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PERSPECTIVES

Conversations Between Breast and Bone: Physiological Bone Loss During Lactation as Evolutionary Template for Osteolysis in Breast Cancer and Pathological Bone Loss After Menopause

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Abstract

Reproduction in mammals is associated with an impressive cycle of bone loss and repair. Lactating mothers lose bone rapidly to supply calcium for milk production. After weaning, bone mass recovers almost as quickly as it was lost. This phenomenon had been well described for many years, but recent studies have begun to paint a clearer picture of the hormonal and molecular regulation of this transient skeletal demineralization. Bone loss occurs as the result of a coordinated conversation between the breast and bone. Suckling induces hypothalamic hypogonadism and the lactating breast secretes parathyroid hormone-related protein into the circulation. Low estrogen levels and elevated PTHrP levels act in combination to liberate skeletal calcium stores. Calcium then feeds back on the breast via the calcium-sensing receptor to regulate calcium transport into milk and PTHrP production, defining a feedback loop between breast and bone. When lactation ends, bone resorption is suddenly halted, but little is currently known of the mechanisms that mediate bone recovery after weaning. These alterations in bone and mineral metabolism during the reproductive cycle are descendants of ancient adaptations that evolved to support egg production in lower vertebrates. Therefore it is interesting to consider if pathological bone resorption in patients with breast cancer and in post-menopausal women may represent the accidental reactivation of circuitry designed to supply calcium for milk production. A better understanding of the skeletal response to weaning may offer clues to new therapies for bone disease. *BoneKEy*. 2007 August;4(8):209-225.

Introduction

All mammals spend a variable period after birth completely dependent on their mothers for survival. During this time, the mother secretes milk, a remarkable fluid that contains all of the energy and raw materials that newborns require for extremely rapid post-natal growth. A great deal of calcium is needed for skeletal expansion during this growth spurt. Not surprisingly, milk from all species is rich in this nutrient. Although critical for the health of the neonate, lactation is metabolically costly for the mother, and maternal physiology must adapt to enable both mother and infant to survive. Given their importance for reproductive success, these adaptations are evolutionarily well conserved. This *Perspective* will first consider the maternal adjustments to calcium and bone metabolism that support milk production. Second, it will explore how bone and mineral metabolism are integrated with calcium handling and milk secretion by the breast. Third, it will describe how bone turnover reverses upon weaning. Finally, it will consider the evolutionary origins of these adaptations and will speculate on how inappropriate activation of these conserved pathways may contribute to bone disease in women.

Calcium and Bone Metabolism During Lactation

The prolonged egress of calcium into breast-milk is a significant stress on maternal calcium metabolism during lactation. Nursing humans secrete between 300 mg to 400 mg of calcium into milk on a daily basis and would be expected to export more than

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80 grams of calcium to their child over 9 months (1-4). In response to this demand, maternal calcium and bone metabolism become significantly altered. In humans, total and ionized calcium levels have been reported to rise slightly during lactation, and serum phosphate levels are elevated (2;3). Parathyroid hormone (PTH) levels are decreased compared to non-lactating controls, while calcitonin and 1,25 dihydroxyvitamin D (1,25(OH)2D) levels, which had been elevated during pregnancy, return to normal (2;3). Some of these changes appear to be species-specific, as calcium and PTH levels in lactating mice are unchanged (5), while calcium has been reported to be decreased and PTH slightly increased in lactating rats (3). Finally, during lactation, circulating levels of parathyroid hormone-related protein (PTHrP) are elevated (5-10). PTHrP is a peptide growth factor ancestrally and

