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REVIEW

Morphological, hormonal, and molecular changes in different maternal tissues during lactation and post-lactation

Gustavo Canul-Medina[1] · Cristina Fernandez-Mejia[1]

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Abstract

Milk supply and quality during lactation are critical for progeny survival. Maternal tissues and metabolism, influenced by hormonal changes, undergo modification during lactation to sustain breastfeeding. Two organs that suffer essential adjustment are the mammary glands and the bone; however, renal calcium conservation and calcium absorption from the intestine are also modified. Lactation leads to a transient loss of bone minerals to provide adequate amounts of minerals, including calcium for milk production. Physiological, metabolic, and molecular changes in different tissues participate in providing nutrients for milk production. After weaning, the histological, metabolic, and hormonal modifications that take place in lactation are reverted, and bone remineralization is a central function at this time. This study focuses on the hormonal, metabolic, molecular, and tissue modifications that occur in mammary glands, bone, intestine, and kidneys in the mother during lactation and post-weaning periods.

Keywords Lactation · Mammary gland · Bone · Intestine

Introduction

Lactation and post-weaning are periods during which several tissues suffer morphologic, metabolic, and hormonal changes. The central function of lactation is to synthesize and release milk; mammary glands change to meet the demand for milk production. In the bone, lactation leads to a transient loss of bone minerals to ensure adequate amounts of minerals, including calcium, for milk production. Other organs such as the gut and the kidney also modify their functions to support the levels of calcium and other nutrient requirements. After weaning, the mammary glands undergo involution, and the modifications in other tissues to sustain breastfeeding are reverted; in particular, bone remineralization is a focal feature during this period.

Calcium is a central protagonist in lactation and post-lactation. Plasma calcium exists in three distinct forms: approximately 15% is bound to organic and inorganic anions, 40% to albumin, and the remaining 45% circulates as free ionized calcium. About 99% of the calcium is stored in the bones and the teeth as hydroxyapatite [1]. The total calcium is maintained within a range of 8.5–10.5 mg/dl (4.3–5.3 mEq/L or 2.2–2.7 mmol) [2]. However, normal values and reference ranges may vary among laboratories by as much as 0.5 mg/dl. In a non-lactating state, parathyroid hormone and calcitriol regulate calcium homeostasis.

In lactation, the hormones produced intervene in calcium metabolism (described below). The mechanisms by which calcium is provided for milk synthesis may differ between species [3]. Reports in rodents indicate a loss of 25–35% of bone mass during lactation. Women lose less bone mass (5–8%) than rodents over the first 6 months after parturition, because humans usually nurse only one child versus multiparous rodents [3]. The main adaptation to provide calcium for milk synthesis is bone demineralization, followed by the renal conservation of calcium [3]. Rodents also have increased intestinal calcium absorption during pregnancy [3].

Mammary gland

Mammary glands are specialized and complex secretory tissues that produce milk to feed the newborn. They contain

✉ Cristina Fernandez-Mejia
crisfern@biomedicas.unam.mx

[1] Unidad de Genética de la Nutrición, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México/Instituto Nacional de Pediatría, Av. del Iman #1, 4th Floor, 04530 Mexico City, Mexico

epithelial cells, adipocytes, vascular endothelial cells, fibroblasts, and immune cells [4].

Mammary gland changes during lactation

Mammary glands change during pregnancy and culminate in lactation [5]. These changes require increased epithelial cell proliferation and differentiation in the milk-producing alveoli secretory gland (alveologenesis). On the other hand, the number of adipocytes decreases and vascularization increases in the mammary gland. At the end of pregnancy, the alveoli occupy most of the adipose space [5]. Lactation in rats is at its peak on day 12 after delivery, the period in which the mammary glands present prominent luminal structures and ducts and few visible adipocytes [5].

For the development of mammary glands during pregnancy, increase in progesterone and estrogen levels is essential. After delivery, the decrease in estrogen and progesterone levels facilitates the effect of prolactin in milk synthesis [3]. Suckling stimulates the hypothalamus to secrete oxytocin and prolactin. The augment of prolactin produced by suckling is pulsatile throughout lactation [3]. Prolactin is also produced by the breast during lactation [3]. The increase of this hormone produces several metabolic changes to promote lactogenesis.

