Published in final edited form as:

Ann N Y Acad Sci. 2010 March ; 1192: 161–169. doi:10.1111/j.1749-6632.2009.05249.x.

Interactions between breast, bone and brain regulate mineral and skeletal metabolism during lactation

John J. Wysolmerski

Yale University School of Medicine

Abstract

Mammalian reproduction requires that nursing mothers transfer large amounts of calcium to their offspring through milk. As a result, lactation is associated with dramatic alterations in bone and mineral metabolism, including reversible bone loss. One theme that has emerged from recent studies examining these adaptations is that the lactating breast actively participates in regulating bone and mineral metabolism. This review will detail our current knowledge of interactions between the breast, skeleton and hypothalamus during lactation and will consider implications that this reproductive physiology has for the pathophysiology of osteoporosis and breast cancer.

Keywords

Osteoporosis; Bone Metastases; Lactation; Estrogen Deficiency; Calcium-Sensing Receptor; Parathyroid Hormone-Related Protein

Introduction

In order to reproduce successfully, vertebrates must supply enough calcium to their developing and dependant offspring to support rapid skeletal growth. Mammals provide calcium to neonates in milk. Producing milk stresses maternal calcium homeostasis and, as a result, lactation is associated with a remarkable set of adaptations, including reversible demineralization of the skeleton. In the past several years, it has become clear that the breast, brain and skeleton all cooperate to provide calcium for milk production. This review will discuss alterations in maternal calcium and bone metabolism that accompany lactation, concentrating on interactions between breast and bone. It will also consider some pathophysiologic implications of these changes for osteoporosis and breast cancer.

Calcium and Bone Metabolism During Lactation

Nursing humans secrete between 300 mg to 400 mg of calcium into milk each day (1, 2). Maternal calcium and bone metabolism must adapt to this extra demand for calcium. In humans, total and ionized calcium levels rise slightly during lactation, parathyroid hormone (PTH) levels are decreased compared to non-lactating controls, and calcitonin and 1,25 dihydroxy-vitamin D levels, which had been elevated during pregnancy, return to normal (1, 2). Some of these changes may be species-specific, as calcium and PTH levels in lactating mice are unchanged, while calcium has been reported to be decreased and PTH slightly increased in lactating rats (1). In both humans and rodents, circulating levels of parathyroid hormone-related protein (PTHrP) are elevated during lactation (1–4). PTHrP is a growth

factor ancestrally and structurally related to PTH (5) and, other than in malignancy, lactation appears to be the only instance in which it circulates.

The calcium secreted into milk comes from several sources. Unlike during pregnancy, 1,25 dihydroxy-vitamin D levels and, presumably, the efficiency of dietary calcium absorption are not elevated during lactation. Nonetheless, suckling induces hyperphagia and some of the extra calcium comes from the diet (1, 6). During lactation, the kidneys retain calcium, and urinary calcium excretion declines to very low levels (1, 2). 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 (1, 2). 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 these three sources of calcium.

As noted above, maternal bone mass declines during lactation (1, 2). In nursing humans, bone mineral density falls 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 (1). Sites of trabecular bone are the most severely affected, although cortical and whole body bone mass also decline. The decline in BMD correlates with the amount of milk produced; women nursing twins and triplets lose more bone than women nursing just one baby (7, 8). 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 (2, 4). A recent study in mice has shown that lactation is associated with changes in bone microarchitecture and bone mineralization as well as bone density (9). Micro-CT measurements demonstrated thinning and perforation of trabecular plates, cortical thinning, increased cortical tunneling and a decrease in tissue density (Fig. 1). As might be expected, studies in rats have demonstrated that these changes in bone mass and architecture diminish bone strength (1, 8) and, occasionally, women present with fragility fractures while nursing (1).

Bone loss during lactation is the result of increased bone turnover. 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 reveal that osteoclast numbers and activity are increased at both trabecular and endocortical sites (1, 2, 4, 9). Osteoblast numbers are also increased and bone formation rates, as measured by double-fluorescent labeling techniques, are elevated in lactating as compared to nulliparous females (1, 2, 4, 9). 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.