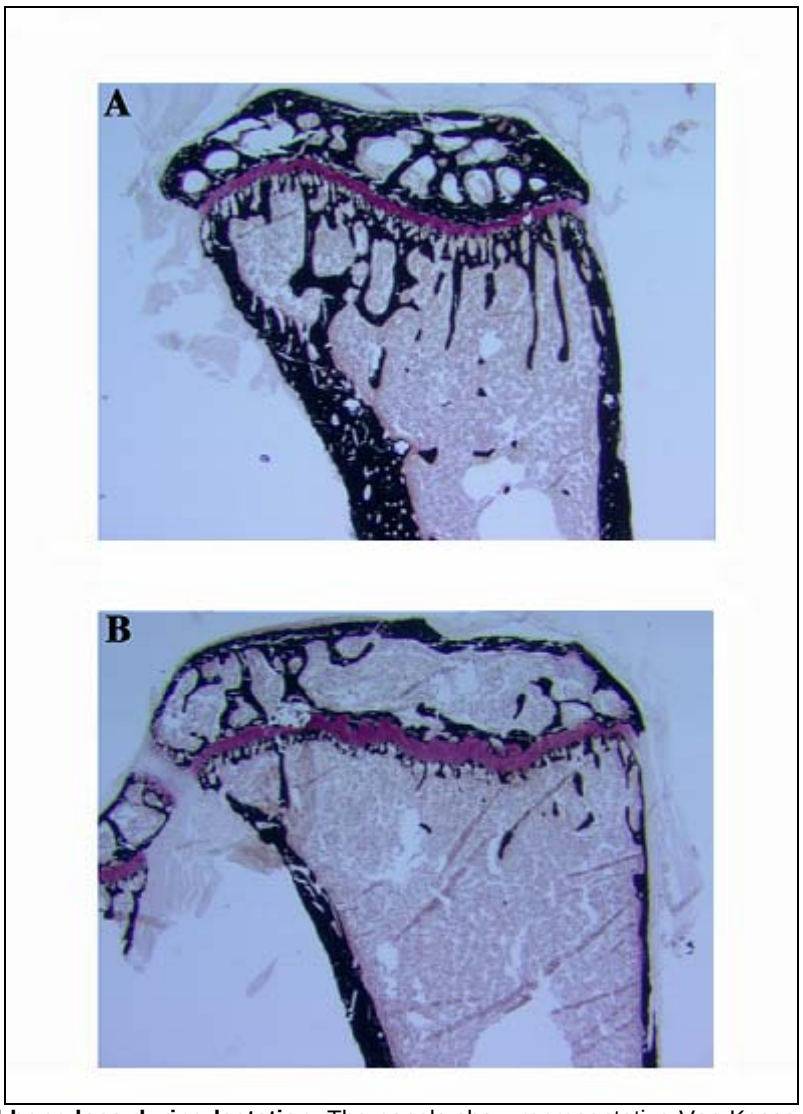

Figure 1. Rapid bone loss during lactation. The panels show representative Von Kossa-stained sections through the proximal tibia of a virgin mouse (A) and a mouse on day 12 of lactation (B). Note the dramatic loss of trabecular elements during lactation as well as the significant thinning of the cortex of the bone.

structurally related to PTH (11;12). It was initially described as a cause of humoral hypercalcemia in patients with cancer. This occurs because it engages the same type I

PTH/PTHrP receptor (PPR1) as PTH, and can mimic the actions of PTH when infused into animals or people (11;12). However, PTHrP generally acts locally in a paracrine and/or autocrine fashion and, other than in malignancy, lactation appears to be the only instance in which PTHrP circulates. During lactation, PTHrP is secreted in a regulated fashion by the breast and, as will be discussed below, it participates in the mobilization of skeletal calcium stores (10;13).

The calcium secreted into milk likely comes from several sources. Unlike what occurs during pregnancy, 1,25(OH)2D levels and, presumably, the efficiency of dietary calcium absorption are not elevated during lactation. However, suckling stimulates appetite centers in the hypothalamus to induce hyperphagia (3;14). Thus, food intake is increased and some of the extra calcium comes from the diet. During lactation, the kidneys retain calcium, and urinary calcium excretion declines to very low levels (3;4). This may be due to the elevated levels of circulating PTHrP, although this hypothesis has not been formally tested. Thus, some calcium is reclaimed from the urine. Finally, lactation is associated with impressive bone loss and it has been assumed that much of the calcium that is used for milk production comes from the skeleton (3;4). However, no studies have addressed the specific contribution of these individual sources, and their relative importance is difficult to discern. Given the importance of milk to reproductive success, it is likely that there is considerable redundancy between the three sources of calcium that support its production.

As noted above, maternal bone mass declines during lactation (2-5;15-22). In nursing humans, bone mineral density has been shown to fall between 6-10% over the first 6 months following delivery. Bone loss occurs rapidly, at an estimated rate of between 1-3% per month, which, by comparison, approximates the yearly rate of bone loss following menopause (3). Sites of trabecular bone are the most severely affected, although cortical and whole body bone mass also decline. The decline in BMD is the result of milk production, as women feeding formula do not lose bone (15;17). It also correlates with the amount of milk produced; women nursing twins and triplets lose more bone than women nursing just one baby (19;22). Consistent with this observation, rodents, which typically nurse many more offspring than humans, lose up to 20-30% of their bone mass over 3 weeks of lactation (5;10;22-25) (Fig. 1). A recent study in mice has shown that lactation is associated with changes in bone microarchitecture and bone mineralization as well as bone density (26). Micro-CT measurements demonstrated thinning and perforation of trabecular plates, cortical thinning, increased cortical tunneling and a decrease in tissue density (Fig. 2). As might be expected, studies in rats have demonstrated that these changes in bone mass and architecture diminish bone strength (22;27) and, occasionally, women present with fragility fractures while nursing (3).

Bone loss during lactation is associated with increased bone turnover. Human and animal studies have demonstrated elevations in both bone resorption and formation, although as evidenced by the rapid bone loss, resorption outstrips formation (3;5;28-32). Biochemical markers of bone resorption have been reported to be 2-3-fold elevated in mice and humans, and markers of bone formation have been demonstrated to be elevated in nursing humans. Histomorphometric data are available for rats, mice and beagles (5;26;33;34). Osteoclast numbers and activity are increased at both trabecular and cortical sites. Cortical bone resorption appears to occur mostly at endocortical surfaces, although periosteal bone resorption has been noted in lactating rodents on calcium-restricted diets. Consistent with these morphometric measurements, bone marrow culture experiments have suggested an increase in the number of osteoclast progenitors in lactating mice (26). In addition, dynamic histomorphometry has demonstrated that bone formation rates, as measured by double-fluorescent labeling techniques, are elevated in lactating as compared to nulliparous females (5;26;33;34). Similarly, there is an increase in the number of osteoblast progenitors in

bone marrow cultured from lactating as compared to nulliparous mice (26). Thus during lactation, the differentiation and activity of both osteoblasts and osteoclasts are accelerated, although that of osteoclasts more so, leading to a form of high-turnover bone loss.

The phenomenon of bone loss during lactation is well described, but we do not fully understand the mechanisms that drive it. Neither PTH nor vitamin D, the two classic calciotropic hormones, triggers this decline in skeletal mass. Studies in animals and experience with patients has suggested that