Metabolic changes

During lactation, numerous changes in maternal metabolism, triggered by hormonal signals, occur to fulfill the nutrient, energy, and mineral requirements for milk production [6]. In the breasts, increased glucose utilization, fatty acid esterification, and lipid and amino acid uptake occur [7, 8]. Prolactin regulates lactogenesis and increases the synthesis of milk proteins, such as β-casein [9], lactoglobulin [10], α-lactoalbumin [11], and whey milk acid protein [12]. It also augments the enzymes and transporters involved in lipid uptake and de novo lipogenesis [13] as well as the enzymes that participate in lactose synthesis [14].

Lactose is the main carbohydrate component of the milk; this disaccharide is synthesized from glucose. Early rat studies from the Dermot Williamson group (1980) [15] estimated that in the mammary gland, the glucose uptake for lactose synthesis is about 23%, and the rest is for lipogenesis [15]. During lactation, the mammary gland is the most active site for both lipid synthesis (five-fold higher than the liver) and fatty acid esterification [7]. Mammary glucose-transport activity raises with a concomitant increase in glucose transporters (GLUT) GLUT-8 and GLUT-1; this later is the predominant isoform that transports mannose and galactose in addition to glucose [16, 17]. Despite the importance of glucose in milk synthesis, there is not a complete understanding of the factors that trigger the transport of glucose by mammary glands. Prolactin together with glucocorticoids and the growth hormone increase glucose transport [18]; however, recent studies have found that GLUT's expression was not affected by lactogenic hormones [19] and that the regulation of GLUT1 and glucose uptake is probably elicited by hypoxia [16]. Other studies suggest that serotonin may participate in the expression of GLUT transporters [20].

Studies by Rudolph et al. [21] examined by microarray analysis the expression of genes contributing to milk synthesis in the mammary gland, comparing the ratio of gene expression at lactation on day 2 to pregnancy on day 17. Important increases were found in lactose synthesis enzymes with a remarkable change of 15-fold in the alpha-lactoalbumin expression [21]. Increased glycolysis, pentose phosphate shunt, fatty acid/malate shuttle, and citric acid cycle enzymes were observed, especially enzymes involved in the regulation of lipogenesis de novo [21], such as aldolase, pyruvate kinase, and citrate kinase. Important increases in glucose transporter GLUT1, fatty acid uptake such as fatty acid plasma membrane transporters, fatty acid translocase, and lipoprotein lipase have also been observed [21, 22]. Accordingly, with the high lipid levels in milk, the triglyceride synthesis enzymes glycerol kinase, long-chain acetyl-CoA synthase, 1-acylglycerol-3-phosphate O-acyltransferase-1, and diacylglycerol O-acyltransferase-1 were also upregulated [21]. The mRNA expressions of amino acid transporters as L-amino acid transporter (LAT-1) and the alanine serine cysteine (ASC) system were enhanced [21]. In rats, the expressions of LAT-1, ASC mRNA, and cationic amino acid transporter (CAT-1) were also increased. In contrast, the expression of the transporters for anionic amino acids EAAC1 and GLAST was low (Fig. 1).

The analysis of the signals regulating the expression of these enzymes and transporters in Rudolph studies [21] suggests that the lipogenic transcription factors C/EBPδ, LXRβ, PPARγ, and SREBP1c play an important role in the upregulation of lipid synthesis, in particular SREBP1c whose increase was paralleled with augments in transcripts of genes known to be regulated by this factor. The mRNA expression of the signaling protein AKT1 was greatly enhanced; this protein is known to upregulate glucose uptake, GLUT1 surface localization, as well as lipogenic enzymes during lactation [23]. Accordingly, with the central action of prolactin, the transcripts of prolactin signaling pathway, STAT5a, STAT5b, and the prolactin receptor (PRLR) were increased [21].

Interestingly, glucose utilization, lipogenesis, and lipid uptake decrease in the white adipose tissue [15], which indicates that adipocyte metabolism is modified to provide substrates for fat and lactose synthesis in the mammary gland.

