Trace Element Transport in the Mammary Gland

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Abstract

The mammary gland has a remarkable capacity to adapt to maternal deficiency or excess of iron, copper, and zinc and to homeostatically control milk concentrations of these essential nutrients. Similarly, it can regulate changes in concentrations of iron, copper, and zinc change during lactation. For iron, this regulation is achieved by transferrin receptor, DMT1, and ferroportin, whereas mammary gland copper metabolism is regulated by Ctr1, ATP7A, and ATP7B. Zinc homeostasis is complex, involving both zinc importers (Zip3) and zinc exporters (ZnT-1, ZnT-2, and ZnT-4). Both transcriptional and post-translational regulation can affect protein abundance and cellular localization of these transporters, finely orchestrating uptake, intracellular trafficking, and secretion of iron, copper, and zinc. The control of mammary gland uptake and milk secretion of iron, copper, and zinc protects both the mammary gland and the breastfed infant against deficiency and excess of these nutrients.

Contents

INTRODUCTION

The newborn infant is dependent on an adequate supply of trace elements for optimal nutrition and health. Iron (Fe) is needed for erythropoiesis and synthesis of Fe-requiring enzymes, and Fe deficiency anemia has been associated with long-term adverse effects on cognitive function and motor development (51). Zinc (Zn) deficiency in infants causes decreased growth, impaired immune function, and increased susceptibility to infection (7, 25). Copper (Cu) deficiency can cause anemia, bone and connective tissue abnormalities, and impaired immune function (48, 61). Deficiencies of these elements are very rare in breast-fed infants, at least for the first six months of life, indicating that breast milk provides adequate amounts of trace elements for the exclusively breast-fed infant. Numerous studies have shown that concentrations of trace elements (Fe, Zn, Cu) in breast milk are remarkably similar among women at a given stage of lactation, even if they consume diets low in trace elements or with low bioavailability (50, 47). Similarly, trace element concentrations in breast milk remain the same even if the mother is receiving trace element supplements at generous levels. Thus, it is evident that the mammary gland has a remarkable capacity to tightly regulate concentrations of trace elements secreted in milk (33). Concentrations of trace elements in milk do vary considerably during the lactation period, starting with high concentrations in early lactation (**Figure 1**), then decreasing as lactation proceeds (15, 28). Obviously, this change in concentrations is also tightly regulated. Recent advances in our knowledge of transporters regulating Fe, Zn, and Cu metabolism at the cellular level have provided a better understanding of how the mammary gland can regulate milk trace element concentrations.

Fe: iron
Zn: zinc
Cu: copper

MAMMARY GLAND IRON METABOLISM

The concentration of Fe in breast milk is often considered as low, both in relation to serum Fe (milk Fe concentration is ~20%–30% of serum Fe) and to estimated Fe requirements of infants. An argument is frequently made that the healthy, term infant is born with ample stores and that these are mobilized and utilized during the first six months of life, making breast milk Fe an "irrelevant" source of Fe. However, infants fed formula that has not been fortified with Fe frequently have poor Fe status, in spite of the fact that formula usually contains up to three times more Fe than does breast milk (65). Thus, the amount of Fe provided by breast milk certainly contributes to meeting the Fe needs of breast-fed infants. It is possible that the relatively low concentration of Fe in breast milk also helps to protect the infant against Fe toxicity. In a recent study on exclusively breast-fed infants given Fe supplements at 1 mg/kg/day (the currently recommended level), a significant adverse effect on linear growth was found in infants with adequate Fe status, and a marginally significant effect on diarrheal disease (16). Although the mechanisms behind these adverse effects are not yet known, from an evolutionary perspective, it may have been advantageous to provide

ample Fe to infants but protect them from excess Fe.

Iron in serum is virtually exclusively bound to transferrin (Tf), and tissue Fe uptake is usually mediated by cellular transferrin receptors (TfRs). Tissue Fe status tightly regulates cellular TfR expression, and circulating TfR (proteolytic cleavage product from cell TfR) is used as an indicator of Fe status (69). No correlation between milk Fe and mammary gland TfR expression has been found in animal models (67), which strongly suggests that regulation of milk Fe concentrations occurs after uptake of Fe by the mammary gland. Diferric Tf binds to the TfR at the mammary epithelial cell surface and is internalized by clathrin-coated vesicles that fuse with acidic endosomes (**Figure 2**). In the endosome, the acidic environment causes release of Fe from the Tf-TfR complex, and Tf is recycled to the plasma membrane with TfR. Iron is most likely exported from the endosome by divalent metal ion transporter 1 (DMT1), as shown for several tissues like liver and placenta (23, 71). DMT1 is also localized to an intracellular compartment in many epithelial cells, but there is not yet any evidence that DMT1 facilitates Fe export from endosomes in mammary epithelial cells. Iron released from the endosome can enter the intracellular chelatable Fe pool and then participate in cellular processes, be sequestered by ferritin for storage, be incorporated into Fe-containing proteins in the endoplasmic reticulum (ER), or be secreted across the luminal membrane into milk. Export of Fe from the mammary gland is most likely achieved by ferroportin (FPN), which is localized to the ER in reticuloendothelial cells, where it is believed to transport Fe into intracellular vesicles prior to secretion (1). We have found that FPN is expressed in the mammary gland of rats (44) and is localized throughout the epithelial cell (32). We therefore believe that FPN in the mammary gland epithelial cell transports Fe into secretory vesicles targeted for export into milk (32).