Bone Recovery After Weaning

Perhaps the most remarkable aspect of the bone loss associated with lactation is its rapid and complete reversibility after weaning (1, 9). In rodents, skeletal mass 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 (1, 10–12). During this burst of anabolic activity in humans, bone is added at an estimated rate of 0.5 – 2% per month (1).

While many studies have documented that bone mineral density recovers after weaning, only a few have attempted to define the mechanisms that govern this response. Miller, Bowman and colleagues performed a series of detailed histologic and histomorphometric studies examining the post-lactation period in rats (13–15). They showed that trabecular number, thickness and connectivity, as well as biomechanical strength, recovered fully 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. At the same time, 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.

Our laboratory recently examined the transition from bone catabolism to bone anabolism at weaning in mice in detail (9). 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. In addition, quantitative micro-CT measurements documented rapid reversal of the changes in skeletal microarchitecture that occur during lactation (Fig. 1). 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. 2). A similar apoptotic response has also been recently noted in osteoclasts in weaned rats (16). 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 (13, 14). Thus, 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.

Systemic Regulation of Bone Metabolism During and After Lactation

The mechanisms that drive bone loss during lactation are only partly understood. Neither PTH nor vitamin D, the two classic calciotropic hormones, trigger this decline in skeletal mass. Studies in animals and experience with patients has suggested that lactational bone loss proceeds in the absence of either of these two hormones (1, 17, 18). A current working model is that bone loss is triggered by the combination of a fall in circulating levels of estradiol and an increase in circulating levels of PTHrP, and is constrained by circulating calcitonin. These three factors will be discussed in the next three paragraphs.

The act of suckling stimulates afferent nerves in the breast that connect through the brainstem to inhibit GnRH production (6, 19). Suppression of GnRH secretion, in turn, leads to hypogonadotropic hypogonadism and a decline in circulating levels of estradiol (see Fig. 3). Additionally, despite adequate fat stores after pregnancy, leptin levels are low during lactation and suckling stimulates prolactin secretion from the pituitary gland (6, 19). 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 (20). In fact, human studies have found a correlation between the degree of bone loss and the duration of amenorrhea post-partum (1, 21). Furthermore, estrogen levels correlate with rates of bone resorption and pharmacologic estrogen replacement leads to a reduction in bone loss in lactating mice (4). 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 (1). Bone loss in lactating rodents also exceeds that seen after ovariectomy (1). 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 (22, 23).

The lactating breast secretes PTHrP both into the systemic circulation and into milk (1, 2, 4, 24). Many studies have now documented that circulating PTHrP levels are elevated in

lactating women and rodents (1, 2, 4, 24). Plasma levels of PTHrP correlate directly with biochemical markers of bone resorption and inversely with changes in bone mass in lactating mice (4). Sowers and colleagues demonstrated a correlation between bone loss and elevated circulating PTHrP levels in nursing women as well (3). 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 (24). Thus, PTHrP, secreted from the breast, contributes to the mobilization of skeletal calcium stores during lactation. Given 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 (25, 26).

Some older reports suggested that calcitonin might act to inhibit osteoclastic bone resorption during lactation (27, 28). Recent studies 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% (29). Other studies have shown that mammary epithelial cells can secrete calcitonin (30), 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. Nevertheless, it would appear that circulating calcitonin protects the skeleton from excessive bone loss during lactation, again reinforcing that bone metabolism during this time is tightly regulated.

In order to test whether low estrogen and elevated PTHrP levels are sufficient to trigger lactational bone loss, we have been attempting to reproduce this physiology in nulliparous mice. We lowered estrogen levels by administering leuprolide to induce hypogonadotrophic hypogonadism similar to that induced by suckling (6). In addition, we infused PTHrP using osmotic minipumps to achieve levels similar to those measured during lactation (4, 24). We have found that the combination of estrogen deficiency and excess PTHrP does cause bone loss, but it does not reproduce the degree of bone loss normally observed in lactating mice (Ardeshirpour and Wysolmerski, unpublished data). These are ongoing studies, but our results suggest that the regulation of bone metabolism during lactation is complex and that there may be other local or systemic components of the response in addition to the changes in circulating levels of estrogen and PTHrP. Suckling affects areas of the hypothalamus that have been shown to regulate bone turnover (6, 31), so one possibility is that there are direct neurological inputs necessary to trigger the full level of bone loss.