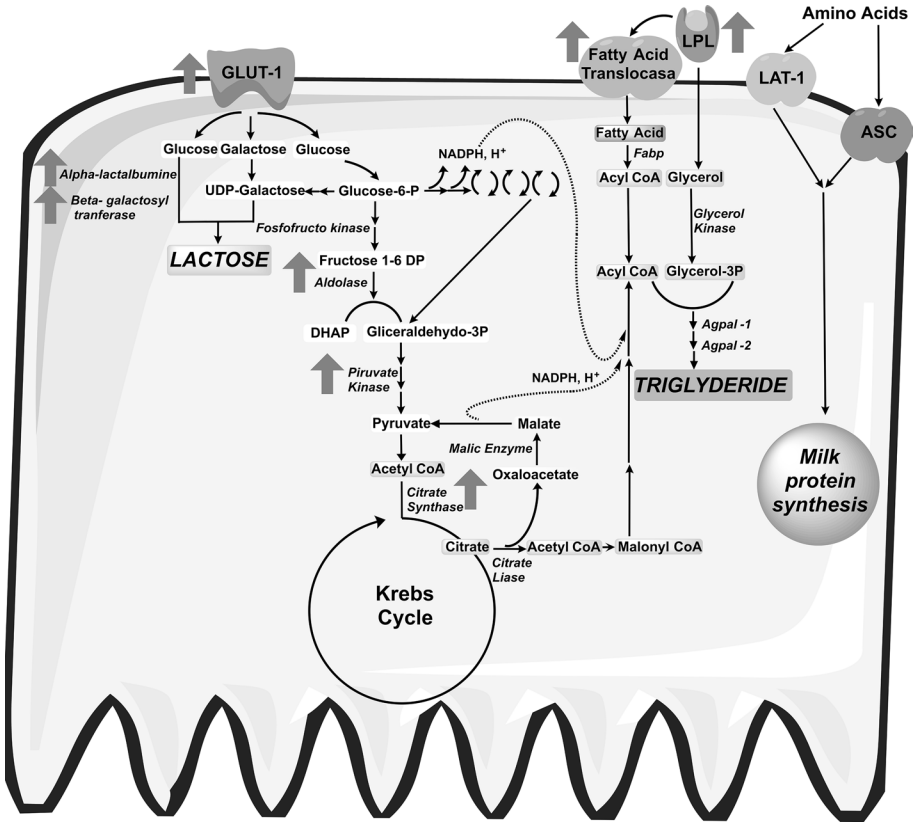

Fig. 1 Enzyme and protein increase in alveoli cells during lactation. *GLUT-1* glucose transporter-1, *LPL* lipoprotein lipase, *LAT-1* L-amino acid transporter system, *ASC* alanine serine cysteine system, *Fabp* fatty acid-binding protein, *DHAP* dihydroxyacetone phosphate, *Agpat-1* 1-acyl glycerol-3-phosphate O-acyltransferase-1, *Agpat-2* diacylglycerol O-acyltransferase-2

Changes in hormones produced by the mammary gland during lactation

Prolactin

Prolactin is a 198-amino acid protein (23 kd) produced in the lactotroph cells of the pituitary gland, which is known for its ability to promote lactation [24]. Prolactin is crucial in the growth and development of mammary glands (mammogenesis), alveologenesis, milk protein synthesis, and maintenance of milk secretion (galactopoiesis) [24]. Prolactin actions are mediated by prolactin receptor (PrlR), which activates Janus kinase 2 (JAK2)/signal transducers and activators of transcription (STAT5), MAP kinase, and phosphoinositide-3 kinase/AKT1 pathways [25].

Growth, development, and survival of mammary cells take place at pregnancy and are mediated through prolactin signaling via PrlR and the Jak2/Stat5A pathway [26], inducing the cytokine receptor activator of nuclear factor κB ligand (RANKL) [27], which via its receptor RANK activates NF-kB, MAPKs, and AKT [28]. In addition to its actions on the hyperplasia of mammary epithelial cells, RANKL also participates in bone resorption [27]. Moreover, the prolactin-induced expression of E74-like factor 5 (Elf5) is also required for the differentiation of alveolar cells [28].

Prolactin in addition to stimulating Jak2–Stat5 signaling also activates the PI3K–Akt pathway [25, 29]. These two signaling cascades mediate milk production. Stat5 regulates milk protein gene transcription, such as whey acidic protein (WAP), β-lactoglobulin, and β-casein genes, via its binding to gamma-activated sequence GAS sequences within promoter regions [25, 26]. On the other hand, Akt1 regulates glucose transport, lactose synthesis, and lipid synthesis. Importantly, prolactin promotes calcium delivery from the bone to the mammary gland via parathyroid hormone-related protein (PTHrP) secretion by the mammary tissue.