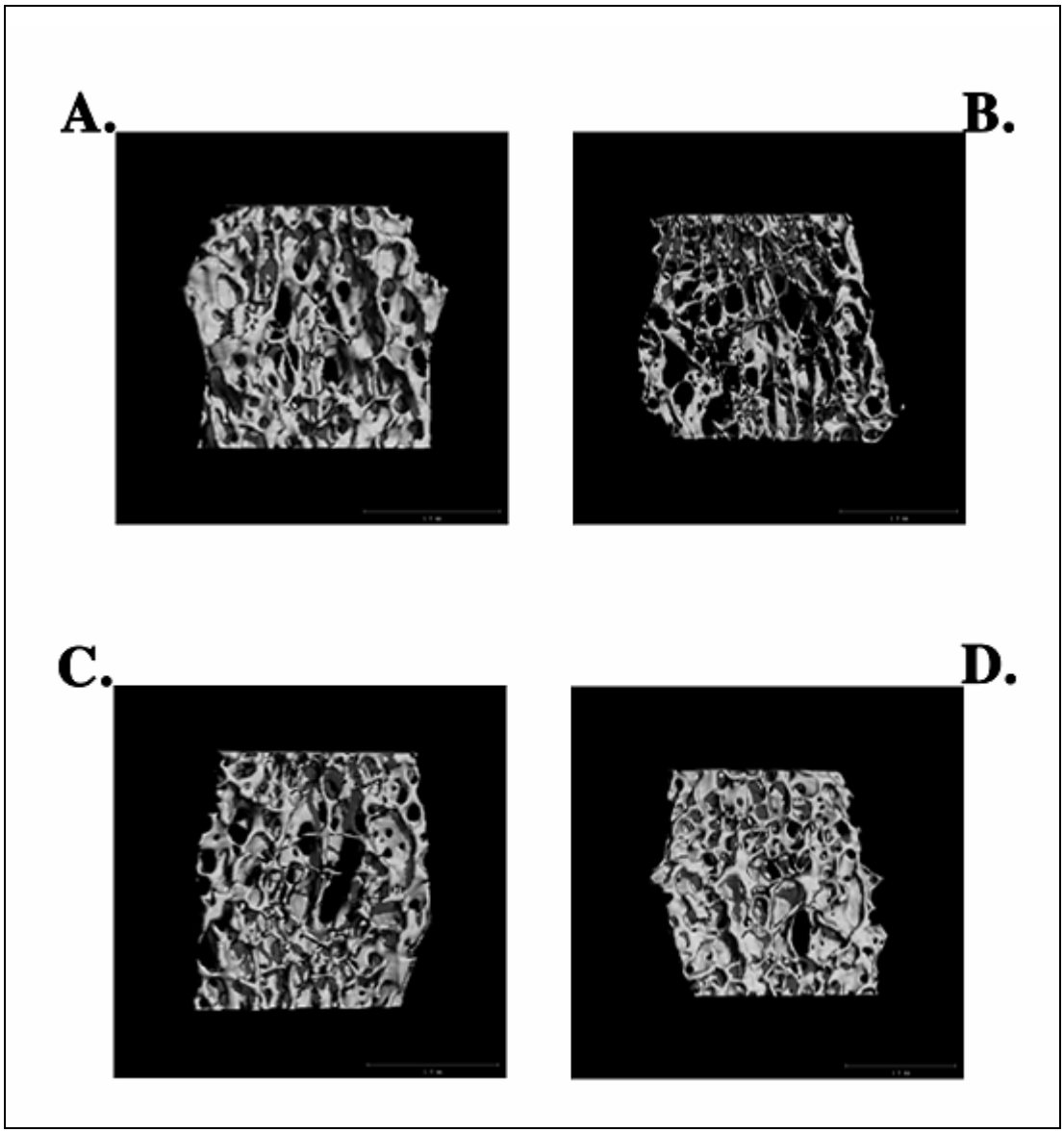

Figure 2. Changes in bone microarchitecture during lactation and after weaning. Three-dimensional reconstructions of representative lumbar vertebrae from nulliparous (A) and lactating (B) mice, as well as mice 7 days (C) and 28 days (D) after weaning. Lactation is associated with trabecular thinning, trabecular perforation and the shift from a plate-like to rod-like appearance of trabeculae (compare A and B). After weaning (C and D), trabecular architecture rapidly reverts back to that seen in nulliparous animals. Reproduced from Ref. 26 with permission.

lactational bone loss proceeds in the absence of either of these two hormones (3;35-37). The current working model is that bone loss is mediated by the combination of a fall in circulating levels of estradiol and a rise in circulating levels of PTHrP. In contrast, circulating calcitonin acts to constrain bone loss during lactation (38). These three factors will be discussed in the next three paragraphs. Note that the breast itself becomes a direct participant in regulating calcium and bone mass during lactation.

The act of suckling stimulates afferent nerves in the breast that connect through the brainstem to stimulate the hypothalamus, which, in response, inhibits GnRH secretion (14;39). Suppression of GnRH secretion, in turn, leads to hypogonadotropic hypogonadism and a decline in circulating levels of estradiol (Fig. 3). Additionally, despite adequate fat stores after pregnancy, leptin levels are low during lactation and suckling stimulates prolactin secretion from the pituitary gland (14;39). Both low leptin and high prolactin levels reinforce the hypogonadism and low estrogen levels. Estrogen deficiency is well known to cause elevations in bone turnover and bone loss, so it is reasonable to assume that estrogen deficiency contributes to lactational bone loss (40;41). Human studies have found a correlation between the degree of bone lost and the duration of amenorrhea post-partum (3;42). Furthermore, estrogen levels correlate with rates of bone resorption and pharmacologic estrogen replacement led to a reduction in bone loss in lactating mice (5). However, estrogen deficiency is not likely to be the sole cause of bone loss post-partum. As Kovacs and Kronenberg have emphasized, the rate of bone loss in nursing women far exceeds that in women rendered estrogen-deficient with GnRH analogues (3). Bone loss in lactating rodents also exceeds that seen after ovariectomy (3). In addition, although estrogen replacement reduced the rate of bone loss in lactating mice by 60%, it did not entirely prevent it, results consistent with clinical observations that nursing women can continue to lose bone after the return of menses (43;44).

