Review

New concepts of breast cell communication to bone

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Lactation is the most extreme case of normal physiological bone loss during a lifetime, and breast cancers have a strong tendency to metastasize to bone. In both the physiological and pathological circumstances, parathyroid hormone-related peptide (PTHrP) plays a central role. Until recently there were no regulatory mechanisms to explain the induction of endocrine PTHrP secretion from breast cells during lactation. The mammary epithelium possesses a local serotonin signaling system which drives PTHrP expression during lactation and in breast cancer cells. The mammary gland serotonin system is highly induced in response to alveolar dilation due to milk secretion. Discovery of serotonergic control of PTHrP suggests that it may be possible to manipulate the breast-to-bone axis by targeting serotonin signaling.

The mammary demand for calcium

During lactation the mammary glands export large amounts of calcium and phosphate into the milk. These minerals, together with proteins to form the osteoid, are essential for skeletal growth of the offspring. The lactating mother mobilizes bone mineral to support the continuous flux of mineral toward the milk [1].

Although it might seem self-evident, it deserves stating that the mammary glands, *per se*, cause calcium regulation during lactation to differ from other physiological states. This was elegantly demonstrated many years ago in dairy cows by Neidermeier and colleagues, who showed that mastectomy prevented changes in calcium homeostasis that are associated with the peripartum (see Glossary) [2]. Studies in humans have confirmed the centrality of a breast-to-bone axis [3].

Lactation places substantial demands on calcium homeostasis and calcium balance. Women nursing a single infant deliver 300–400 mg of calcium daily via breast milk, and lose 5–10% of their skeletal mass during 6 months of breastfeeding [4]. Total calcium loss is fourfold greater during 6 months of lactation than during the entire pregnancy. The relative calcium demands during lactation are greater still in laboratory rodents and dairy cows. In mice the daily demand for calcium results from the large number and rapid skeletal growth of the pups, and can be in the range of 85 mg/day (for comparison, this is about 60-fold greater than women on a metabolic body-size basis). This causes loss of up to 30% of bone mineral despite

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Glossary

5-HT (5-hydroxytryptamine) or serotonin: is a monoamine neurotransmitter that is derived from tryptophan. It is primarily found in the gastrointestinal tract, in platelets, and the central nervous system.
Aromatic amine decarboxylase (AADC): also known as dopa-decarboxylase, is a lyase that catalyzes the second, non-rate-limiting, step in catecholamine and indoleamine (e.g., 5-HT) biosynthesis.
Calcium-sensing receptor (CaSR): a G protein-coupled receptor and member of the metabotropic glutamate receptor family C (GPCRC), originally identified from parathyroid gland chief cells. It serves as a physiological sensor of extracellular Ca^{2+}.
Low-density lipoprotein receptor-related protein 5 (Lrp5): a member of a family of plasma membrane wnt coreceptor proteins, which also has been proposed to inhibit TPH-1 in osteoblasts and enterochromaffin cells.
Osteoblastic: refers to processes related to or involved with the formation of bone, or with the osteoblast cell type.
Osteoid: the unmineralized, organic portion of the bone matrix, which makes up about 50% of bone volume and 40% of bone weight. It forms before the maturation of bone tissue. It is composed of fibers, the predominant fiber-type being type I collagen, and ground substance composed of chondroitin sulfate and osteocalcin.
Osteolysis/osteolytic: refers to the dissolution of bone; especially the removal of the calcium from bone. Often assumes a pathological context, but more generally applies to any bone mineral loss process or state.
Peripartum: occurring during the physiologically dynamic interval that extends from shortly before birth to shortly after, with reference to the mother. In humans, generally assumed to be the last month of gestation through the first few months after delivery.
Plasma membrane Ca^{2+}-ATPase (PMCA): a calcium transporter in the plasma membrane of cells that functions to remove calcium (Ca^{2+}) from the cell; encoded by four genes (*ATP2B1–4*), with *ATP2B2* (*PMCA2*) being highly expressed in the lactating mammary gland epithelium.
Runt-related transcription factor 2 (Runx2, previously Cbfa1): a transcription factor that is essential for osteoblast differentiation and skeletal development. Also implicated in cancer metastasis.
Sarcoplasmic-endoplasmic reticulum Ca^{2+}-ATPase (SERCA): a calcium transporter that transfers Ca^{2+} from the cytosol into the lumen of the endo/sarcoplasmic reticulum, encoded by any of three vertebrate genes (*ATP2A1–3*).
Secretory pathway Ca^{2+}-ATPase (SPCA): a calcium transporter in the membranes of the Golgi apparatus, increasing in density from the *cis*- to *trans*-Golgi.
Serotonin reuptake transporter (SERT, sometimes 5-HTT): an integral membrane protein of the sodium-dependent symporter type (encoded by the *SLC6A4* gene), which transports 5-HT, typically into the cell.
Serotonin-selective reuptake inhibitor (SSRI): any of a large class of drugs that preferentially inhibit the activity of SERT, epitomized by fluoxetine (Prozac®).
Tryptophan hydroxylase-1 (TPH-1): the non-neuronal isoform of vertebrate enzyme (synonymous with tryptophan-5-monooxygenase) that catalyzes the rate-limiting step in the synthesis of indoleamines (serotonin, melatonin).

increased food intake [5,6]. High-producing dairy cows export 80 g/day of calcium into milk, compared with a total plasma steady-state pool of only about 3 g [7,8].