Parathyroid hormone-related protein

PTHrP is a hormone with homology to parathyroid hormone. It plays an essential role in regulating calcium homeostasis during lactation via bone resorption [30, 31]. The source of the parathyroid hormone-related protein during lactation is the mammary gland, where its mRNA and protein synthesis are upregulated [30, 31]. At the end of lactation, the fall in progesterone and estradiol, which occurs after delivery, accelerates parathyroid hormone-related protein production [32]. Suckling also induces its mRNA and protein expression [30, 33]; this effect is mediated by prolactin [34]. Some studies suggest that serotonin produced by the mammary

gland during lactation may also stimulate parathyroid hormone-related protein synthesis and release [35].

Serotonin

Serotonin (5-hydroxytryptamine; 5HT) is produced in a variety of peripheral tissues, including the gut, bone, and mammary gland. It is synthesized by tryptophan hydroxylase-1 from L-tryptophan [20]. In the mammary gland, two actions of serotonin have been described: a negative feedback on milk synthesis and secretion, and a positive effect on parathyroid hormone-related protein synthesis and secretion [36]. Suckling is an important mechanism that regulates and maintains low levels of serotonin in milk. When the mammary gland is filled with milk, serotonin provides a negative feedback that inhibits milk synthesis in the mammary epithelium [37]. The expression of β-casein is suppressed in the mammary epithelial MCF-12A cells and is associated with the serotonin-7 receptor (5HT/) expression [37]. Furthermore, during lactation, serotonin has a negative control over the β-casein expression in the mammary gland through the serotonin-7 receptor (5HT7) expression [37], and increased mRNA abundance of calcium transporters *Ncx1*, *Serca2*, *Spca2*, *Pmca*2, and *Sglt1,* but not the calcium-sensing receptor expression in the mammary gland [35], and increased osteoclasts and resorption of bone [35]. However, these effects can also be mediated by parathyroid hormone-related protein.

Changes in hormones and signals that modify morphology and function in the mammary gland during lactation

Glucocorticoids

Glucocorticoids trigger differentiation of the secretory epithelium and milk production [38]. In the last part of the gestation, and in parturition, maternal cortisol increases sharply [39]. Although this increase in circulating glucocorticoids is not the primary trigger of lactogenesis, it has a permissive action for the prolactin effects on α-lactalbumin and casein synthesis [39]. During lactation, glucocorticoid receptors act as a survival signal in the mammary gland [40] and inducer of milk protein gene expression and milk secretion [41, 42]. In addition, glucocorticoids and prolactin activate the prolactin-inducible protein (PIP) and exert an antiapoptotic effect on the mammary gland during lactation [43].

Oxytocin

Oxytocin is a peptide hormone synthesized in the hypothalamus, where it acts as a neurotransmitter. It is also released into the bloodstream via the posterior pituitary gland functioning as a hormone in peripheral targets [44]. During lactation, oxytocin increases in maternal circulation in response to suckling [45]. The hormone participates in milk ejection through the contraction of myoepithelial cells within the mammary tissue. It can also regulate osteoblast and osteoclast functions during lactation [46].

Insulin

Early studies revealed that in addition to insulin, prolactin and corticoids are also required to maintain the synthesis of milk components [47]; nevertheless, insulin is an important signal for milk production. Other investigations based on circulating concentrations of insulin and glucose at different days of lactation showed contrasting results. Women on the 3rd–4th day postpartum exhibited increased insulin levels [48]. In lactating humans and rats, suckling increases the insulin release [49, 50]. In contrast, compared with non-lactating rats, 11–13 day lactating rats presented lower glucose and plasma insulin levels [8, 51], which can be explained by the high rate of glucose utilization and increased insulin sensitivity [8, 51] in the mammary gland. Molecular studies helped disclose the role of insulin, demonstrating that insulin receptor substrate-1 expression and its transduction pathway via Akt1 increase dramatically in the mammary glands in lactation [21, 52–54], supporting the important role of this hormone in the mammary gland during lactation.