The lactating breast secretes PTHrP both into the systemic circulation and into milk (10;45;46). A great many studies have now documented that circulating PTHrP levels are elevated in lactating women and rodents (5;7;9;10;13;47-50). Plasma levels of PTHrP correlate directly with biochemical markers of bone resorption and inversely with changes in bone mass in lactating mice (5). Sowers and colleagues demonstrated a correlation between bone loss and an elevation in circulating PTHrP levels in nursing women as well (9). Finally, selective disruption of the PTHrP gene in mammary epithelial cells at the onset of lactation reduced circulating levels of PTHrP, reduced rates of bone turnover and reduced bone loss by 50% in mice (10). Thus, PTHrP, secreted from the breast, contributes to the mobilization of skeletal calcium stores during lactation. Given several studies that have shown that estrogen deficiency amplifies the catabolic effects of continuous PTH infusion on the skeleton, it is likely that during lactation, PTHrP synergizes with estrogen withdrawal to cause bone loss (51-56).

There may also be specific mechanisms that protect the skeleton from excessive bone loss during lactation. A series of older reports suggested that calcitonin might act to inhibit osteoclastic bone resorption during lactation (57;58). Recent studies from the Kovacs laboratory have confirmed this hypothesis by showing that calcitonin-deficient mice lose up to 50% of their trabecular bone mass during lactation, as compared to the usual 20-30% (38). Other recent studies have shown that mammary epithelial cells can secrete calcitonin (59), although it is not known if the breast, thyroid gland or other tissue is the major contributor to the circulating pool of calcitonin during lactation.

Crosstalk Between Breast and Bone: A Newly Appreciated Feedback Loop

The lactating breast secretes PTHrP, which circulates and activates bone resorption in the skeleton in order to mobilize calcium to make milk. Moreover, recent experiments suggest that PTHrP production is regulated in response to calcium delivery to the breast, defining a classic negative feedback loop

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between breast and bone during lactation. The Brown laboratory was the first to show that the calcium-sensing receptor (CaR) was expressed in normal and malignant human breast tissue (60). Subsequent studies from this laboratory also demonstrated that the CaR was expressed on human breast cancer cell lines and that stimulation of the CaR in breast cancer cells stimulated PTHrP production (61). In light of these publications, our laboratory became

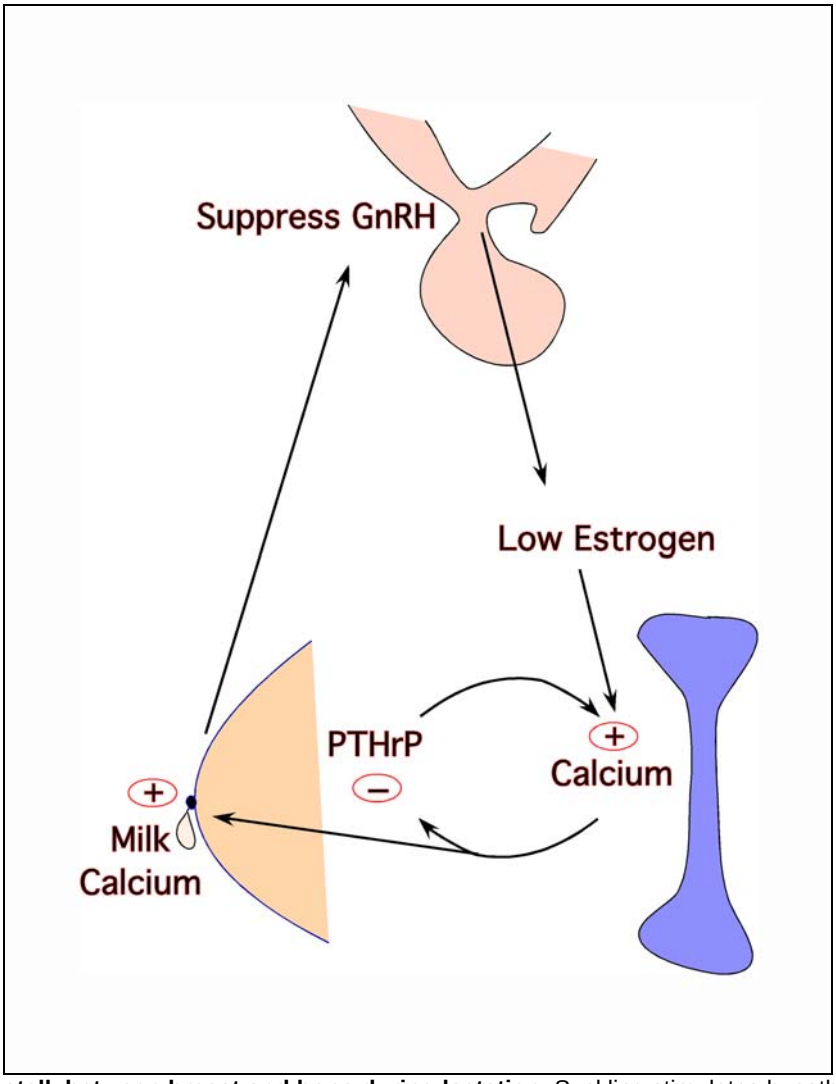

Figure 3. Crosstalk between breast and bone during lactation. Suckling stimulates hypothalamic centers that then suppress GnRH secretion. This leads to hypothalamic hypogonadism and low estrogen levels, which stimulates bone resorption. The breast secretes PTHrP into the circulation, which also stimulates bone resorption. The bone resorption caused by increased PTHrP and low estrogen levels liberates calcium from the skeleton into the circulation. When calcium is delivered to the lactating breast, it stimulates the calcium-sensing receptor, which promotes calcium transport into milk and inhibits PTHrP secretion from the breast, defining a classic negative feedback loop between breast and bone. If calcium delivery to the breast falls, calcium usage (transport) is decreased and more PTHrP is produced to increase bone resorption, liberating additional skeletal calcium and preventing hypocalcemia. The calcium-sensing receptor allows mammary epithelial cells to monitor their calcium supply and to coordinate their demand for calcium and maternal bone metabolism accordingly.