We know very little about the regulation of bone recovery after weaning. Studies from our laboratory have found that RANKL mRNA levels are elevated in bones from lactating mice as compared to those from nulliparous mice (9). After weaning, we found that 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. We and others have also noted several systemic changes in the initial 48–72 hours following weaning, including a rapid increase in circulating estrogen levels, a decline in circulating PTHrP levels, a transient rise in circulating calcium levels and a spike in circulating calcitonin concentrations (9, 16, 29). Individually, all of these changes could contribute to osteoclast apoptosis and the inhibition of bone resorption after weaning but the full response may require all of them to occur simultaneously. Ongoing experiments are examining these possibilities.

Interactions Between Breast and Bone During Lactation

One theme that has emerged from recent studies of lactational bone loss is that the breast becomes an active participant in maternal bone and mineral metabolism. As noted previously, the lactating breast secretes PTHrP, which circulates and activates bone

resorption in the skeleton in order to mobilize calcium for milk production. In addition, calcium delivery to the breast regulates mammary gland PTHrP production, defining a classic negative feedback loop between breast and bone during lactation (Fig. 3) (32). The Brown laboratory initially showed that the calcium-sensing receptor (CaR) was expressed in normal and malignant human breast tissue (33). Subsequently, we found that the CaR is expressed at low levels in the mammary glands of virgin and pregnant mice, but its expression is greatly increased during lactation where it is expressed on the basolateral surface of the epithelial cells (32). Stimulation of the CaR suppresses PTHrP production by normal mammary epithelial cells *in vitro* and *in vivo* (32). In keeping with these findings, mammary epithelial cells from CaR^{+/-} mice increase PTHrP production by approximately two-fold (34). Therefore, as demonstrated in Fig. 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 the 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 bone metabolism.

In addition to regulating calcium delivery to the lactating mammary gland, the CaR also adjusts calcium consumption by mammary epithelial cells. Activation of the CaR on these cells stimulates the trans-epithelial transport of calcium into milk (32, 34) by stimulating the enzymatic activity of a specific calcium pump, the plasma membrane calcium-ATPase type 2bw (PMCA2), located on the apical membrane of mammary epithelial cells (35). 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. 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).

Bone Loss During Lactation as Evolutionary Template for Post-menopausal Osteoporosis

The need to provide calcium for the rapid skeletal growth of offspring is not unique to mammals. 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 (36). Calcium metabolism during egg production resembles a hybrid of the patterns described for pregnancy and lactation in mammals and the calcium used for egg production comes, in part, from the mobilization of internal stores from bones, scales and, in turtles, shells (36). Thus, it is important to note that the mobilization of skeletal calcium stores for the sake of reproduction 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 (36). Therefore, bone loss during lactation results from a series of ancient adaptations that have been highly conserved during evolution.

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 (1, 36). 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, 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 (36). 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 possibility that we might learn to manipulate this response in order to heal osteoporosis fully.

G-protein Switching and the CaR; Implications for Osteolytic Bone Metastases in Breast Cancer

As described previously and as depicted in Fig. 3, activation of the CaR normally inhibits PTHrP production by mammary epithelial cells (32, 34). This is similar to the function of the CaR in regulating PTH production by parathyroid cells and it is responsible for setting up a classic negative feedback loop between breast and bone during lactation. However, the initial report of CaR expression in breast cancer cells suggested that activation of the CaR stimulated, rather than inhibited, PTHrP production (33, 37). We recently studied the mechanisms underlying the opposing actions of calcium in normal as compared to malignant breast cells (38). First, we confirmed that in immortalized mouse mammary epithelial cells and in human breast cancer cells, stimulation of the CaR increased both PTHrP mRNA expression and PTHrP secretion. Second the opposite affects of calcium correlated with opposing effects of CaR activation on cAMP levels, which, in turn, were responsible for regulating PTHrP production by breast cells. Third, we found that in benign breast cells, the CaR coupled to G_i and inhibited adenyl cyclase, while in transformed breast cells, the CaR coupled to G_s and stimulated adenyl cyclase. These results were somewhat surprising in that the CaR had previously only been described to couple to G_i , $G_{q/11}$ and $G_{12/13}$ (39). However, in breast cells the receptor can utilize G_s as well. This also appears to be the case in some pituitary cells as well (Mamillapalli and Wysolmerski, unpublished data), so coupling of his receptor to G_s may be a more general phenomenon.

