Chapter

Lactose Synthesis

Lorena Mardones and Marcelo Villagrán

Abstract

This chapter is related to lactose synthesis, its chemistry, regulation, and differences between species, especially in cattle. Lactose synthesis takes place in the Golgi apparatus of mammary epithelial cells (MEC) by the lactose synthase (LS) enzyme complex from two precursors, glucose and UDP-galactose. The enzyme complex is formed by galactosyltransferase, and it is associated with α-lactalbumin. Importantly, the lactose secreted determines the volume of milk produced, due to its osmotic properties. Milk contains 5% lactose and 80% water, percentages that remain constant during lactation in the different mammalian species. The low variation in milk lactose content indicates that lactose synthesis remains constant throughout the period of lactation and that is highly conserved in all mammals. Lactose synthesis is initiated during the first third of the pregnancy, increasing after birth and placenta removal. Different glucose transporters have been involved in mammary glucose uptake, mainly facilitative glucose transporters GLUT1, GLUT8, and GLUT12 and sodium-glucose transporter SGLT1, with more or less participation depending on mammal species.

Keywords: lactose, glucose, glucose transporter, mammary epithelial cells

1. Introduction

The mammary gland plays an essential role during the early postnatal life of young mammals, providing them nutrients, water and electrolytes, and immune protection until they reach the size and maturity to survive independently. The mammary gland has a stroma rich in adipose cells and glandular epithelium that origins a lobule-alveolar system, in which terminal there are alveolar epithelial cells involved in milk production. The mammary gland development, as well as its fundamental structure is very similar among different species, with little differences in function, architecture, and number of glands. For example, in rodents, the branches are few and disperse, whereas ruminants have more branches and they are concentrated in the terminal of alveoli [1]. This gland undergoes cyclic changes that make it reach its maximal development in lactation. This is a unique model of cyclic morphogenesis in adults, with four characteristic steps replicated in each pregnancy and which ends with its involution in menopause. The four phases of mammary gland maturity in adulthood are proliferative phase, secretory differentiation phase, secretory activation phase, and lactation phase [2, 3]. Although breast development begins during embryogenesis, it is during pregnancy when terminal maturation of the gland occurs, developing a lobule-alveolar system characterized by branching of the galactophorous ducts and the differentiation of terminal buds to alveoli. Growth of mammary gland is stimulated during pregnancy by the mammotrophic combination of steroids (estrogen, progesterone, and corticosteroids) and polypeptide hormones (prolactin, growth

hormone, and placental lactogen). The four phases of breast differentiation that occur after conception have clear histological differences, as can be seen in **Figure 1**. In the first phase, there is an increase in the amount of glandular tissue, associated with an increase in the number of acini. The second phase is defined by the beginning of lipid synthesis and by its accumulation inside mammary epithelial cells (MEC). The third phase is characterized by the presence of differentiated MEC, capable of producing and secreting all the constituents of milk, which results in a dilation of the alveoli and the presence of an eosinophilic secretion that occupies the acinar lumen. The final phase of mammary gland development is called the lactation phase, and it is the stage in which breastfeeding is established [2]. At this stage, milk secretion is continuous, which is associated with a greater degree of dilation of the alveoli and the presence of milk secretion in the acinar lumen. The process of mammary gland involution starts after weaning and develops in two stages. The first step is a reversible first phase, which lasts a few days, and is due to the release of local breast factors that trigger apoptosis of the secretory epithelia. The second phase, the remodeling, involves the replacing of the lobule-alveolar structures by adipose tissue, degradation of the extracellular matrix and its basal lamina, and the remodeling of adipose tissue [4].

