a process by which osteocytes function like osteoclasts and resorb mineral from their surroundings.[11,12] Widened lacunae around osteocytes were first recognized by Rigal and Vignal in 1881,[13] followed by von Recklinghausen's 1910 proposal that osteocytes digest the perilacunar matrix in conditions associated with reduced mineral availability.[14] It wasn't until the 1960s that Belanger coined the term osteocytic osteolysis and found that the enlarged osteocytic lacunae have reduced mineral and protein content.[15,16] He also found that in rodents, osteocytic osteolysis was suppressed by calcitonin or a high-calcium diet, and increased by a low-calcium diet, parathyroid hormone (PTH), and pregnancy.[15,16] He estimated that osteocyte lacunae have about 10 times the surface area as trabecular bone in the human skeleton, which implies that osteocytic osteolysis has the potential to mobilize skeletal calcium more rapidly than resorption of trabeculae by osteoclasts.[15]

Unfortunately, osteocytic osteolysis was largely disbelieved and disregarded for decades. In an elegant essay, Parfitt reminded us that calcium doesn't leave bone solely by

osteoclast-mediated resorption, but even he overlooked osteocytic osteolysis.[17] The Bonewald lab revived interest in osteocytic osteolysis and found that osteocytes express osteoclast-related genes and enzymes (including cathepsin K) and resorb the mineral and proteinaceous matrix that fills their lacunae.[18,19] They confirmed that this process occurs during lactation in mice and is dependent upon osteocytes expressing the PTH/PTHrP receptor.[12,18,19] It remains unclear whether osteocytes free the calcium solely through chemical dissolution (reducing the pH in their lacunae), by resorbing the proteinaceous matrix similar to the actions of osteoclasts, or by both methods.

In this issue of *JBMR*, Kaya and colleagues used several techniques to infer the potential effects of lactation-induced osteocytic osteolysis on cortical bone of mice from the C57BL/6 strain.[20] After 2 weeks of lactation, they obtained cross sections of the mid-diaphyseal cortex of femora and used microindentation to find a 10% to 13% decrease in elastic modulus. Using varied imaging techniques, they demonstrated that lacunar and canalicular areas increased by 7% and 15%, respectively, no intracortical resorption was observed, and there were no alterations in bone mineralization, mineral/matrix ratio, or crystallinity.[20] The authors concluded that the implied porosity from the increased lacunar and canalicular areas may explain the 10% to 13% reduction in elastic modulus.[20] To put this in proper perspective, it would have been useful for the authors to have included a 3-point bend test of the opposite femora and the ash weight and mineral content of the same bone. Because the load to failure of the femora or tibias have been shown to decrease by 25% to 50% in mice or rats after 2 to 3 weeks of lactation,[1] this suggests that structural changes in osteocyte canaliculi may have only a minor effect on femoral bone strength compared with the microarchitectural changes induced by osteoclast-mediated resorption.

What Stimulates Osteoclasts and Osteocytic Osteolysis During Lactation?

Two factors that have been clearly established in rodents include systemic low estradiol and increased parathyroid hormone-related protein (PTHrP) concentrations.[1] Increased prolactin concentrations and suckling both inhibit the gonadotropin-releasing hormone pulse center in the hypothalamus, leading to low gonadotropins and estradiol. Simultaneously, lactating mammary epithelial cells become potent sources of PTHrP. Pharmacological treatment with estradiol blunts skeletal resorption during lactation.[21] Deletion of the gene encoding PTHrP from mammary tissue at the onset of lactation blunts skeletal resorption and the systemic rise in PTHrP.[22] Deletion of the PTH/PTHRP receptor selectively from osteocytes also blunts skeletal losses during lactation.[18]

Breastfeeding women also have low estradiol and increased PTHrP concentrations, which synergistically stimulate skeletal resorption.[1] Higher PTHrP levels correlate with greater lactational loss of BMD at the lumbar spine and femoral neck, even after accounting for the effects of estradiol levels, PTH, and breastfeeding status.[23] The secretion of PTHrP from lactating breast tissue is sufficient to upregulate bone turnover and normalize calcium homeostasis in hypoparathyroid women.[1] Occasionally, lactation causes even higher PTHrP concentrations that lead to more markedly increased bone resorption and hypercalcemia (pseudohyperparathyroidism); weaning usually normalizes these findings.[1]

What Happens After Lactation Ceases?

In animal models, osteoclast numbers plummet—widespread apoptosis of osteoclasts occurs within 24 hours of forced weaning—whereas osteoblasts and their precursors surge in numbers, and up to 75% of bone surfaces become covered with thick osteoid seams.[24–26] Furthermore, osteocytes now function like osteoblasts, as confirmed by the post-weaning appearance of tetracycline labels and a return of lacunar size to normal.[19] Biochemical markers of bone resorption fall to low levels, whereas bone formation markers increase, suggesting an uncoupling of bone turnover in favor of formation.[1]