Calcium-sensing receptor

This receptor is a G-protein-coupled receptor that recognizes and responds to small changes in the extracellular ionized calcium concentration [55]. Currently, it is known that this receptor is present in the mammary gland [56] and bone [57]. The calcium-sensing receptor expression augments during lactation in the mammary gland [58] and functions as a feedback regulator during breastfeeding [58]. Hormonal changes at the start of lactation induce parathyroid hormone-related protein secretion that acts on bone cells to promote bone resorption and liberate calcium in circulation [59]. Circulating calcium, in turn, helps to control parathyroid hormone-related protein secretion in the mammary gland through the calcium-sensing receptor [59]. If the systemic calcium levels decline and calcium influx in the mammary gland decreases, then the calcium-sensing receptor expression in the mammary gland is reduced. These changes lead to increased parathyroid hormone-related protein secretion in the maternal circulation by the mammary epithelial cells to increase the release of calcium from bone reserves [59].

Bone

Bone is formed from different cell types: preosteoblasts, osteoblasts, bone-lining cells, osteocytes, preosteoclast, and osteoclast [60]. Osteocytes represent 90% of the total cells in the normal skeleton [61]. During lactation, bone plays a critical role in the release of minerals for milk synthesis.

Bone changes during lactation

Bone resorption and demineralization take place during lactation due to an increase in the number and activity of osteoclast [61]; nevertheless, osteocytes and osteoblast also participate in bone mass decrease. Osteocytes express the osteoclast-related genes that are upregulated during lactation and contribute to bone resorption during lactation (osteocytic osteolysis) [62]. Osteoblasts also increase; however, the bone turnover is in favor of bone mass loss, because the osteoclast and osteocyte activity is higher compared to osteoblasts [63].

Hormonal signal changes that modify bone morphology during lactation

The mechanisms that initiate bone loss during lactation are not fully understood; several hormones may participate in this process. Studies in rats demonstrated high prolactin levels induced bone density loss [64, 65]; however, prolactin induces bone density loss only during the late lactating period [64]. Both suckling and prolactin inhibit the pulse center of the gonadotropin-releasing hormone that suppresses the luteinizing and follicle-stimulating hormone. These hormonal changes result in diminished levels of progesterone and estradiol [66]; this decrease of steroid hormones along with serotonin-induced parathyroid hormone-related protein secretion by prolactin promotes bone resorption. Nevertheless, parathyroid hormone-related protein and decreased steroid hormone levels are not the only participants in the accelerated bone loss during lactation [67]. Among other factors that may participate is the fibroblast growth factor 21 (FGF21), a molecule that increases during lactation and whose ablation results in lack of bone resorption during lactation [68].

Parathyroid hormone-related protein

PTHrP secreted by the mammary gland during lactation is determinant for calcium milk supply through bone resorption [30, 31]. Several tissues produce parathyroid hormone-related protein; however, this cannot be detected in the serum of healthy non-lactating individuals, suggesting this hormone functions locally in an autocrine or paracrine manner and acts as an endocrine factor only during lactation [3]. Parathyroid hormone-related protein is generated locally in bone and is essential for endochondral bone formation in neonates and maturity for bone remodeling [69, 70]. As a hormone, parathyroid hormone-related protein-mediated actions are produced by binding to the G protein-coupled receptor [69]. In the bone, parathyroid hormone-related protein acts via the RANL/RANK signaling pathway, stimulating the tissue resorption and liberating skeletal calcium stores [69].

Calcitonin

Calcitonin is a hormone that regulates calcium homeostasis in vertebrates via osteoclast-mediated bone resorption and Ca^{2+} excretion by the kidney [71]. The hormone is produced primarily by the C cells of the thyroid gland [72]. Interestingly, the mammary epithelial cells are also an essential source of calcitonin during lactation [73]. However, it is unknown whether the breast, thyroid gland, or other cells are the source of the hormone during lactation. The effect of calcitonin is mediated by its receptors (CTRs), a member of the G-protein-coupled receptor (GPCR) [71]. Calcitonin levels during lactation are increased [3] and it might have an essential role in preventing excessive bone resorption [74].

Intestinal changes during lactation

Intestinal calcium absorption is normal in lactating women, but increased in rodents [3]. In rats, lactation induces morphological changes in the intestine [75], including increased length and weight [76, 77]. Wongdee et al. [75] found that lactating rats had higher villous heights in the ileum, duodenum, and jejunum compared with nulliparous rats. In addition to calcium, leucine and glucose absorption increased during lactation, reaching their peak at the 10th day of lactation [78]. Other changes in the gut of lactating rats include increased disaccharidase activities [79].