From a clinical perspective, the switching of G-protein usage by the CaR in breast cancer may contribute to the pathophysiology of osteolytic bone metastases. The secretion of PTHrP by breast cancer cells has been shown to accelerate bone resorption and support the formation of osteolytic bone metastases (40). Inhibition of PTHrP production by calcium would tend to limit the amount of PTHrP available to participate in this process. However, switching the coupling of the CaR to G_s would allow calcium liberated during bone resorption to stimulate more PTHrP secretion by tumor cells, thereby accelerating the osteolytic process. Since bone resorption also liberates other growth factors that stimulate breast tumor growth (40), such a shift in G-protein usage by the CaR may represent an important event during breast cancer progression. Given these considerations, defining the mechanisms through which malignant transformation of mammary epithelial cells alters G-protein coupling to the CaR may suggest new therapeutic targets for breast cancer therapy.

Conclusions

Reproduction is associated with a remarkable cycle of bone loss and recovery. This is an ancient response that has been strongly conserved through evolution. During lactation, the breast becomes a calcium-sensing organ that actively participates in the regulation of mineral and bone metabolism. Lactation-associated bone loss is triggered, in part, by the

suckling-induced fall in circulating estradiol levels and rise in circulating levels of breast-derived PTHrP. However, we do not yet have a full understanding of the integrated regulation of bone and mineral metabolism during this period. We believe that this physiology has several implications for the pathophysiology of osteoporosis and breast cancer. Thus, it is our hope that a better understanding of the conversations between breast, bone and brain during lactation may suggest new approaches to these common diseases.

References

1. Kovacs CS, Kronenberg HM. Maternal-fetal calcium and bone metabolism during pregnancy, puerperium, and lactation. *Endocr Rev.* 1997; 18:832–872. [PubMed: 9408745]
2. VanHouten J. Maternal calcium and bone metabolism during lactation. *Current Opinion in Endocrinology and Diabetes.* 2005; 12:477–482.
3. Sowers MF, Hollis BW, Shapiro B, Randolph J, Janney CA, Zhang D, Schork A, Crutchfield M, Stanczyk F, Russell-Aulet M. Elevated parathyroid hormone-related peptide associated with lactation and bone density loss. *Jama.* 1996; 276:549–554. [PubMed: 8709404]
4. VanHouten JN, Wysolmerski JJ. Low estrogen and high parathyroid hormone-related peptide levels contribute to accelerated bone resorption and bone loss in lactating mice. *Endocrinology.* 2003; 144:5521–5529. [PubMed: 14500568]
5. Wysolmerski, JJ. Parathyroid hormone-related protein. In: Rosen, CJ., editor. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism.* Seventh Edition ed. Washington, DC: American Society for Bone and Mineral Research; 2008. p. 127-133.
6. Smith MS, Grove KL. Integration of the regulation of reproductive function and energy balance: lactation as a model. *Front Neuroendocrinol.* 2002; 23:225–256. [PubMed: 12127305]
7. Laskey MA, Prentice A, Hanratty LA, Jarjou LM, Dibba B, Beavan SR, Cole TJ. Bone changes after 3 mo of lactation: influence of calcium intake, breast-milk output, and vitamin D-receptor genotype. *Am J Clin Nutr.* 1998; 67:685–692. [PubMed: 9537615]
8. Peng TC, Garner SC, Kusy RP, Hirsch PF. Effect of number of suckling pups and dietary calcium on bone mineral content and mechanical properties of femurs of lactating rats. *Bone Miner.* 1988; 3:293–304. [PubMed: 3228614]
9. Ardeshirpour L, Dann P, Adams DJ, Nelson T, VanHouten J, Horowitz MC, Wysolmerski JJ. Weaning triggers a decrease in receptor activator of nuclear factor-kappaB ligand expression, widespread osteoclast apoptosis, and rapid recovery of bone mass after lactation in mice. *Endocrinology.* 2007; 148:3875–3886. [PubMed: 17495007]
10. Karlsson C, Obrant KJ, Karlsson M. Pregnancy and lactation confer reversible bone loss in humans. *Osteoporos Int.* 2001; 12:828–834. [PubMed: 11716185]
11. Laskey MA, Prentice A. Bone mineral changes during and after lactation. *Obstet Gynecol.* 1999; 94:608–615. [PubMed: 10511368]
12. Polatti F, Capuzzo E, Viazzo F, Colleoni R, Klersy C. Bone mineral changes during and after lactation. *Obstet Gynecol.* 1999; 94:52–56. [PubMed: 10389717]
13. Bowman BM, Siska CC, Miller SC. Greatly increased cancellous bone formation with rapid improvements in bone structure in the rat maternal skeleton after lactation. *J Bone Miner Res.* 2002; 17:1954–1960. [PubMed: 12412802]
14. Miller SC, Bowman BM. Rapid improvements in cortical bone dynamics and structure after lactation in established breeder rats. *Anat Rec A Discov Mol Cell Evol Biol.* 2004; 276:143–149. [PubMed: 14752853]
15. Vajda EG, Bowman BM, Miller SC. Cancellous and cortical bone mechanical properties and tissue dynamics during pregnancy, lactation, and postlactation in the rat. *Biol Reprod.* 2001; 65:689–695. [PubMed: 11514329]
16. Miller SC, Bowman BM. Rapid inactivation and apoptosis of osteoclasts in the maternal skeleton during the bone remodeling reversal at the end of lactation. *Anat Rec (Hoboken).* 2007; 290:65–73. [PubMed: 17441199]