interested in the function of the CaR in the normal mammary gland. We found that the CaR gene is expressed at low levels in the mammary glands of virgin and pregnant mice, but that CaR mRNA expression was greatly increased during lactation where it could be detected on the basolateral surface of the epithelial cells (13). In contrast to malignant breast cells, stimulation of the CaR by either calcium or calcimimetic agents suppresses PTHrP production by normal mammary epithelial cells *in vitro* and *in vivo* (13). In keeping with these findings, mammary epithelial cells from CaR(+/-) mice increase PTHrP production by approximately two-fold (62). Therefore, as demonstrated in Figure 3, during lactation the breast becomes a calcium-sensing organ that uses PTHrP to regulate bone resorption and to ensure a steady supply of calcium for milk production. If calcium delivery to the gland falls, more PTHrP is produced, which, in turn, increases efflux of calcium from the maternal skeleton. This calcium then feeds back on the mammary gland in order to suppress further PTHrP production. In this manner, the lactating breast acts like an accessory parathyroid gland, using PTHrP instead of PTH to regulate (or even co-opt) bone metabolism for its own purposes.

In addition to its role in regulating calcium delivery to the lactating mammary gland, the CaR also regulates calcium consumption by mammary epithelial cells. We have found that activation of the CaR on these cells stimulates the trans-epithelial transport of calcium into milk (13;62). Some calcium is transported into milk through the secretory pathway, bound to casein in macromolecular complexes known as micelles (63). However, the majority of calcium in milk is transported across the apical membrane of mammary epithelial cells through the actions of a specific calcium pump, the plasma membrane calcium-ATPase type 2bw (PMCA2) (64;65). We have recently found that activation of the calcium receptor on the basolateral surface of breast cells stimulates the enzymatic activity of the apical PMCA2, thus increasing calcium transport into milk (65). Therefore, in the lactating breast, the CaR serves to coordinate calcium supply and calcium demand. Acting in similar fashion to its ancestral periplasmic proteins, the CaR serves as a nutrient sensor (66). If calcium is plentiful, CaR signaling inhibits PTHrP production and stimulates calcium transport and milk production. However, if calcium becomes limiting, then calcium transport and consumption are decreased, and PTHrP production is increased, calling forth more calcium from skeletal stores (see Fig. 3). We hypothesize that these actions of the CaR help prevent the development of hypocalcemia during lactation, especially when dietary calcium becomes limiting. A full test of this idea awaits the development of genetic models disrupting the CaR gene specifically in mammary epithelial cells.

Recovery of Bone Mass After Weaning

Perhaps the most remarkable aspect of the bone loss associated with lactation is its rapid and complete reversibility after weaning (27;67-69). In rodents, the 20-30% of skeletal mass that is lost is almost completely restored within 4 weeks after suckling ceases. In humans, bone mineral density is restored to baseline within 6-12 months after nursing has stopped (3;16;18;70). During this burst of anabolic activity in humans, bone is added at an estimated rate of 0.5-2% per month (3). Therefore, just as lactation represents the most rapid period of bone loss in the adult skeleton, post-weaning is the period of the most robust increase in bone mass in the adult skeleton. Epidemiological data in women have shown that neither the duration of lactation nor the number of offspring nursed increases the risk for lower bone mass or for osteoporotic fracture later in life (17;71). The lack of any cumulative damage to the skeleton from multiple cycles of lactation underscores the efficient nature by which bone mass is restored after each individual bout of reproduction.

Many studies have documented that bone mineral density recovers after weaning, but only a few have attempted to define the mechanisms that govern this response. Miller, Bowman and colleagues have reported a series of detailed histologic and histomorphometric studies examining the post-lactation period in rats (27;67;68). They have shown that bone mass increases

back to baseline values over a period of 4-8 weeks after lactation ceases. Their studies revealed full recovery of trabecular number, thickness and connectivity as well as biomechanical strength by 4-6 weeks following weaning. They reported a substantial increase (up to 800%) in bone formation rates at 2 weeks, with a return to baseline rates of bone formation by 6 weeks (67;68). Coincidently, the numbers of osteoclasts and the extent of the eroded surface decreased, suggesting that the elevated rate of bone resorption so typical of lactation decreases after weaning (67;68).