Prolactin stimulates intestinal calcium absorption in rodents [65, 75, 80]; however, hyperprolactinemia per se cannot explain the intestinal adaptive changes of lactation [81], suggesting that other factors may participate in this effect. Intestinal calcium absorption occurs through two different mechanisms: (1) the paracellular transport pathway, which is the result of passive diffusion, and (2) the active transcellular pathway, which is crucial during lactation when there is a high calcium demand. In intestine-like Caco-2 monolayer cells, prolactin increases the protein of the first step of the active transcellular pathway: voltage-dependent L-type calcium channel (Cav) 1.3 [82]. Lactating mice increase the intestinal expression of the active transcellular transport proteins: the 1,25(OH)2D-inducible epithelial

calcium-selective channel (TRPV6) and the S100 calcium-binding protein G (S100G) [83]. However, no alterations were found in the passive diffusion protein expression of cation-permeable claudin-2, claudin-12, or claudin-15 [83]. Fibroblast growth factor (FGF)-23 is a negative regulator of calcium absorption and is increased perhaps as a compensatory mechanism to prevent calcium hyper-absorption [75, 80].

Renal changes during lactation

During lactation, urinary calcium excretion is decreased in rodents and humans to maintain calcium levels for milk production. This effect is mediated by both prolactin and parathyroid hormone-related protein, which stimulate calcium reabsorption [3, 59]. At the molecular level, the expression of transient receptor potential cation channel subfamily V member 5 (Trpv5) and calbindin 1 (Calb1) was found to increase in the kidney of lactating mice; however, no changes were observed in claudins involved in Ca^{2+} and Mg^{2+} transport (claudin-2, claudin-14, claudin-16, or claudin-19) [83].

Post-lactation

The metabolic and histological changes needed for milk production are reverted after weaning. The mammary gland and the skeleton undergo rapid morphological changes, and the physiological actions shift toward restoration in the bone.

In rodents, studies conducted immediately after weaning reveled high plasma calcium levels [3, 84]. The transient hypercalcemic levels seen after weaning are likely a consequence of the decreased outflow of calcium into the mammary gland with concurrent bone reabsorption and release of calcium into circulation. One week after weaning, the plasma calcium levels returned to the normal range and remained steady afterward [3].

Mammary gland in post-lactation

After weaning, the mammary gland morphology remodels to reach the pre-pregnant state. These processes are mediated by local signals and changes in circulating levels of hormones [85]. Mammary gland involution initiates with the apoptosis of epithelial cells, the collapse of alveolar structures, a decrease in milk synthesis, and a rise in fat cells [85]. Involution of the mammary glands can be divided into two different stages: the first starts immediately upon weaning, lasts for about 48 h, and is reversible [86]. This phase is regulated by local factors within the gland [85]. The second phase is controlled by hormonal factors [87]. The first involution stage is protease-independent and is triggered by milk stasis (milk synthesis, which remains in the mammary gland and cannot come out) within the alveolar lumen and the decrease in lactogenic prolactin levels [85]. In mice, the rapid involution during the first 2 days is related to the shedding of apoptotic bodies derived from the alveolar epithelial cells in alveolar lumens. The process is followed by a gradual regression in which macrophages phagocytose the apoptotic bodies within the epithelium [86]. In rats, glandular involution is more gradual and uniform with the shedding of the apoptotic epithelial cells in alveolar lumens being a lesser noticeable process [86]. The second phase of involution is characterized by proteolysis of the mammary gland basement membrane and tissue remodeling [85]. The mechanism is triggered by matrix metalloproteinases, the phagocytic clearance of apoptotic bodies, and the re-differentiation of adipocytes.

Metabolic changes

Since the increase in nutrient synthesis and transport are no longer required for milk production, the expression of GLUT1 [17], fatty acid-binding proteins [22], amino acids uptake [88], and the biochemical machinery for milk synthesis are decreased. Milk stasis has an essential role in this process [85, 88].

Several studies have focused on the signaling pathways that participate in mammary gland involution. As described above, in lactating rodents, the signaling pathway was governed by phosphorylated STAT5 for lobule-alveolar development and milk protein gene expression [89]. This pathway decreases within 3–6 h of milk stasis [90]. Then, STAT3 phosphorylation increases and its activity consequently enhances [90]. Expression and secretion of proinflammatory cytokine leukemia inhibitory factor (LIF) activate STAT3 [91]. STAT3 induces mammary epithelial cell death and suppression of cell survival signals through PI3K-AKT-mediated survival signaling [92]. It also increases insulin-like growth factor binding protein-5 (IGFBP5) that affects proliferative IGF1 signaling [93]. Besides, the activation of nuclear factor-κB (NF-κB), the increase of nitric oxide synthase 2 gene expression, and the subsequent increase in nitric oxide levels drive the decrease in milk levels and increase in the cleavage of caspase-3, promoting apoptosis [94, 95].