To date, no genetic defects in the transfer of Fe into milk have been found. The hypotransferrinemic mouse (13), which is lacking Tf in serum, dies at a young age, most likely because it was compromised during fetal life, but there have been no studies on milk Fe in this mutant mouse model. These mice survive if given iron intravenously during early life, which suggests that alternative mechanisms exist for tissue Fe uptake. However, once Fe enters the mammary gland it is likely that the homeostatic mechanisms described above regulate milk Fe export.

The decline in milk Fe concentration that occurs during lactation parallels decreases in TfR and FPN expression (44), which suggests that Fe uptake by the mammary gland and its secretion into milk is functionally decreased and not due to tissue Fe depletion. In contrast, mammary gland Fe concentration and DMT1 expression remained constant during lactation, which suggests that DMT1 plays a role in maintaining cellular Fe (44) and does not directly participate in the secretion of Fe into milk. Maternal Fe deficiency in lactating rats did not significantly affect milk Fe concentration, although mammary gland Fe stores were reduced. The maintained milk Fe levels were associated with a decrease in DMT1 expression, whereas TfR and FPN expression did not change, which suggests that the primary regulators of milk Fe secretion are TfR and FPN. These observations suggest that milk Fe concentration is maintained during Fe deficiency due to an uncoupling of the regulatory mechanisms "normally" responding to tissue Fe status, possibly protecting the newborn from excessive Fe transfer into milk during Fe deficiency (33).

The major Fe-binding protein in human milk is lactoferrin (Lf), which, similar to Tf, can bind two ferric ions per molecule (49). Lf is synthesized by the ER and likely is incorporated early into secretory vesicles. It is not yet known whether Fe is incorporated into Lf during its synthesis or if Fe transported into the secretory vesicles becomes bound to Lf in the vesicle, due to the very high affinity

Tf: transferrin

TfR: transferrin receptor

DMT1: divalent metal ion transporter 1

FPN: ferroportin

of Lf for Fe (K_{ass} $\sim 10^{24}$). It should be noted that although Lf binds a significant proportion of Fe in human milk, it is only saturated to 5%–10% (20) and therefore is capable of picking up any "free" Fe transported into the secretory vesicles. Iron in human milk is also bound to xanthine oxidase (22), which is part of the milk fat globule membrane, and as Fe is in the heme form, it is likely incorporated during xanthine oxidase biosynthesis.

Ctr1: copper transporter 1

MAMMARY GLAND COPPER METABOLISM

The concentration of Cu in human milk is about 20%–25% of that in serum. The major part of Cu in serum is tightly bound to ceruloplasmin, whereas a minor fraction is loosely associated to serum albumin, amino acids, and low-molecular-weight chelators (**Figure 3**). It is not yet known whether membrane-associated Cu transporters accrue Cu from the low-molecular-weight "accessible" Cu pool or if the ceruloplasmin-bound Cu can be made available, either by an endocytotic pathway or by release of Cu at the plasma membrane. However, during early lactation plasma Cu is high, and Cu is primarily bound to serum albumin and amino acids and has been shown to be directly taken up by the mammary gland (18). In contrast, during late lactation plasma Cu is low, and Cu is primarily bound to ceruloplasmin, which suggests that milk Cu levels may reflect the availability of "loosely bound Cu" for uptake by the mammary gland.

The newborn, term infant has ample stores of Cu, primarily in the liver, and these are mobilized during early life (48, 61), similar to Fe. Thus, a case is often made that the amount of Cu provided by breast milk is insignificant with regard to meeting the Cu requirement of infants. However, infants fed unfortified cow milk or infant formula, which contain only half the concentration of Cu in human milk, develop Cu deficiency if fed such diets for extended periods (12), which strongly suggests that human milk Cu contributes significantly to the Cu needs of breast-fed infants.

The mammary gland has been found to have three Cu-specific transporters; Ctr1, ATP7A, and ATP7B (3, 35, 54). Of these, Ctr1 has been found in all tissues examined (42) and is believed to be essential for cellular Cu import as Ctr1 knockout mice die at an early embryonic stage (43). Studies in cells transfected with CTR1 suggest that this protein imports Cu^{+} with high affinity (41, 73). This high affinity may be needed to acquire Cu from the circulation in amounts adequate to meet cellular needs. Ctr1 forms a multimeric complex containing a barrel structure with a central Cu channel (37). It has been shown that Ctr1 is vesicular and is endocytosed and proteolytically degraded in response to increased Cu levels, thereby providing a means of regulating cellular Cu uptake (63). We have found that Ctr1 in the rat mammary gland is localized to both the cell membrane and intracellular vesicles (30), which is similar to other cell types.