Multiple studies in animal models suggest that skeletal ash weight, bone mass, and strength are fully restored after lactation.[1] Within 14 to 28 days post-weaning, as assessed by ash weight or DXA, the spine, hindlimb, and whole body achieve a bone mineral content that equals or exceeds the prepregnancy value.[1] Remarkably, mice that lack the gene encoding calcitonin and calcitonin gene-related peptide lose 55% of trabecular and 30% of cortical BMC during lactation but restore it within 18 days of weaning.[27] Histomorphometric assessment of rodents has shown a return of bone mass, cortical thickness, and cortical area to virgin values.[2] However, micro-CT and electron microscopy studies have found that bone mass and microarchitecture improve at differing rates for each skeletal site.[1] Trabecular bone parameters are quickly and fully restored within the vertebrae.[28] The femora and tibias show significant improvements in trabecular number and thickness, bone volumes, mineralization, cortical thickness, porosity, and stiffness, with the values restored to that of age-matched virgins in some studies or with permanent deficits in others.[1] In rats, the proximal and distal femora regained the mineral content of nulliparous controls, whereas the femoral midshaft and trabecular microarchitecture did not fully recover, which indicates that the extent of recovery can vary within the same bone.[29] Because multiple (but not all) studies have shown that vertebral, femoral, and tibial bone strength parameters return to virgin values,[1,2] it appears that these persistent changes in skeletal microarchitecture may not affect bone strength.

The study by Kaya and colleagues, which found increased osteocytic lacunae and canalicular spaces in mice after 2 weeks of lactation, also reported that these spaces had completely filled in by 1 week post-weaning and became equivalent to that of age-matched virgins.[20] This reaffirms that osteocytes can also act like osteoblasts and that skeletal recovery after weaning (both improved mineralization and strength) may be due in part to osteocytes restoring mineral to their immediate surroundings.

In breastfeeding women, the lactational decreases in BMD (as assessed by DXA) appear to be completely reversed by 12 months after weaning,[1] although the speed and completeness of recovery also differ by skeletal site. There are no histomorphometric studies, and few HR-pQCT studies have been performed. In the aforementioned study by Brembeck and colleagues, women were followed up to 18 months postpartum.[8] In women who had weaned by 9 months and experienced at least 9 months of recovery, the altered cortical microarchitecture was largely restored, including that cortical thickness and area reached *higher* than baseline values.[8] There

were persistent deficits in trabecular thickness but not trabecular volumetric BMD.[8] This contrasts with the study by Bjornerem and colleagues,[7] in which women lactated for a mean of 8 months and were followed for a range of 1 to 4.8 years after cessation of lactation. BMD of the spine and hip increased significantly between baseline and follow-up, whereas none of the structural changes in the distal tibia or radius induced by lactation showed improvement over the follow-up interval.[7] The authors concluded that lactation induces irreversible changes in skeletal microarchitecture at the distal tibia and radius. Bjornerem and colleagues also reported that there was no change in the cross-sectional diameter of the distal tibia.[7] This contrasts with the greater cortical thickness and area reported by Brembeck and colleagues and two other studies that reported that parity and lactation increase the size (circumference or cross-sectional diameter) of the femur and tibia.[30,31] A post-weaning increase in bone size could explain restoration of bone strength despite persistent deficits in skeletal microarchitecture.

What Stimulates Post-weaning Skeletal Recovery?

That lactational bone loss is rapidly and fully recovered at some skeletal sites distinguishes it from the sequelae of most other causes of bone loss in adults. Studies in animal models have shown that parathyroid hormone, PTHrP, calcitonin, vitamin D, the vitamin D receptor, and estradiol are not required for recovery of ash weight, BMC, or bone strength.[1] Adequate calcium is required because a calcium-restricted diet prevented skeletal recovery until after the diet was normalized 3 weeks later.[29] When lactational bone loss was prevented by treatment with osteoprotegerin (OPG), there was no post-weaning improvement in skeletal parameters.[32] This may simply mean that without skeletal resorption during lactation, there is no signal to upregulate bone formation or mineralization after weaning.

In women who have weaned, resumption of menses, rising estradiol levels, and falling PTHrP levels should contribute to a reduction in the rate of bone loss but will not directly stimulate bone formation. A hypoparathyroid woman showed a post-weaning 40% increase in lumbar spine BMD and a 7.5% increase in femoral neck BMD,[33] which is consistent with the animal data that PTH is not required for post-weaning recovery.

Overall, it remains unclear what factors stimulate post-weaning skeletal recovery; hundreds of genes are differentially expressed in murine bone and marrow during the post-weaning interval.[26]

The Long-Term Outlook on Skeletal Health

Bjornerem and colleagues concluded their report in this issue by stating that further study is needed to determine whether the changes they observed in skeletal microarchitecture of the distal tibia and radius have any long-term effects on skeletal fragility. But there is already a substantial body of data suggesting that the skeleton recovers full strength after weaning. In most (but not all) animal models, vertebral crush and 3-point bend tests show no differences between those that went through post-weaning recovery compared with prepregnancy or age-matched virgins.[1] More than 5 dozen epidemiological studies have found a neutral or protective effect of lactation on peak bone mass, bone density, or fracture risk.[1] This includes a large study of twins who were discordant for lactation history.[34] NHANES III found that among 819 women aged 20 to 25 years, those who breastfed as adolescents had higher BMD than women who had not breastfed or were nulliparous.[35] Comparatively few studies have reported that lactation increases long-term fracture risk,[1] and so there may be certain women or clinical circumstances in which skeletal strength is permanently compromised by breastfeeding.

However, the bulk of available data suggest that adolescents and adults do not suffer long-term adverse consequences from lactation, and might even be protected against future fractures. Skeletal strength may be fully restored after lactation for most women, despite the appearance at the microstructural level that the skeletal storehouse was plundered during lactation and not fully replenished afterward.

Disclosures

The author states that he has no conflicts of interest.

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