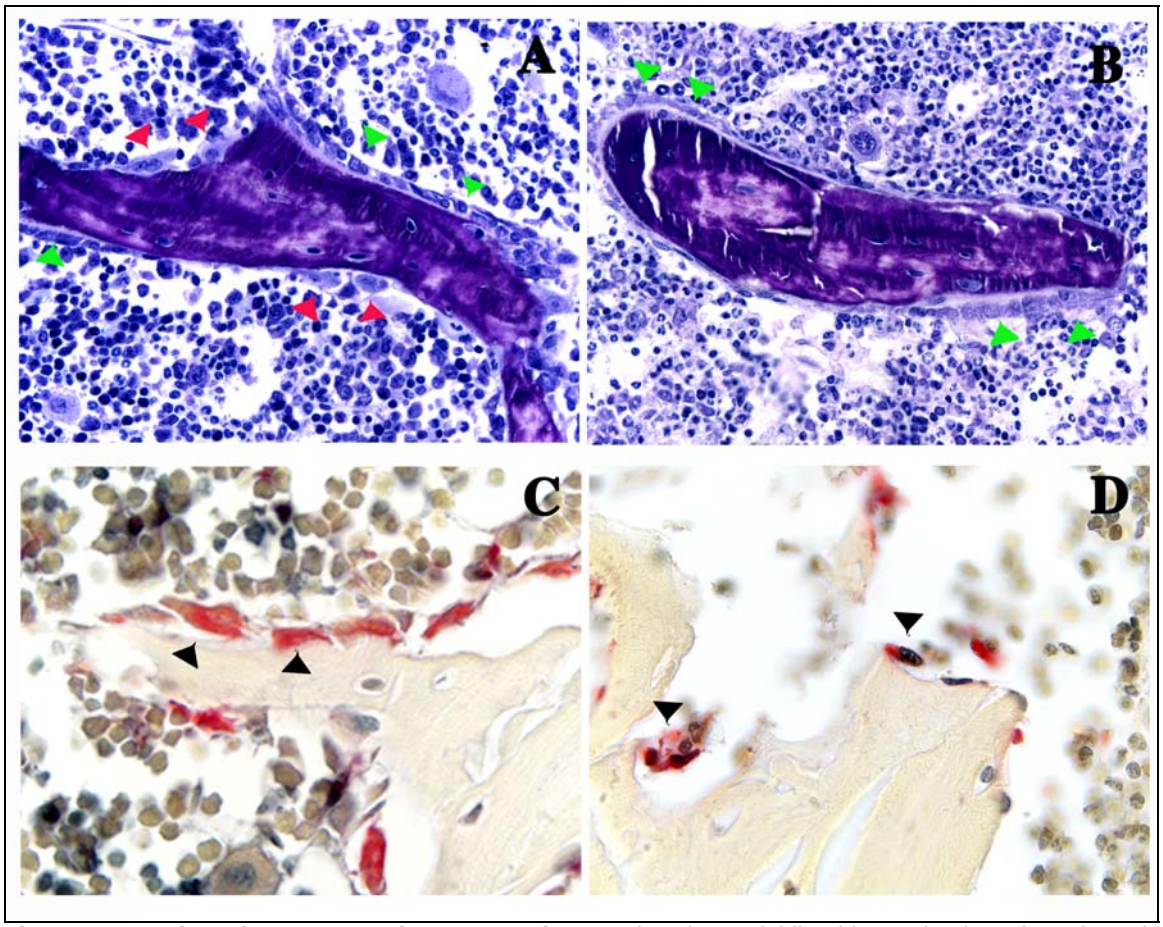

Figure 4. Weaning triggers osteoclast apoptosis. A and B show toluidine blue-stained sections through the proximal tibia from mice at 12 days of lactation (A) and 3 days after weaning of pups (B). During lactation, both osteoclasts (red arrowheads) and osteoblasts (green arrowheads) are plentiful on trabecular surfaces. However, 3 days after weaning (B), osteoclasts are much reduced in number and osteoblasts surround many individual trabeculae. C and D demonstrate sections of bone that have been stained for both acid phosphatase activity and subjected to TUNEL assay. Osteoclasts stain red and apoptotic nucleii stain black. On day 12 of lactation (C), acid phosphatase-positive osteoclasts are abundant and are TUNEL negative (black arrowheads). One can appreciate TUNEL positive cells in the bone marrow, which serve as an internal, positive control. 48-hours after weaning (D), the overall numbers of acid phosphatase-positive cells are reduced. In addition, acid phosphatase-positive cells are frequently separated from the bone surface, appear fragmented and are TUNEL positive (black arrowheads), consistent with the occurrence of widespread osteoclast apoptosis. Reproduced from Ref. 26 with permission.

Our laboratory recently examined the transition from bone catabolism to bone anabolism at weaning in mice in detail (26). Using serial DEXA measurements, we found that bone mineral density begins to increase immediately after weaning and by 28 days

after lactation had increased by 37% in the spine, 27% at the femur and by 25% for the whole body. Underscoring the rapidity of the response, bone density was statistically significantly higher as early as 3 days after weaning in the spine and total body, and by 7 days after weaning in the femur. These findings were corroborated by quantitative micro-CT measurements, which documented rapid reversal of the previously discussed changes in skeletal microarchitecture that occur during lactation (Fig. 2). Measurement of bone turnover markers and static and dynamic histomorphometry revealed a sudden halt to bone resorption due to a coordinated wave of osteoclast apoptosis occurring between 48 and 72 hours after forced weaning (Fig. 4). A similar apoptotic response has also been recently noted in osteoclasts in weaned rats (72). In contrast, osteoblast numbers and bone formation rates, which are already elevated during lactation, were maintained after weaning, although not increased as had been reported in rats (67;68). We also cultured osteoprogenitor cells from the long bones of lactating and weaned mice. Mirroring the changes seen on histomorphometry, as compared to lactation, cultures from weaned mice produced few osteoclasts, but there appeared to be a further increase in the numbers of osteoblasts (26). These results demonstrate that weaning results in dramatic changes in bone turnover and bone cell differentiation. Osteoclasts suddenly disappear and bone resorption halts, while accelerated osteoblast activity continues, resulting in relatively unopposed bone formation and the rapid restoration of bone mass.

The above data suggest that, in mice, a key event in halting bone loss and beginning the anabolic recovery of the skeleton is the sudden death of osteoclasts. Osteoclasts are derived from monocytes under the regulation of several hormones, including PTHrP and estrogen, each of which influences the local production of cytokines that, in turn, regulate osteoclast differentiation, activity and survival (41;73-75). Perhaps the most significant of these factors are RANKL and osteoprotegerin (OPG) (41;73-75). We found that RANKL mRNA levels are elevated in bones from lactating mice as compared to those from nulliparous mice. After weaning, RANKL mRNA expression decreased but OPG expression remained constant. Therefore, weaning precipitates a significant decline in the RANKL/OPG ratio, which may explain the observed wave of osteoclast apoptosis as well as the decline in committed osteoclast precursors noted in the bone marrow culture experiments.