It is likely that Cu uptake by the mammary gland is mediated by Ctr1 (30, 39). Mammary gland Cu uptake was highest during early lactation and increased in response to suckling, but increased Ctr1 abundance does not appear to explain this. In fact, Ctr1 abundance decreased in response to suckling and hyperprolactinemia, possibly due to negative feedback of increased prolactin on prolactin receptor abundance (35). Further, Ctr1 abundance was not affected by prolactin treatment of HC11 cells, a finding that suggests Ctr1 abundance in the mammary gland may be regulated by proteasomal degradation (63) through mechanisms unrelated to prolactin signaling pathways. Our results (35) suggest that minimal Ctr1 is located at the serosal membrane until stimulated by prolactin or suckling. Colocalization of Ctr1 with transferrin receptor suggests that Ctr1 may traffic within recycling endosomes, as proposed by Petris et al. (63).

ATP7B, which belongs to the P-type ATPase family of transmembrane proteins, is involved in mammary gland Cu metabolism

(54). ATP7B was found to be defective in patients with Wilson disease, a genetic disorder of Cu toxicity (14). Wilson's patients have mutations in the ATP7B gene, resulting in an inability of the protein to properly localize to an intracellular compartment in the liver, leading to reduced Cu incorporation into ceruloplasmin, bile secretion, and hepatotoxicity. ATP7B is always localized proximal to the luminal membrane of secretory mammary epithelial cells in the lactating rat, most likely associated with the trans-Golgi network and late endosomes (30, 54), and neither its expression nor localization change during lactation or in cell culture (35, 40). It is possible that Cu transported into late endosomes is recycled back to the trans-Golgi and then incorporated into ceruloplasmin for secretion into milk. Mice with a mutation in ATP7B, which results in this Cu transporter being mislocalized in the mammary gland, have impaired Cu secretion into milk (~50% of normal) (54). In normal mice, ATP7B localization changes from being perinuclear to a cytoplasmic, diffuse location, whereas in mutant mice, ATP7B stays perinuclear, probably impairing the secretion of Cu into milk. This "toxic milk" (*tx*) mutation results in neonatal death due to severe Cu deficiency, which strongly suggests that ATP7B is essential for mammary gland secretion of Cu into milk. However, *tx* mice still have some Cu in their milk and most milk Cu is not ceruloplasmin bound (18), which suggests that Cu can be secreted by other mechanisms also.

Another P-type ATPase, ATP7A, which is homologous to ATP7B, was discovered as the protein being defective in Menkes disease (9, 46), a disorder of Cu accrual resulting in severe tissue Cu depletion (27). Several types of mutations of ATP7A have been found, all associated with impaired cellular Cu export. Thus, newly absorbed Cu becomes "trapped" in the enterocytes of the small intestine and never reaches the circulation. ATP7A is expressed ubiquitously and it is localized to both a vesicular and a perinuclear compartment in the mammary gland of mice and humans in the nonlactating state (3, 24). During lactation, however, expression of ATP7A increases and the protein relocalizes to the cell membrane (3), which suggests that ATP7A actively participates in mammary gland Cu transport during lactation. We have found that both suckling and hyperprolactinemia cause increased secretion of ^{67}Cu into milk and relocalization of ATP7A to the plasma membrane during both early and late lactation (35). However, this response is stronger during early lactation, which suggests that it is due to the higher prolactin concentration during this time. In cultured HC11 cells we found ATP7A associated with both the endoplasmic reticulum and late endosomes. It is therefore likely that what we observed in lactating rats was due to increased ATP7A-containing vesicles at the apical membrane.

The concentration of Cu in milk declines during lactation in humans and rodents (15, 21, 28). We have used the rat as a model to study mechanisms regulating milk Cu levels during lactation (35). It is possible that the decrease in serum Cu that occurs during lactation is partially responsible for decreasing milk Cu levels by reducing the supply of Cu to the mammary gland. However, there is also a modest decline in ATP7B protein levels during this time, possibly reflecting Cu export into milk (35). Our results indicate that mammary gland uptake of Cu and its secretion into milk are higher during early lactation, which is mediated by post-translational relocalization of Ctr1 and ATP7A to the cell membrane in response to suckling. We hypothesize that ATP7B is responsible for constitutive Cu secretion into milk via ceruloplasmin, whereas Ctr1 and ATP7A transiently increase Cu uptake into the gland and secretion into milk to ensure adequate transfer of Cu to the nursing infant.