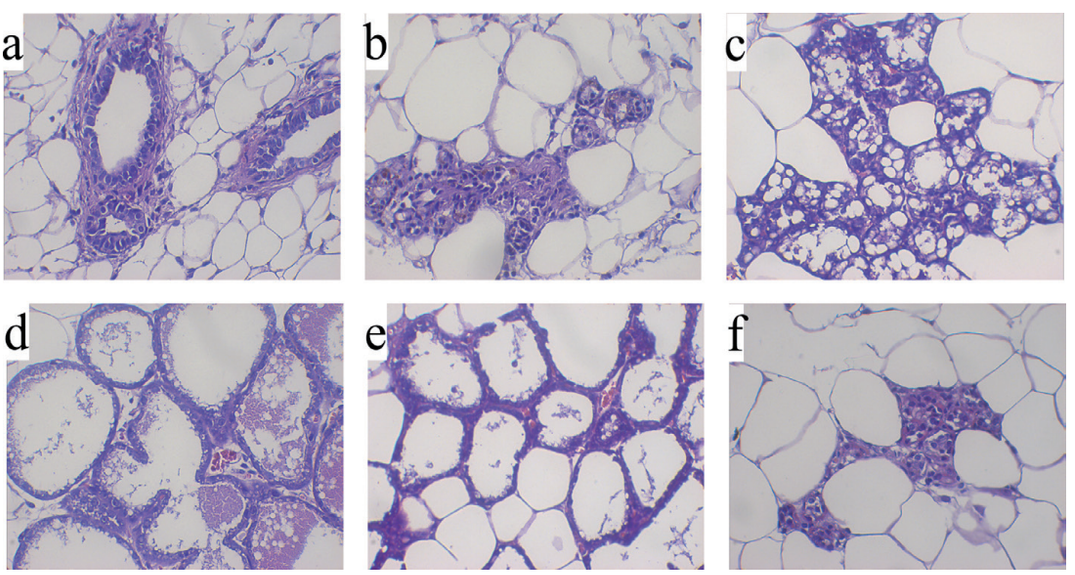

Figure 1.
Histological characteristics of mammary morphogenesis in adults. (a) virgin; (b) proliferative phase; (c) secretory differentiation phase; (d) secretory activation phase; (e) lactation phase; and (f) early involution. Hematoxylin and eosin staining, bar 50 μm [5].

2. Glucose uptake in mammary epithelial cells

Glucose supply to the mammary gland is pivotal to maintain the high rate of proliferation of glandular epithelium in pregnancy and the continuous production of lactose, fat acids, and proteins during lactation [6]. Studies in cows demonstrate that between 60 and 85% of plasmatic glucose is distributed to the mammary gland during lactation and that duodenal glucose injection increases mammary gland glucose uptake and lactose synthesis glucose supply to the mammary gland during lactation, whereas the inhibition of this process or the renal reabsorption of glucose decreased them [7, 8]. On the other hand, in rodents, glucose uptake of the mammary gland duplicates 2 days before delivery, and it remains high during all lactation period, and in humans, 30% of glucose intake is used to lactose production in established lactation [9]. The whole organism adapts to the synthesis of milk; the initial negative energy balance is reversed by greater hepatic gluconeogenesis and decreased peripheral glucose use [10].

The necessity of glucose supply for the proliferation of MEC is very important to lactation persistency, being, together with secretory activity, the two factors that define the lactation curve and peak [4, 11]. In rodents, milk production is mainly associated with a high proliferation of MEC, whereas in cows it is principally due to the increase in secretory activity per cell [12]. In general, MEC proliferation is low in virgin and early pregnancy (5%) in mice, where it has been associated with stem cell renewing, but it persists throughout lactation period, associated with cell replacement, with low net growth, associated with the expression of Ki67 [5, 11]. For example, in cows, the MEC replacement reaches 50%, whereas in rodents it is lower than 25% [11]. When lactation declines, proliferation decreases, and it is exceeded by apoptosis rate, but also the secretory activity by cell decreases [11]. In particular, in humans, mammary growth for lactation starts at week 20 of pregnancy, whereas in mice it starts at day 12 of pregnancy. Studies in mice have established that DNA content increases from the middle of pregnancy until day 5 of lactation, doubling every 6 days, maintaining a net proliferation rate of only 0.3% during lactation, due to parallel apoptosis and loss of cells in milk [13].