Post-lactation changes in hormones and signals produced by the mammary gland

The second phase of mammary gland involution involves the decay of hormones produced during lactation. Prolactin, a central orchestrator of mammary gland, changes during lactation and drops to the non-lactating serum levels, and, consequently, prolactin-induced parathyroid-related protein

mammary production decreases [30, 31]. The expression of the calcium-sensing receptor decreases after the cessation of lactation [96]. In contrast, the increased serum serotonin levels observed during lactation remain high after 21 days of lactation [68]. Because mammary glands involute after lactation, these data suggest that other tissues may participate as serotonin sources during post-lactation.

Post-lactation changes in hormones and signals that modify morphology and function in the mammary gland

A decrease in glucocorticoids is required for breast involution and apoptosis. There is a significant decrease in glucocorticoid hormones 3 days after the end of lactation (34–14 nM) [87]. In contrast, serum insulin levels increase compared with those observed at day 7 of lactation [68]. Interestingly, these studies have also found that serum IGF-I levels, which remain constant during lactation, increased 21 days after the end of lactation [68].

Bone in post-lactation

In the post-lactation period, intense remineralization occurs with substantial increases in bone formation to reconstruct bone [3, 97, 98]. In mice, bone mineral content is recovered within 2–4 weeks after weaning and 4–8 weeks in rats [3]. In humans, dual-energy X-ray absorptiometry (DXA) studies reveal bone recovery 12 months after weaning [3].

Remineralization is a rapid process that starts with decrease of osteoclast population within 24 h after removal of pups [97]. At the molecular level, the expression of the receptor activator of nuclear factor κB (RANK) decreases and osteoclast apoptosis increases 1 day after weaning. Osteocytic osteolysis also drops, and osteocytes start to express osteoblast-specific genes [62]. Furthermore, a trigger in the osteoblast number and an increase in bone density also contribute to the recovery of bone mass [97, 99]. In both animal and human studies, morphology data indicate that the skeleton is restored to its prior mineral content despite the marked trabecular microarchitectural deterioration during lactation [100].

The mechanisms and factors that stimulate bone recovery after weaning are still debatable. The reversed hormonal milieu produced by the decrease of prolactin—and increased to normal levels of gonadotropin-releasing hormone, luteinizing hormone, follicle-stimulating hormone, and estrogen—may participate in bone mineralization. A recent study found that estrogen increases osteoclasts apoptosis and decreases bone resorption through Fas/FasL pathway and receptor-interacting protein 140 (RIP140) [101]. However, the hypothalamic–pituitary–gonadal system is not determinant for bone remineralization [102]. Studies of the hormone profile in rats found that after 24 h of weaning, the maternal concentrations of prolactin decreased; and serum calcium, estrogen, and calcitonin were increased [97]. The increase of calcitonin does not seem to be required for bone remineralization, since bone mass is fully restored within 18 days after weaning in calcitonin/calcitonin gene-related peptide-alpha (*Ctcgrp*) null mice [98]. Other studies have revealed that skeletal recovery after lactation does not require osteoblast-derived parathyroid hormone-related protein [103], parathyroid hormone [104], or vitamin D [105]. Compatible with osteoblast activity, the bone formation markers osteocalcin and procollagen type-1 amino-terminal propeptide (P1NP) were increased during post-weaning versus a pre-pregnancy baseline, pregnancy, and lactation [104].

Tibial microarray studies in mice comparing 7 days after weaning versus pre-pregnancy found that more than 700 genes had been differentially expressed. Some of these are related to the proliferation and activity of osteoblasts and the inhibition of osteoclasts [98]. There were decreased levels in cathepsin K—a lysosomal cysteine protease involved in bone remodeling and resorption—as well as in IGF-binding protein 2, which stimulates bone resorption. Downregulation of Wnt family inhibitors was also observed. Protein transcripts involved in energy production pathways such as peroxisome proliferator-activated receptor coactivator-1 alpha (PGC1-alpha), pyruvate dehydrogenase kinase, isoenzyme-4, and insulin-responsive glucose transporter-4 (GLUT-4; key transporter for glucose in osteoblasts) were increased to ensure energy for skeletal restoration [98].