Prolactin is responsible for regulating milk protein synthesis and maintaining lactation (52). During lactation, circulating prolactin concentrations fall, but they increase in response to suckling episodes (60). We investigated the mechanisms behind the increased

ZIP: zinc import protein

ZnT: zinc transporter

Cu secretion into milk in response to suckling by using a mouse mammary epithelial cell line (HC11) that is unique in that it expresses functional prolactin receptors (8) and therefore differentiates into a secretory phenotype. We found that prolactin treatment of HC11 cells increases Cu secretion from monolayers of differentiated cells (35). This increased Cu secretion is not achieved by changes in Ctr1 (see above) or ATP7A expression, but by transient relocalization of Ctr1 to the plasma membrane and of ATP7A from a perinuclear location to a vesicular compartment, possibly resulting in increased Cu secretion from the cell.

MAMMARY GLAND ZINC METABOLISM

In contrast to Fe and Cu, whose concentrations in human milk are a fraction of those in serum, milk Zn concentrations are considerably higher than in serum, at least for the first several months of lactation. Thus, there must be effective mechanisms ensuring uptake of Zn into the mammary gland and its subsequent secretion into milk. In fact, more than 0.5–1.0 mg of Zn is taken up by the mammary gland and secreted into milk per day. This amount is almost twice that of Zn transferred across the placenta to the fetus during late pregnancy (36), which illustrates the remarkable capacity of the mammary gland to transport Zn. Since milk Zn concentrations are similar in women with low Zn status and those who receive daily supplements (38, 56–58), it is apparent that homeostasis of milk Zn transfer is tightly regulated.

Cellular Zn transporters belong to two families with distinct properties (19). Those belonging to the zinc import protein (ZIP) family (ZIP1–14) are Zn importers and were discovered by gene sequence homology to known Zn transporters in yeast and plants (Zrt1, Irt-like proteins). Zip1 is found in all tissues studied, whereas Zip2–4 are tissue specific (72). Zip3 is found in tissues with high Zn requirements, such as pancreas, thymus, brain, and eye, and we have found it in the mammary gland (34), where it is located on the epithelial cell plasma membrane. By gene silencing, we reduced Zip3 expression in cultured mouse mammary cells (HC11) by ~80% and found significantly decreased Zn uptake, showing that Zip3 facilitates Zn import by mammary epithelial cells (**Figure 4**). The decreased cell viability following Zip3 knockdown demonstrated the essentiality of Zip3 for the mammary epithelial cell and possibly reflects the high Zn requirement of this highly specialized cell type (34).

The zinc transporter (ZnT) family (ZnT-1–9) of transporters belongs to the larger cation diffusion facilitator family, and its members are primarily responsible for Zn export (19). They have six transmembrane-spanning domains and a histidine-rich region that is believed to play a key role in Zn binding. ZnT-1 and ZnT-2 are expressed in the mammary gland (29, 31, 45), where they are localized to the luminal membrane of the mammary epithelial cell (31), which suggests that they are responsible for mediating Zn secretion into milk. ZnT-4 is most likely a key transporter in milk Zn secretion, as a mutation in this transporter in mice, the lethal milk (*lm*) mouse, results in early death of their offspring due to severe Zn deficiency (2, 26). Their milk, however, contains ~50% of normal milk Zn concentrations, and as maternal Zn supplementation improves pup survival, it is evident that the mammary gland can utilize other Zn transport mechanisms for Zn secretion into milk.

A disorder similar to that in the *lm* mouse is known to exist in humans. Some women who produce breast milk abnormally low in Zn cause this "transient neonatal Zn deficiency." The condition has been described in numerous case reports (4, 5, 64, 70), and milk Zn concentrations cannot be increased by maternal Zn supplementation. Term breast-fed infants of such women usually experience severe eczema and decreased growth by 2–3 months of age; premature infants experience

eczema and decreased growth earlier due to their lower Zn stores at birth (5). Oral Zn supplementation quickly alleviates the symptoms, as does the introduction of infant formula, which is fortified with Zn at generous levels. In contrast to the *lm* mouse, ZnT-4 was not found to be responsible for the condition in humans (55). We found a family with several women having had exclusively breastfed infants who during early infancy experienced classical signs of Zn deficiency, which was undiagnosed (11). Two of these women were still lactating and had abnormally low breast-milk Zn concentrations. We were able to obtain genetic material and found a point mutation (His→Arg) in the *ZnT-2* gene in the mothers of infants suffering from transient neonatal Zn deficiency. We also showed in HEK293 cells that site-directed mutagenesis of the same residue resulted in ZnT-2 mislocalization to a perinuclear, aggresomal compartment, which strongly suggests that ZnT-2 plays a major role in milk Zn secretion (11). We used gene silencing to reduce *ZnT-2* expression in cultured mouse mammary epithelial cells (HC11) by ~75%, which caused a reduction in Zn secretion by ~59%, demonstrating that ZnT-2 is partially responsible for Zn export into milk. It is not yet known how common this mutation is in various populations, but the abundance of published case reports suggests that it is not rare.