17. Garner SC, Boass A, Toverud SU. Parathyroid hormone is not required for normal milk composition or secretion or lactation-associated bone loss in normocalcemic rats. *J Bone Miner Res.* 1990; 5:69–75. [PubMed: 2309581]
18. Halloran BP, DeLuca HF. Skeletal changes during pregnancy and lactation: the role of vitamin D. *Endocrinology.* 1980; 107:1923–1929. [PubMed: 7428698]
19. McNeilly AS, Tay CC, Glasier A. Physiological mechanisms underlying lactational amenorrhea. *Ann N Y Acad Sci.* 1994; 709:145–155. [PubMed: 8154698]
20. Riggs BL. The mechanisms of estrogen regulation of bone resorption. *J Clin Invest.* 2000; 106:1203–1204. [PubMed: 11086020]
21. Kalkwarf HJ, Specker BL. Bone mineral loss during lactation and recovery after weaning. *Obstet Gynecol.* 1995; 86:26–32. [PubMed: 7784018]
22. Funk JL, Shoback DM, Genant HK. Transient osteoporosis of the hip in pregnancy: natural history of changes in bone mineral density. *Clin Endocrinol (Oxf).* 1995; 43:373–382. [PubMed: 7586609]
23. Sowers M, Corton G, Shapiro B, Jannausch ML, Crutchfield M, Smith ML, Randolph JF, Hollis B. Changes in bone density with lactation. *Jama.* 1993; 269:3130–3135. [PubMed: 8505816]
24. VanHouten JN, Dann P, Stewart AF, Watson CJ, Pollak M, Karaplis AC, Wysolmerski JJ. Mammary-specific deletion of parathyroid hormone-related protein preserves bone mass during lactation. *J Clin Invest.* 2003; 112:1429–1436. [PubMed: 14597768]
25. Cosman F, Shen V, Xie F, Seibel M, Ratcliffe A, Lindsay R. Estrogen protection against bone resorbing effects of parathyroid hormone infusion. Assessment by use of biochemical markers. *Ann Intern Med.* 1993; 118:337–343. [PubMed: 8430979]
26. Grey AB, Stapleton JP, Evans MC, Tatnell MA, Reid IR. Effect of hormone replacement therapy on bone mineral density in postmenopausal women with mild primary hyperparathyroidism. A randomized, controlled trial. *Ann Intern Med.* 1996; 125:360–368. [PubMed: 8702086]
27. Stevenson JC, Hillyard CJ, MacIntyre I, Cooper H, Whitehead MI. A physiological role for calcitonin: protection of the maternal skeleton. *Lancet.* 1979; 2:769–770. [PubMed: 90862]
28. Toverud SU, Harper C, Munson PL. Calcium metabolism during lactation: enhanced effects of thyrocalcitonin. *Endocrinology.* 1976; 99:371–378. [PubMed: 954638]
29. Woodrow JP, Sharpe CJ, Fudge NJ, Hoff AO, Gagel RF, Kovacs CS. Calcitonin plays a critical role in regulating skeletal mineral metabolism during lactation. *Endocrinology.* 2006; 147:4010–4021. [PubMed: 16675524]
30. Tverberg LA, Gustafson MF, Scott TL, Arzumanova IV, Provost ER, Yan AW, Rawie SA. Induction of calcitonin and calcitonin receptor expression in rat mammary tissue during pregnancy. *Endocrinology.* 2000; 141:3696–3702. [PubMed: 11014224]
31. Wong IP, Zengin A, Herzog H, Baldock PA. Central regulation of bone mass. *Semin Cell Dev Biol.* 2008; 19:452–458. [PubMed: 18761098]
32. VanHouten J, Dann P, McGeoch G, Brown EM, Krapcho K, Neville M, Wysolmerski JJ. The calcium-sensing receptor regulates mammary gland parathyroid hormone-related protein production and calcium transport. *J Clin Invest.* 2004; 113:598–608. [PubMed: 14966569]
33. Cheng I, Klingensmith ME, Chattopadhyay N, Kifor O, Butters RR, Soybel DI, Brown EM. Identification and localization of the extracellular calcium-sensing receptor in human breast. *J Clin Endocrinol Metab.* 1998; 83:703–707. [PubMed: 9467597]
34. Ardeshirpour L, Dann P, Pollak M, Wysolmerski J, VanHouten J. The calcium-sensing receptor regulates PTHrP production and calcium transport in the lactating mammary gland. *Bone.* 2006; 38:787–793. [PubMed: 16377269]
35. VanHouten JN, Neville MC, Wysolmerski JJ. The calcium-sensing receptor regulates plasma membrane calcium adenosine triphosphatase isoform 2 activity in mammary epithelial cells: a mechanism for calcium-regulated calcium transport into milk. *Endocrinology.* 2007; 148:5943–5954. [PubMed: 17823248]
36. Wysolmerski JJ. The evolutionary origins of maternal calcium and bone metabolism during lactation. *J Mammary Gland Biol Neoplasia.* 2002; 7:267–276. [PubMed: 12751891]
37. Sanders JL, Chattopadhyay N, Kifor O, Yamaguchi T, Butters RR, Brown EM. Extracellular calcium-sensing receptor expression and its potential role in regulating parathyroid hormone-