The glucose transporters already identified in mammary epithelial cells of rodents, humans, and ruminants are facilitative glucose transporters GLUT1, GLUT8, and GLUT12, SGLT1, and the bidirectional sugar transporter SWEET1 (sugars will eventually be exported transporter) [7, 8, 14–16] (**Figure 2**). The GLUT transporters were initially identified due to the capacity of MEC to transport 3-O-methyl-D-Glucose and inhibition of this by cytochalasin B [17–19]. There are differences in the location and magnitude of peak expression of glucose transporters due to differences between species in the prevalence of cell proliferation or secretory activity [8, 20, 21]. In cows, the increase in the expression of GLUTs is in order of magnitude greater than in rodents, which reflects that its secretory activity

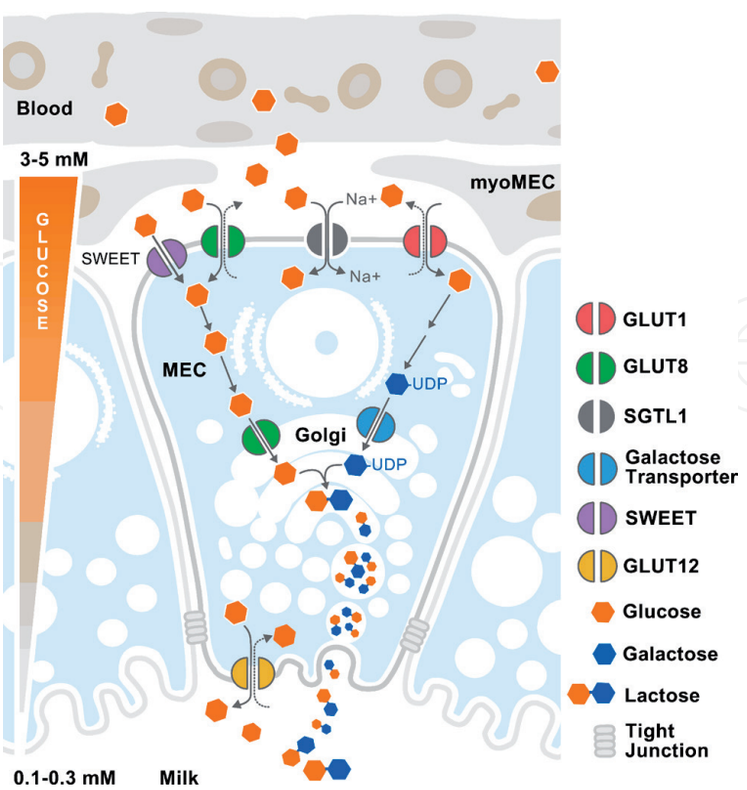

Figure 2.
Glucose uptake in mammary epithelial cell. Distribution of glucose transporters and glucose concentration in different compartments is detailed. MEC, mammary epithelial cells; myoMEC, mammary myoepithelial cells; GLUT, facilitative glucose transporter; SGLT, sodium-glucose cotransporter; SWEET, sugars will eventually be exported transporter.

is highly dependent on the expression of glucose transporters [6, 14]. The maximum expression of GLUT1 observed was between late pregnancy and late lactation, reaching an increase of 5-fold at the protein level and 50-fold at mRNA level [5, 15, 17, 18, 22]. The increase in GLUT8 expression is lower, but it follows the same pattern, associated with cytokeratin 18 and Ki67 expression and MEC proliferation [5, 8]. However, some studies also found an increase of GLUT1 expression in MEC in early pregnancy, which could be related to the start of lipid synthesis in secretory activation phase, when sterol regulatory element-binding protein (SREBP), a transcription factor, appears, or to stem cell renewal [2, 5, 10, 23]. Although the majority of authors found over 60% of GLUT1 expression in plasma membrane in rat lactating gland, some studies found it almost exclusively at intracellular level [5, 19, 20, 24]. Interestingly, in early weaning of BalB/BalC mice, GLUT1 is also concentrated intracellularly, but, due to a decrease in lactation at this step, that could be associated with its accumulation in proteasomal compartment or to apoptotic bodies phagocyted by other epithelial cells acting as nonprofessional phagocytes [5, 25].