During lactation in both humans and rats, milk Zn concentrations decline (15, 21, 28) whereas plasma Zn increases. We have used the lactating rat as a model to study mechanisms underlying developmental changes in milk Zn secretion. When plasma Zn increases during lactation, mammary gland Zn levels and ZnT-1 and ZnT-2 expression increase, whereas ZnT-4 and Zip3 expression peaks during early lactation and then decreases, but remains higher than during initiation of lactation (31). ZnT-1, ZnT-2, and ZnT-4 were all localized to both the luminal and the serosal membrane, but during early lactation, they were primarily localized to the luminal membrane. As lactation proceeds, their intensity at the luminal membrane decreases and ZnT-4 is relocalized to a homogenous intracellular distribution. Thus, the decreased abundance of these transporters at the luminal membrane and the relocalization observed likely explain the decline in milk Zn concentration that occurs during lactation.

Milk Zn concentration is maintained over a wide range of Zn intake (see above). We have investigated how maternal low Zn intake affects mammary gland Zn transporters and their localization in the rat (30, 31). We found that, similar to observations in humans, plasma Zn is reduced, but milk Zn concentration is maintained. We believe that milk Zn is homeostatically regulated by a combination of decreased Zn efflux across the serosal membrane into the maternal circulation mediated by decreased ZnT-1 expression, and increased milk Zn secretion achieved by increased ZnT-4 expression. It should be noted, though, that there is most likely a threshold for the mammary gland being able to respond to maternal Zn deficiency as milk Zn does decrease with further severity of Zn deficiency, most likely due to decreased expression of Zip3, ZnT-1, ZnT-2, and ZnT-4. However, although such a severe deficiency can be achieved in experimental animals, it is unlikely to occur in lactating women.

Prolactin also affects Zn transport during lactation. In a study on lactating rats, we found that maternal Zn deficiency increases circulating prolactin levels, which suggests that Zn deficiency may have secondary effects on lactogenic hormone-signaling pathways, which are involved in the regulation of mammary gland Zn homeostasis (10). We used cultured mouse mammary epithelial cells (HC11) to investigate mechanisms by which prolactin regulates Zip3 and ZnT-2 and found that prolactin exposure transiently enhanced both serosal Zn uptake and luminal Zn export. This increased Zn transport was associated with increased ZnT-2 but not Zip3 expression, which suggests that increased Zn transporter levels is not the only mechanism used by the mammary gland to regulate Zn transport. Using

confocal microscopy we found that Zip3 is localized to the serosal membrane (34) in HC11 cells, which is similar to what we found in lactating rats (31). Prolactin transiently induces the movement of Zip3-associated vesicles to the serosal membrane, thereby likely increasing cellular uptake of Zn. In contrast, ZnT-2, which usually is associated with the Golgi, relocalizes to a dispersed vesicular compartment in response to prolactin. Thus, alterations in hormone signaling play a significant role in the regulation of milk Zn secretion.

INTERACTIONS BETWEEN MICRONUTRIENTS IN THE MAMMARY GLAND

Although it is known that maternal excess intake or deficiency of Fe, Cu, and Zn do not affect concentrations of that particular trace element in milk, there are known examples of how an underlying deficiency of one micronutrient can affect the concentration of another micronutrient in milk.

Effect of Maternal Zinc Deficiency on Milk Copper

Low Zn status is common among pregnant and lactating women because of high Zn requirements during these periods. We have investigated the effects of maternal Zn deficiency in rats on mammary gland Zn metabolism and milk Zn concentration (6, 31). In these studies, we noted significantly increased milk Cu levels; therefore, we also explored the effects of maternal marginal Zn deficiency on mammary gland Cu metabolism (30). We found that the Zn deficiency did not affect maternal tissue Zn or Cu, or milk Zn concentration, but that plasma ceruloplasmin activity was higher in dams fed the Zn-deficient diet. They also had high mammary gland Ctr1, ATP7A, and ATP7B levels, milk ceruloplasmin activity, and Cu concentration. Immunohistochemistry and differential centrifugation showed that Zn deficiency also altered Ctr1 and ATP7A localization in the mammary gland. We found a larger proportion of monomeric Ctr1 proteins, but no increase in the larger dimeric or multimeric complexes believed to be the functional form of Ctr1 (41). This suggests that mature Ctr1 in the mammary gland may undergo endocytosis in response to a low-Zn diet, as has been shown to occur in response to Cu exposure (63). However, our results do not suggest that Ctr1 is degraded as a consequence of internalization in response to low Zn intake, as was observed in response to Cu exposure (63). It has been shown previously that Zn can affect Cu transport in Caco-2 cells as they respond to high Zn levels by increasing Cu uptake and decreasing export, a finding that suggests that Zn may play a role in the regulation of Cu transporters (66).