- related peptide secretion in human breast cancer cell lines. *Endocrinology*. 2000; 141:4357–4364. [PubMed: 11108243]
38. Mamillapalli R, VanHouten J, Zawulich W, Wysolmerski J. Switching of G-protein usage by the calcium-sensing receptor reverses its effect on parathyroid hormone-related protein secretion in normal versus malignant breast cells. *J Biol Chem*. 2008; 283:24435–24447. [PubMed: 18621740]
39. Ward DT. Calcium receptor-mediated intracellular signalling. *Cell Calcium*. 2004; 35:217–228. [PubMed: 15200145]
40. Guise TA, Mohammad KS, Clines G, Stebbins EG, Wong DH, Higgins LS, Vessella R, Corey E, Padalecki S, Suva L, Chirgwin JM. Basic mechanisms responsible for osteolytic and osteoblastic bone metastases. *Clin Cancer Res*. 2006; 12:6213s–6216s. [PubMed: 17062703]

Figure 1. Reversible bone loss during lactation

Three-dimensional reconstructions of representative lumbar vertebrae from aged-matched, nulliparous (A), lactating (B) and weaned (C, 28 days post weaning) mice. Lactation is associated with significant bone loss as well as structural changes such as trabecular thinning, trabecular perforation and a shift from a plate-like to rod-like appearance of trabeculae (compare A&B). After weaning (C), bone mass and trabecular architecture rapidly revert back to that seen in nulliparous animals.

Figure 2. 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 nuclei 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.

Figure 3. Crosstalk between breast, bone and brain 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.