The intracellular concentration of glucose in the MEC is mainly determined by its incorporation by GLUT1 transporters in the basolateral membrane and by the activity of the cytosolic hexokinases, which transform glucose into glucose-6-phosphate [2, 23]. The induction of the expression of hexokinase II in the cytoplasm of MEC during the period of breastfeeding is essential in the determination of intracellular glucose levels, because this enzyme has low glucose affinity (K_m 0.3 mM) [26]. On the other hand, glucose is also transported to the lumen of the alveolus, through GLUT12, reaching a concentration of 1.5 mM in milk, equivalent to the concentration found in the cytoplasm of the MEC [20, 26].

3. Lactose synthesis in the Golgi of MEC

3.1 Lactose complex

The first evidence of lactose synthesis in the Golgi of mammary alveolar cells dates back to 1980, when it was associated with the activity of galactosyltransferase and the presence of lactalbumin and bivalent metals such as manganese and calcium [27]. The lactose synthase (LS) synthetizes lactose (beta 1,4-galactoglucose) from UDP-galactose and glucose, and it is located specifically in trans-Golgi. The LS is an enzymatic complex of galactosyltransferase and LALB. LALB is only found in mammary epithelial cells, allowing galactosyltransferase to be specific for the formation of this disaccharide, making galactosyltransferase add galactose to glucose at even low concentration of glucose, increasing its affinity to this carbohydrate 1000-fold [23, 27]. In others cells, galactosyltransferase adds galactose to N-acetylglucosamine glycoconjugates, but in MEC, LALB changes substrate specificity from N-acetylglucosamine to glucose. In fact, the lactose synthesis depends directly on the amount of LALB associated with the galactosyltransferase that is inserted in the inner face of the Golgi apparatus membrane [28]. The LALB knockout produces a viscous, low-lactose milk difficult to remove from the mammary gland, highlighting the osmotic role of lactose in milk yield [29].

LALB expression increases immediately after delivery in pig and rodents and is regulated by lactogenic hormones [30–32]. LS has a K_m of 1.5 mM for glucose and 60 μM for UDP-galactose; thus, the limiting stage in lactose synthesis is the availability of glucose in the Golgi [2, 23, 27]. Lactose synthesis begins in the first third of pregnancy but increases considerably after childbirth, as levels of placental sex steroids decrease, which has an inhibitory effect on lactose synthesis [1]. Lactose production remains relatively constant throughout the entire lactation process thanks to

the action of prolactin and other lactogenic hormones and the stimulation associated with mammary gland emptying. The lactose is secreted in the milk together with LALB, α, β, and κ caseins, β-lactoglobulin, others nutrients, and immunomodulatory molecules [1]. Lactose represents around 5% of the milk content in all species, which revealed that is a highly conserved process. Also lactose is an osmotically active molecule, defining the water content in milk, which is in average 80% [1, 7]. In cows, in particular, milk is 5.0% lactose, 3.4% fat, and 2.3% protein. In seals, the lactose content of milk is minimal, and fat is predominant (50%), followed by 6.0% protein. This could be explained because pups need to double their weight in only 4 days to survive adverse environmental conditions [1]. On the other hand, human milk has a similar fat content to cow milk (3.7%), less protein content (1.0%), and more lactose (7.0 v/s. 5.0%). Donkeys presents similar content of macronutrients in milk to human, with 7.4% lactose, 2.0% protein, and 0.4% fat [9].

3.2 Golgi's glucose transporters

The first studies related to glucose transport to the Golgi of MEC concluded that this was mediated by GLUT and SGLT transporters, since the transport of monosaccharides was inhibited by phloretin and phlorizin, known inhibitors of both types of transporters [33]. These vesicles present stereospecificity for several monosaccharides, such as D-glucose, L-glucose, D-xylose, 2-deoxy-D-glucose, and D-fructose. Moreover, the vesicles showed low permeability for glucosamine, a substrate of the GLUT1 transporter; for this reason, we assume that another glucose transporter is involved in the incorporation of glucose into this organelle. A decade later, another GLUT was identified in Golgi vesicles of MEC of late-lactating mice through Western blotting and binding studies of cytochalasin B, co-localizing with 110-kDa coatomer-associated protein β-COP [19, 24]. Interestingly, the results revealed that there is a second cytochalasin B-sensitive glucose transporter, which could correspond to GLUT8, cloned after such studies [34, 35]. In our last study, we were able to identify GLUT8 in the Golgi of lactating MEC in mice, co-localized with LALB, 58 K Golgi protein, and Golgi membrane-associated protein 130 [5]. Additionally, SGLT1 was identified in the Golgi of MEC from lactating cows, but no functional studies have been performed [23]. As SGLT is an active transporter that mobilize glucose thanks to electrochemical sodium gradient at the plasma membrane, more studies related to ion gradient between cytoplasm and inside the Golgi should be performed to really know the contribution of this transporter to glucose uptake into the Golgi of MEC. In **Figure 3**, we show glucose transporters present in the Golgi of mammary epithelial cell and their association with lactose synthesis.