We also found that ATP7A abundance increased in response to the low-Zn diet, and its localization changed away from smaller vesicles to larger vesicles (30), which may have contributed to the increase in milk Cu. This relocalization of ATP7A is similar to that observed with high Cu exposure (62), which suggests that the mammary gland is responding to the higher serum Cu levels. Abundance of ATP7B also increased in response to the low-Zn diet, but its localization did not change. Since ATP7B is responsible for incorporation of Cu into ceruloplasmin, it is likely that the high abundance of ATP7B was responsible for the higher milk Cu and ceruloplasmin activity.

In summary, our results show that marginal maternal Zn intake during pregnancy and lactation increases the abundance of mammary gland Cu transporters and alters their localization, resulting in high milk Cu concentration, possibly in response to transiently elevated plasma Cu levels. The mechanisms behind these observations are not yet known, but it is possible that the Zn deficiency affects prolactin secretion (described above) and that prolactin affects milk Cu secretion. It is also possible that Zn deficiency directly affects the expression and/or localization of Cu transporters.

It is interesting to note that we found significantly higher milk Cu concentrations in women from Honduras than in Swedish lactating women (17). The Honduran women had lower plasma Zn concentrations than the Swedish women, most likely due to low Zn intake and a corn-based diet with low Zn bioavailability. Thus, marginal Zn deficiency appears to increase milk Cu concentrations in humans also, although the mechanism(s) behind this observation is not yet known.

Effect of Maternal Vitamin A Deficiency on Milk Iron

Vitamin A deficiency is common in developing countries and is known to cause a secondary Fe deficiency and anemia (53, 68). A positive correlation between maternal Fe status and milk Fe has been observed in lactating women supplemented with both vitamin A and Fe but not with Fe alone (59), which suggests there is an effect of vitamin A on mammary gland Fe transport. We investigated the potential mechanisms behind these observations in lactating rats fed a diet marginal in vitamin A (0.4 RE/g) or a control diet (4 RE/g) (32). Milk and liver vitamin A and Fe, and mammary gland Fe concentrations, were lower in rats fed the low–vitamin A diet as compared with control rats. Liver TfR expression was higher, whereas mammary gland TfR expression was lower in rats fed the low–vitamin A diet as compared with controls, which suggests that vitamin A deficiency increased liver Fe acquisition at the expense of the mammary gland. Liver and mammary gland ferritin, DMT1, and FPN protein levels were lower in the low–vitamin A rats, which indicates that there are tissue-specific responses to vitamin A deficiency and that a diet marginally low in vitamin A results in specific effects on Fe transporters. These results suggest that the mammary gland and the liver respond differently to low vitamin A intake during lactation and that milk Fe is significantly decreased due to effects on mammary gland Fe transporters, putting the nursing offspring at risk for Fe deficiency. Whether this occurs in human populations is not yet known, but deficiencies of vitamin A and Fe often coexist in infants, young children, and lactating women.

CONCLUSIONS

It is evident that the mammary gland has a unique capacity to tightly regulate milk secretion of Fe, Cu, and Zn and thereby protect the offspring from maternal deficiency or excess of these trace elements. By up- and down-regulation of transporters and altering their localization within the mammary epithelial cell, homeostasis can be achieved. Lactogenic hormones are involved in this regulation, and their roles, particularly that of prolactin, in this regulation are being unraveled. The consequences of micronutrient interactions on mammary gland trace element homeostasis need to be studied further.

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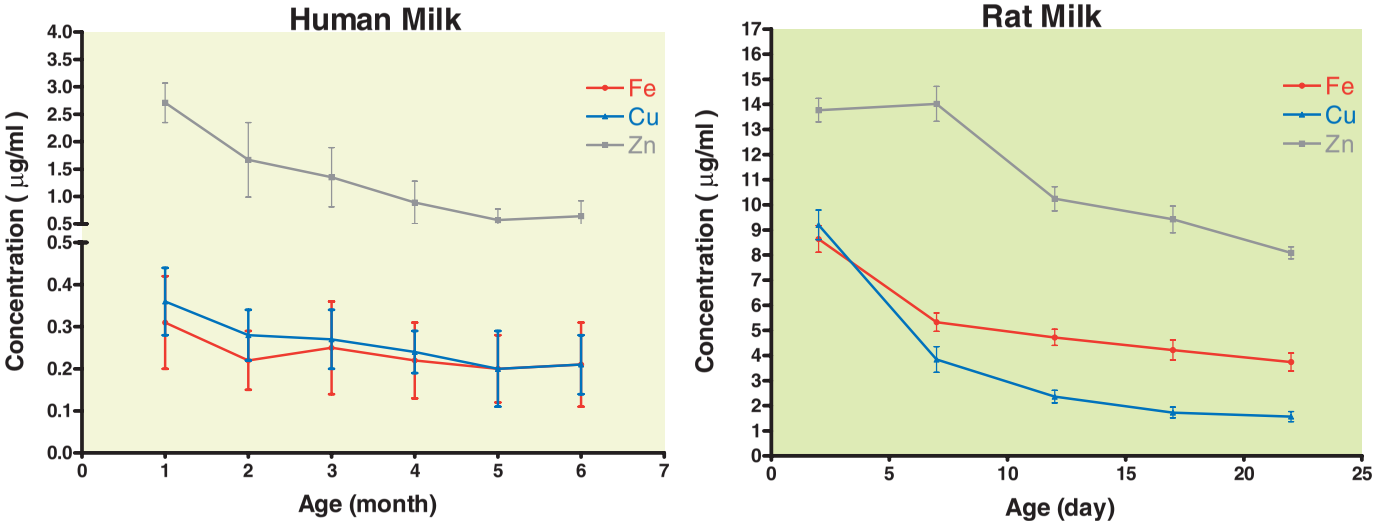