The mobilization of maternal bone is not simply a matter of transferring calcium (and phosphate) directly to the milk. Maintaining a state of high bone mineral flux during lactation increases the effective size of the metabolically active pool of mineral that is available to the breasts. This expansion of the calcium pool minimizes the excursions of circulating calcium as demand rises and falls with milk secretion. All of the physiological resources that are available to regulate calcium homeostasis and calcium balance in non-lactating animals are also available during lactation. These include the major calcium-regulatory organs (bone, gut, kidney, blood) and calciotropic hormones: parathyroid hormone (PTH), calcitonin (CT), and vitamin D hormone (1,25-OH_2-vitamin D, calcitriol). However, these systems are directly responsive only to maternal cues (mainly plasma free [Ca^{2+}]) [9], and therefore are poorly deployed to provide for the massive flow of calcium into the milk and the offspring.

Four lactation-specific mechanisms are deployed to respond to the special demand for calcium delivered through the milk. Although there are important differences among species, in general these mechanisms are: (i) parathyroid hormone-related peptide (PTHrP), which drives calcium conservation and extraction of bone calcium; (ii) prolactin (PRL) and growth hormone (GH), which support intestinal calcium uptake; (iii) low estrogen levels, which may be suppressed by suckling; and (iv) the efficient export of calcium into the milk.

Low estrogen levels, which increase osteoclastic bone resorption and other bone-loss mechanisms [10], can be important for calcium mobilization in lactation, depending on the degree of gonadal suppression. In women who breastfeed often enough to maintain lactational amenorrhea, low estrogen should be important [11,12]. However, in women who cycle while breastfeeding (or take oral contraceptives), and in animals that have mated shortly after giving birth (e.g., continuously-mated mice, and dairy cows), estrogen levels do not stay low enough to be a main cause of bone loss.

Recent studies reviewed here point to serotonin (5-hydroxytryptamine, 5-HT) as an essential link in mammary gland signaling to the bone. In addition, the pathways for the ultimate secretion of Ca^{2+} into milk, and its regulation, are now better understood.

These findings elucidate previously unexplainable features of breast-to-bone communication, and point to fertile directions for basic research and clinical interventions.

Endocrine control of calcium during lactation

There are multiple layers of endocrine factors that regulate calcium homeostasis and long-term calcium balance, and these hormones integrate with the unique physiology of lactation. Under non-lactating conditions the major calciotropic hormones are PTH, CT, and 1,25-OH_2-D3. During lactation two additional hormones, PRL and PTHrP, reorganize calcium homeostasis and control overall calcium balance, making the mammary glands the central contributor to calcium homeostasis (Figure 1).

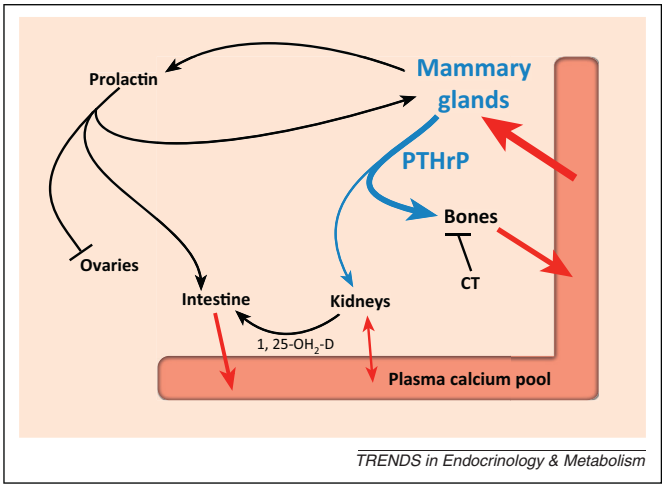

Figure 1. Organismal regulation of calcium flux during lactation. The diagram displays the major calcium regulatory relationships between the mammary glands and other important organs during lactation. PRL (prolactin) is the ultimate endocrine driver of lactation, with multiple integrating roles including suppression of the ovaries, supporting intestinal calcium absorption, and driving mammary gland differentiation and secretion. The differentiated mammary glands secrete parathyroid hormone-related peptide (PTHrP), and drive PRL secretion via the nipple stimulation reflex. The bones are the major target organs for PTHrP. In the bones, PTHrP-induced calcium mobilization is resisted by calcitonin (CT). Abbreviation: D, vitamin D.

A unique feature of bone mobilization during lactation is that bone resorption and bone formation are coupled normally during lactation, despite the rapid bone loss [13]. In other states of bone loss, such as immobilization or malignancy, bone resorption is uncoupled from formation (i.e., there is a net increase in markers of resorption over formation). These pathological circumstances lead to bone loss that is very difficult to reverse. By contrast, lactation-associated bone loss is very rapidly 'repaired' after weaning, and does not result in any long-term negative bone consequences [4]. Two possible explanations have been posited for the coupling of bone turnover during lactation: either osteoblasts proliferate but then pause before completely differentiating, or bone osteoid is secreted but not mineralized [13]. Either way, it is likely that sustaining the physiological coupling between osteoblasts and osteoclasts during lactation allows females to mobilize minerals into the milk while maintaining bone health.

CT levels in women are elevated during pregnancy and early lactation, but return to normal after the first 6 weeks of breastfeeding [4]. CT-deficient mice lose substantially more bone (2×) during lactation compared to normal mice [14]. Therefore, CT, which may be secreted from the placenta and mammary glands in addition to thyroid C cells, appears to be important for preserving the maternal skeleton in the face of heightened calcium demand. PTH levels are generally low in lactating females, and parathyroidectomized rats lose bone similarly to intact animals [15–18]. Circulating [Ca^{2+}] is normal, or slightly elevated, in lactating females compared to males or non-lactating females. This level of blood [Ca^{2+}] is presumably the feedback that suppresses PTH secretion from the chief cells of the parathyroid glands. Supplying calcium to the mammary glands, despite low PTH, appears to depend on the combined effects of PRL and PTHrP.