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COMMENTARY

Mammals and minerals: a story of lactation and lacunae

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Commentary on: Qing H, Ardeshirpour L, Pjevic PD, Dusevich V, Jähn K, Kato S, Wysolmerski J, Bonewald LF. Demonstration of osteocytic perilacunar/canalicular remodeling in mice during lactation. J Bone Miner Res. 2012; **27**:1018–1029.

Pregnancy and lactation demand a significant calcium transfer from the mother to the fetus and neonate, a topic which has been well reviewed by Kovacs.[1] Whereas the fetal calcium demand seems to be met largely by increased calcium absorption across the gut, milk calcium is sourced by a 'temporary borrowing of calcium from the skeleton,' together with renal calcium conservation.[1] The mechanism controlling calcium efflux from the skeleton during lactation (280–400 mg per day in humans) is not well understood but has been assumed to be carried out by osteoclastic resorption, and calcitonin has a critical role in its regulation.[2] Qing *et al.*[3] have now found evidence, in an elegant series of experiments and analytical approaches, that liberation of calcium from maternal bone during lactation is also contributed to by osteocytic osteolysis. They have further shown involvement in this process of the parathyroid hormone receptor 1 (PTHR1) on osteocytes, and identified a number of genes that are elevated in osteocytes during lactation, which are also utilised by bone resorbing osteoclasts. Interestingly, osteocytic osteolysis occurred in lumbar vertebrae, tibiae and femora, but not in the calvaria. Earlier attempts to implicate osteocytes in mobilising calcium during lactation came to different conclusions, possibly due to less informative technology. Thus, although Rasmussen[4] found that a calcium-depriving regimen in female rats, which included lactation, caused removal of bone seemingly without osteoclast involvement, no signs of osteocytic osteolysis were observed. Similarly, osteocyte lacunar volume showed no increase in lactating rats, in a study reported by Mercer and Crenshaw.[5]

The Qing *et al.* study raises several questions: these include the nature of the mineral removal process by osteocytes; the molecular mechanisms involved; and the drivers of lactation-induced osteocytic osteolysis. First, how is the mineral removed, if slowly over the course of the lactation period, would this contribute significantly to total calcium in the milk, or is mineral removed and replaced continuously? If the former is the case, then the detection of osteocytic osteolysis might depend on when one looked. If the latter, then detection by electron backscatter microscopy would require the peri-lacunar mineral to be somehow different from that in non-lactating animals. Whatever the case, the Qing *et al.* findings are consistent with a recent report, which described in some detail the micro-architectural changes in bone during lactation and weaning, and which found a 10% loss and recovery in central trabecular tissue mineral density in lactating and recovered mice.[6] Changes in mineral density deep in the trabeculae are more likely attributable to loss of mineral by a mechanism other than bone remodelling.

Second, how might lactation-induced osteocytic osteolysis work at a molecular level? The gene microarray expression analysis suggests that similar mechanisms might apply as those found in resorbing osteoclasts. Thus, proton transporters and matrix-degrading enzymes were increased in osteocyte-enriched bone from lactating animals compared with post-lactation animals. The increase observed in matrix metalloprotease (MMP)-13 expression is of interest following a recent report, suggesting that MMP-13 expression by osteocytes has an essential role in the remodelling of cortical bone matrix and the maintenance of bone quality.[7] These authors found that deletion of the *MMP-13* gene results in significant changes in the tissue mineral distribution and the collagen organisation of the bone matrix, independently of changes in osteoclast-mediated bone resorption and osteoblast-mediated bone formation.[7]

Osteocyte expression of the PTHR was shown to be critical for lactation-induced osteocytic osteolysis because its deletion attenuated the decrease in bone mineral density due to lactation and prevented the increase in osteocyte volume seen in wild-type animals. Interestingly, Barrett *et al.*[8] found that PTH elicited a receptor-mediated increase in extracellular acidification in SaOS-2 cells, which, at least in terms of their sclerostin expression,[9] have some characteristics of osteocytes. This action of PTH was not dependent on the cyclic AMP/protein kinase A signalling pathway or the Na/H^+ exchanger. It is not known whether it involved a member(s) of the carbonic anhydrase family, two of which were shown by Qing *et al.* to be upregulated in bone during lactation.