Figure 1

Changes in milk concentrations of iron (Fe), copper (Cu), and zinc (Zn) during lactation in humans and rats (adapted from References 15 and 28).

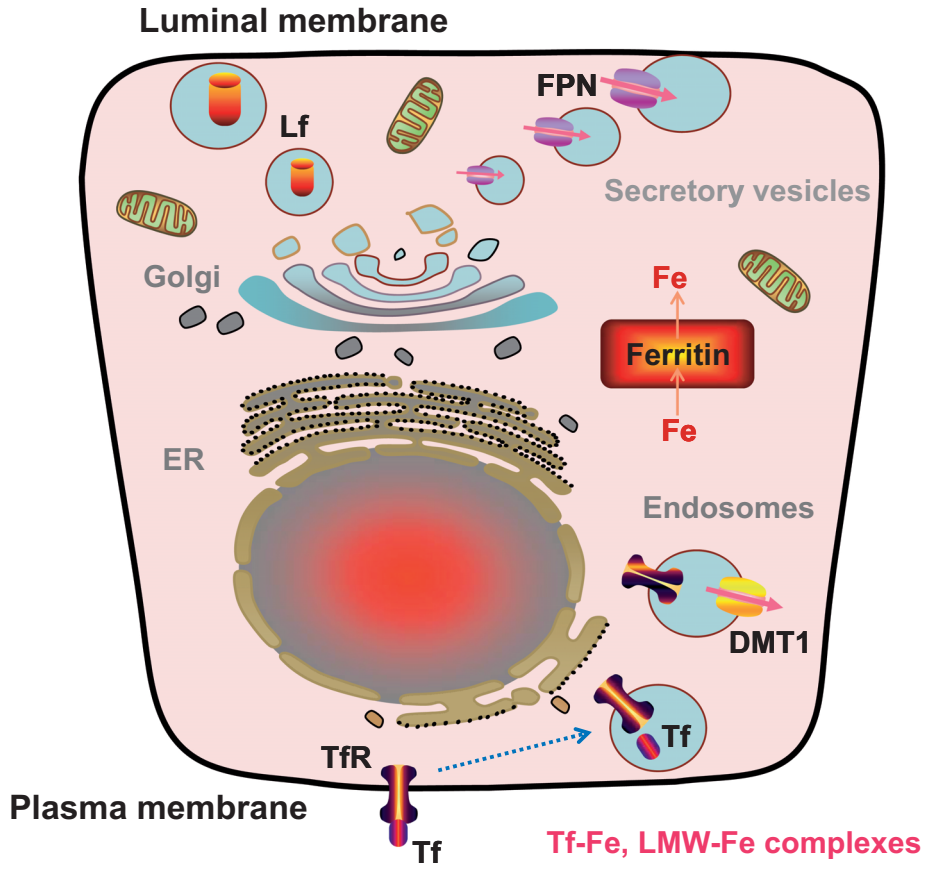

Figure 2

Transport of iron (Fe) in the mammary gland epithelial cell. DMT1, divalent metal transporter 1; ER, endoplasmic reticulum; FPN, ferroportin; LMW, low molecular weight; Tf, transferrin, TfR, transferrin receptor.