Evolutionary Origins of Calcium and Bone Metabolism During Lactation

The need to provide calcium for the rapid skeletal growth of offspring is not a burden unique to mothers in *mammalia*. Instead of providing this calcium via the placenta and in milk, lower vertebrates transfer large amounts of calcium into the yolk and shell of their eggs, where it is gradually utilized by the developing embryo (76). During egg production, elevations in circulating levels of estrogen drive hepatic production of vitellogenins, phosphate-rich proteins, which bind calcium avidly (77;78). These calcium and vitellogenin complexes are transported into the developing yolk, after which more calcium is deposited into the shell as the egg matures. This latter stage is especially prominent in birds since they produce eggs with a thick shell containing abundant calcium carbonate (77). Calcium metabolism during egg production resembles a hybrid of the patterns described for pregnancy and lactation in mammals. Although the particulars vary with species, calcium used for egg production comes from a combination of increased dietary absorption, increased reclamation by the kidneys and the mobilization of internal stores from bones, scales and, in turtles, shells (76). Thus, for the current discussion, it is important to note that the mobilization of skeletal calcium stores for the sake of reproduction is a very ancient adaptation that predated the development of milk production in mammals. Furthermore, the mobilization of skeletal calcium during egg production is regulated by changes in circulating levels of estrogens and, during shell calcification in laying hens, by PTH (79). Therefore, during the evolution of mammals, a series of alterations in maternal

hormones and bone and mineral metabolism that had already developed in response to the calcium demands of egg production was adapted to meet similar challenges during pregnancy and lactation.

Are There Echoes of Lactation in Bone Diseases?

The previous section underscored the fact that transient demineralization of the skeleton in response to hormonal changes is an important aspect of reproduction that is conserved from fish to human beings. Furthermore, the active participation of the breast in regulating bone metabolism during lactation suggests that the ability to cause bone resorption is an intrinsic characteristic of the differentiated mammary epithelial cell. During normal reproduction, calcium taken from the skeleton during lactation is promptly replaced after lactation ceases, and nursing does not lead to permanent bone loss. However, there may be several instances in which this strongly conserved physiology is activated inappropriately, leading to pathological bone resorption and disease.

Several authors have suggested that pathological bone loss after the menopause may represent the inappropriate reactivation of the mechanisms designed to provide for the physiological bone loss of lactation (3;76;80). This idea is based on several similarities between the two conditions. First, both states are characterized by elevated, yet relatively unbalanced rates of bone turnover, such that bone resorption outstrips bone formation and leads to net bone loss. Second, both forms of bone loss are more pronounced in trabecular bone. Third, estrogen deficiency is an important contributing factor to both processes. In fact, as discussed previously, the emergence of estrogen receptors in bone during evolution appears to have coincided with the first use of the maternal skeleton as a source of calcium for egg formation in lower vertebrates (76). Furthermore, lactation represents the only naturally occurring period of estrogen deficiency during a mammal's reproductive lifespan. Thus, the ability to store and mobilize calcium for reproductive purposes may represent one of the main reasons for the skeleton's estrogen responsiveness, and post-menopausal osteoporosis is likely a post-reproductive consequence of the evolutionary program activating bone resorption in response to low estrogen levels during lactation. If this construct is true, then the complete recovery of bone mass after weaning holds forth the tantalizing possibility that we might learn to manipulate this response in order to heal osteoporosis fully.

Primary hyperparathyroidism is a disease principally of post-menopausal women, which can lead to progressive bone loss. Given their use of the same PTH1R, it is not surprising that the catabolic response of the skeleton in response to continuous exposure to PTHrP during lactation is similar to the catabolic response of the skeleton to continuous exposure to PTH in primary hyperparathyroidism. Our laboratory believes that estrogen deficiency interacts with PTHrP to amplify bone loss during lactation. This hypothesis is based in part on experimental observations in animals and humans showing that estrogen deficiency can amplify the bone resorption caused by continuous infusions of PTH (51-56). Thus, it is interesting to speculate that the more severe bone loss noted in hyperparathyroid patients who are also estrogen-deficient (53) may result from a more complete reactivation of circuitry that normally triggers bone loss during lactation.

A final disease whose pathophysiology may reflect bone loss during lactation is breast cancer. Lactation represents the only instance, other than malignancy, in which PTHrP circulates to act in a "PTH-like" fashion. This function likely contributed to the evolutionary pressure that maintained the common use of the PTH1R between these two peptides, allowing PTHrP to mimic the actions of PTH when secreted by malignant cells (11). In breast cancer patients, hypercalcemia and osteolytic bone metastases are particularly common clinical complications, which contribute importantly to patients' morbidity and mortality (81). A series of studies from several groups have demonstrated that in both instances, PTHrP is a critical mediator of a conversation between breast cancer cells and bone cells

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(82-84). In the case of humoral hypercalcemia of malignancy (HHM), PTHrP is secreted into the circulation and in the case of osteolytic bone metastases, PTHrP is released into the local microenvironment. In either situation, the breast cancer cells are simply mimicking the normal behavior of breast epithelial cells, which secrete PTHrP to activate bone resorption during lactation (10). During lactation, widespread bone resorption does not lead to hypercalcemia, because the circulating calcium is transported into milk and calcium feeds back to suppress PTHrP secretion (13). However, in the setting of HHM or