The Qing *et al.* study suggests that the lactation effect on osteocytes is due to activation of the PTHR. It has been reported recently that lactation increases expression of bone receptor activator of nuclear factor-κB ligand (RANKL) mRNA[10] and that RANKL induction was reduced in mice lacking the distal control

region enhancer in the *RANKL* gene that mediates regulation by PTH. However, despite the attenuated RANKL response to lactation in distal control region knockout mice, loss of bone, measured in terms of changes in bone mineral density, was similar to wild-type mice. These authors did not investigate the cellular origin of RANKL in lactation, although the same group have also shown the physiological importance of osteocyte-derived RANKL.[11] Suggesting a role for RANKL in lactation-mediated mineral release from bone, Onal *et al.*[10] observed a lactation-induced decrease in osteoprotegerin expression in both distal control region knockout mice and wild-type mice, suggesting an increased bioavailability of RANKL. They also found that circulating RANKL decreased in both genotypes, in the face of increased RANKL mRNA levels in bone, and speculated that lactation may reduce RANKL shedding, again increasing the bioavailability of RANKL in bone to support osteoclast formation and activation. Curiously, neither RANKL nor osteoprotegerin changes during lactation were revealed in the Qing *et al.* paper. There is also no mention of lactation-induced changes in the *SOST* gene expression, although a reduction in SOST/sclerostin expression might be expected if the effects on osteocytes in lactation are PTHR1-mediated.[12] Introduction of a constitutively active PTHR1 into osteocytes increased bone turnover and concomitantly decreased the expression of sclerostin, and increased the expression of RANKL and macrophage colony-stimulating factor.[13] The effect on osteocytes *per se* was not reported. Paradoxically, we found that treatment of osteocytes with exogenous sclerostin increased their expression of RANKL and their capacity to promote osteoclastic bone resorption.[14] Thus, the molecular mechanisms of lactation-mediated osteocyte osteolysis remain to be elucidated. Related questions also to be addressed are those of how calcium release from bone in lactation is partitioned between osteoclast and osteocyte action, what is the relative contribution of each cell type and are the bone-resorbing activities of osteoclasts and osteocytes interdependent? More generally, how is osteocytic osteolysis contained compared with the more extensive osteoclastic resorption, which is presumably limited by osteoclast survival?

Third, the Qing *et al.* paper suggests that activation of the PTHR1 receptor is the driver of lactation-mediated osteocyte osteolysis. The endocrinology of calcium control in lactation has received a great deal of previous attention. Circulating prolactin is obviously higher in lactation, estradiol is lower and 25(OH)vitamin D_3 and 1,25$(OH)_2$vitamin D_3 levels are reportedly unchanged.[15] Calcitonin may have an important role in protecting bone during lactation, as deletion of the combined calcitonin/*CGRP* gene in mice resulted in dramatically more bone loss during lactation than in wild-type mice;[2] daily treatment with calcitonin from the onset of lactation, but not calcitonin gene-related peptide, normalised the bone losses in the double-knockout animals. PTH levels are apparently stable during lactation and lactation-induced bone loss has been reported not to be dependent on either vitamin D or PTH.[16] In contrast, Vanhouten *et al.*[17] have reviewed a series of studies that suggest a role for PTH-related protein (PTHrP) in lactation-induced bone loss. As PTHrP expression is induced in the lactating breast, and as PTHrP levels are high in milk and increased in the circulation during lactation, these authors investigated the result of specifically deleting the *PTHrP* gene in the mammary gland. They reported that: 'in the absence of mammary gland-derived PTHrP, plasma PTHrP levels are lower [during lactation], bone turnover is lower, and bone mass is preserved. These results suggest that the mammary gland actively participates in the regulation of maternal bone loss during lactation by releasing PTHrP into the circulation to act as an endocrine mediator of bone resorption'. They did not distinguish between osteoclast and osteocyte-mediated bone loss.

In summary, the Qing *et al.* paper provides strong evidence for the physiological relevance of osteocytic osteolysis during lactation. Physiologically, this process may apply only to bone loss that is required to contribute to calcium homeostasis, because bone loss due to unloading did not result in changed osteocyte lacunar volume. It now remains to elucidate the mechanisms of osteocytic osteolysis. Defining other physiological and pathological bone loss pathways that invoke osteocytic osteolysis represents an exciting area for future research. Specifically, it will be important to determine whether osteocytic osteolysis might have a role in cancer-associated hypercalcemia, in which PTHrP circulates at high levels in the majority of patients.[17]

Conflict of Interest

The authors declare no conflict of interest.

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