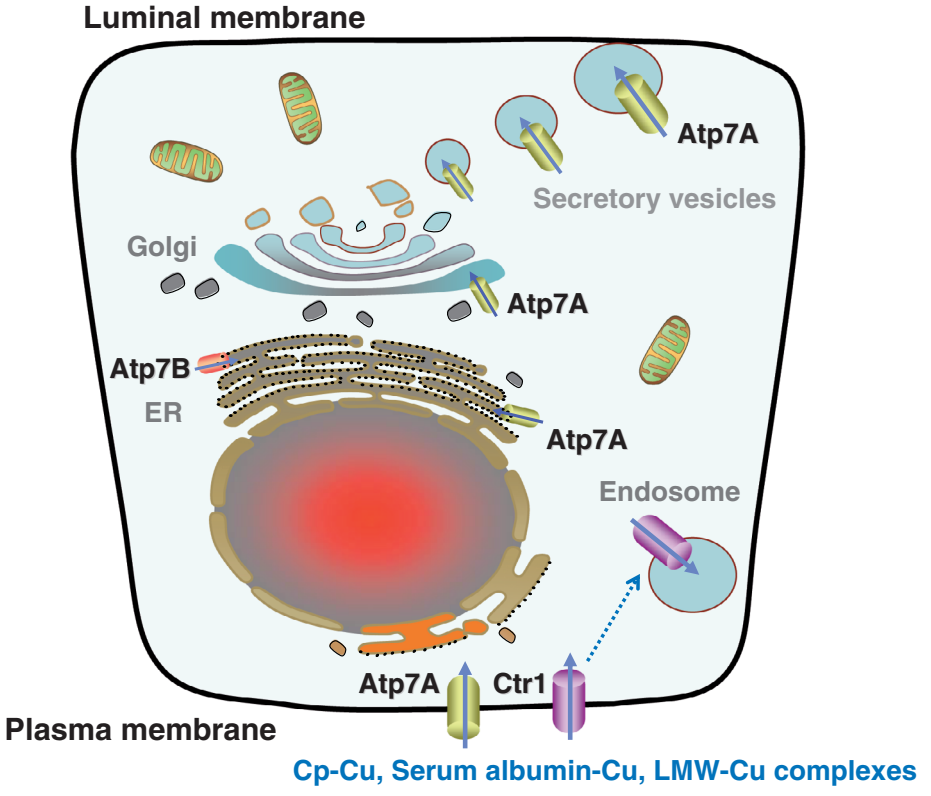

Figure 3

Transport of copper (Cu) in the mammary gland epithelial cell. Atp, adenosine triphosphate; Ctr1, copper transporter 1; ER, endoplasmic reticulum; LMW, low molecular weight.

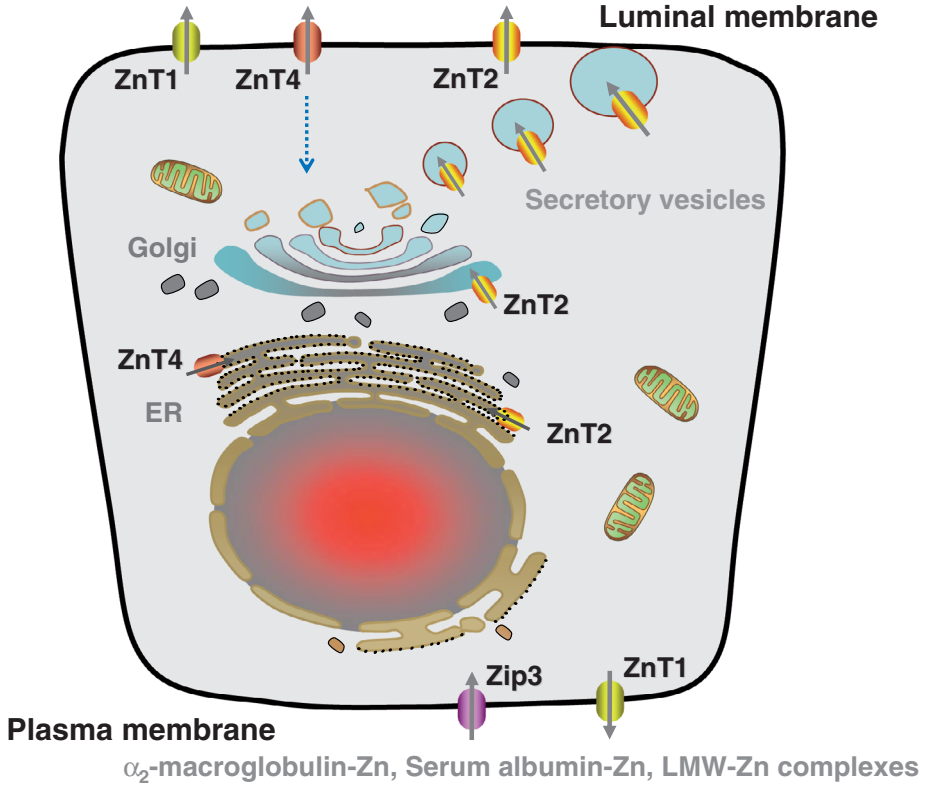

Figure 4

Transport of zinc (Zn) in the mammary gland epithelial cell. ER, endoplasmic reticulum; LMW, low molecular weight; Zip, zinc importer; ZnT, zinc transporter.

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Contents

Indexes

Errata

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