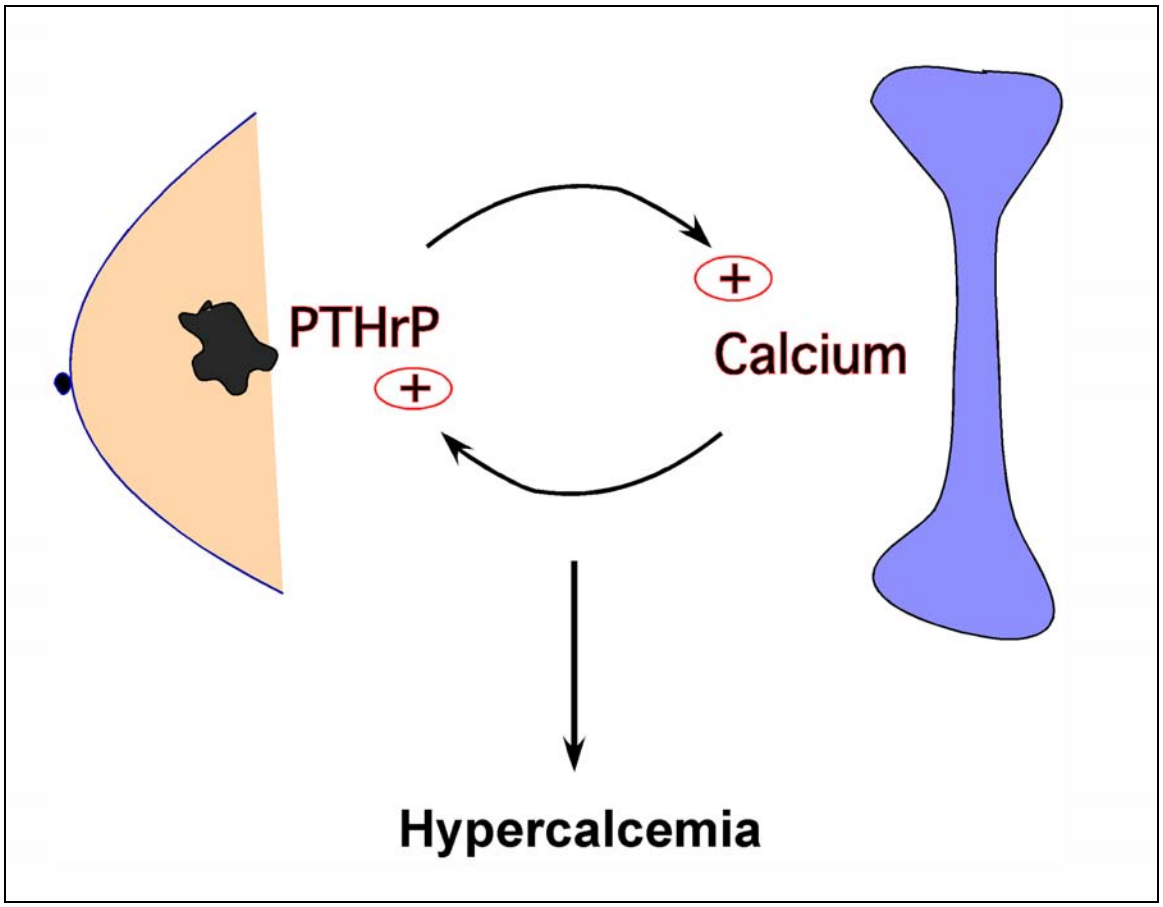

Figure 5. HHM mimics lactation in breast cancer. When breast tumors secrete PTHrP into the circulation, they recapitulate the normal function of the lactating breast to increase bone resorption and to liberate calcium from the skeleton. However, calcium is not transported into milk in cancer patients. Furthermore, increased extracellular calcium normally feeds back to suppress PTHrP production by lactating breast cells. However, in breast cancer cells, activation of the CaR stimulates PTHrP production, potentially worsening bone resorption. When the efflux of calcium from the skeleton exceeds the capacity for renal calcium excretion, hypercalcemia ensues.

widespread bone metastases in breast cancer, hypercalcemia develops because no milk is being produced and thus calcium is not removed from the circulation in this manner. Furthermore, while calcium feeds back to suppress PTHrP production by normal breast cells, in breast cancer cells the opposite happens and calcium actually stimulates PTHrP production (61). This switch in the regulation of PTHrP production appears to result from alteration in downstream intracellular signaling events upon CaR activation that occur early in the process of malignant transformation

(Mamillapalli and Wysolmerski, unpublished results). The resulting augmentation of PTHrP secretion by CaR signaling may then contribute to the development of a vicious cycle that accelerates bone resorption and osteolysis in breast cancer patients (84) (Fig. 5).

Conclusions

Reproduction is associated with an impressive cycle of maternal bone loss and repair. Lactation causes rapid bone loss at least in part due to estrogen deficiency and elevations in circulating levels of PTHrP. During this period, the breast itself becomes an active participant in the regulation of calcium and bone metabolism in order to ensure a steady supply of calcium for milk production. Sensory nerves in the nipple stimulate the hypothalamus to suppress GnRH secretion in response to suckling, thus lowering circulating levels of estrogen. In addition, breast cells secrete PTHrP during lactation, resulting in elevated circulating levels. The combination of low estrogen levels and high PTHrP levels contribute to increase bone turnover and induce bone loss. In addition, the lactating breast expresses the CaR, allowing it to adjust calcium usage and PTHrP secretion in response to calcium delivery. Thus, the breast mimics the parathyroid gland during lactation, although it uses PTHrP rather than PTH to adjust bone metabolism. Remarkably, after weaning, the skeleton is repaired almost as rapidly as it was demineralized during lactation. Initially, this is associated with coordinated osteoclast apoptosis that occurs immediately after the cessation of milk production and is associated with an abrupt fall in RANKL expression in bone cells. Very little is known about the hormonal or molecular regulation of the recovery of bone mass after lactation. Nonetheless, the pattern of transient demineralization of the skeleton for the purposes of reproduction is an ancient one. It is likely that the pathological bone resorption that occurs after the menopause, in patients with hyperparathyroidism and in patients with breast cancer, all involve the inappropriate activation of conserved pathways meant to provide for calcium needs during lactation. Thus, a better understanding of this cycle of bone loss and repair could provide us with clues for better treatment of bone diseases. In particular, understanding how nature reverses skeletal catabolism after weaning may point towards new anabolic strategies to treat osteoporosis.

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