Intestinal changes in post-lactation

Although the increased intestinal length produced during lactation partially diminishes after weaning, the gut does not completely regress by day 30 post-lactation [77, 78]. Other studies in rats have shown that calcium transport in the intestine is increased in post-lactation, failing to attain control values by 3 weeks post-weaning [106]. Given the active remineralization and osteoblast proliferation in post-lactation, this suggests that increased absorption is likely used during this period to supply metabolites for bone regeneration.

Conclusion

Lactation is an evolutionary adaptation that gives mammals the opportunity to provide reliable nurturing to their offspring in face to uncertain access to food. A highly integrated maternal tissue network modifies its function to supply the different components needed for milk production in the mammary gland. In particular, skeletal demineralization is a refined strategy that assures calcium supply. The current literature principally documented the

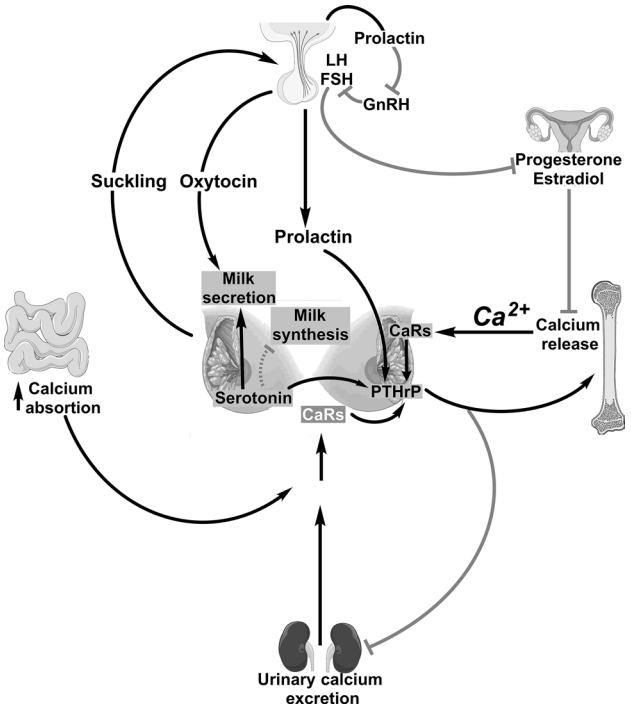

Fig. 2 Communication between different organs in lactation. Suckling stimulates the hypothalamus to secrete oxytocin and prolactin. Both suckling and prolactin inhibit the pulse center of gonadotropin-releasing hormone (GnRH), which suppresses luteinizing hormone (LH) and follicle-stimulating hormone (FSH). This decreases the levels of progesterone and estradiol. In the mammary glands, prolactin stimulates milk synthesis and secretion. Prolactin increases parathyroid hormone-related protein (PTHrP) secretion by mammary epithelial cells and its release in maternal circulation. The decrease of steroid hormones along with serotonin-induced PTHrP secretion by prolactin promotes bone resorption and liberates calcium in circulation. Circulating calcium in turn acts on the mammary gland calcium-sensing receptor to control PTHrP secretion. If the delivery of calcium to the gland decreases, then the resulting lower systemic calcium levels reduce receptor expression. This leads to increased PTHrP secretion by mammary epithelial cells and secretion into the maternal circulation to increase the supply of calcium from skeletal stores. PTHrP promotes renal calcium conservation. In lactating rodents, intestinal calcium absorption is increased. Continuous line arrows induced pathways; dotted lines inhibited pathways

changes produced in the mammary gland and in the skeleton; less information exists on the changes to the gut and kidney. In spite of the various models used, an integrated picture of the morphological, hormonal, and molecular changes that occur in lactation has emerged (Fig. 2). In contrast, most studies in post-lactation focus on mammary gland involution and morphological changes in bone structure, and there is little information on the factors and molecular mechanisms that take place in post-lactation. Identifying the factors involved in bone remineralization in post-lactation will help to develop therapeutic strategies for illness with bone loss.

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Author contributions GCM and CFM: revised the literature, and conceived and designed the manuscript and the figures. GCM: drew the figures. CFM: drafted and critically revised the article.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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