In summary, the reports highlight a variable increase in the expression of GLUT1, GLUT8, and GLUT12 in pregnancy and/or lactation in different models, including rodents and ruminants, but their responsibility in glucose uptake in the Golgi of mammary epithelial cells, an essential step to lactose synthesis, is not clear [8, 24, 36]. Moreover, although GLUT8 has been co-localized with Golgi proteins in MEC and in different compartments of endomembrane system in other cell types, GLUT1 has been found in the Golgi of MEC only in some of the species and strains studied, and its intracellular localization had been associated with mitochondria, which in not part of endomembrane system [5, 24, 33, 37]. In particular, GLUT8 has been found in late endosome and reticulum.

3.3 Lactose synthesis regulation

The lactose synthesis depends principally on lactogenic hormones and glucose uptake in the Golgi of MEC. It starts in the first third of pregnancy, increasing

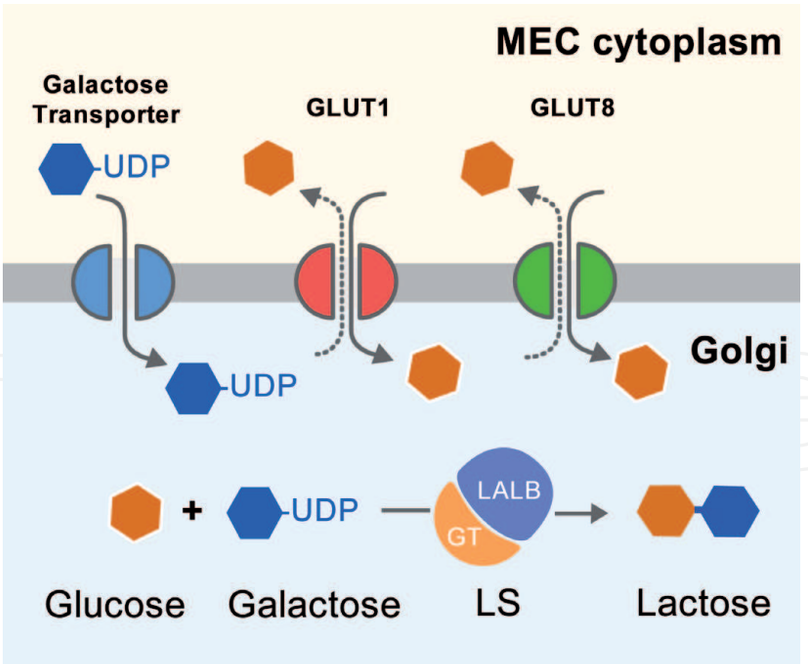

Figure 3.
Glucose transporters in the Golgi of mammary epithelial cell associated with lactose synthesis. Distributions of glucose transporters in this organelle are detailed. MEC, mammary epithelial cells; GLUT, facilitative glucose transporter; LS, lactose synthase; LALB, lactalbumin; GS, galactosyltransferase.

considerately after parturition, when placental sexual steroid hormones decrease and lactogenic hormones increase [30–32]. The principal lactogenic hormone is prolactin, which is stimulated by suckling. The milk production also has been associated with the removal of an inhibitory agent of secretory activity of MEC in milk. Other factors involved in milk production are light and sexual hormones. The increase in light photoperiod from 16 to 18 h increases milk production, due to prolactin via insulin-like growth factor (IGF-1) and somatotropin, whereas pregnancy decreases milk production, due to an increase in estrogens [8]. There is still controversy over the role of LS in milk production. In particular, LALB knockout mice were unable to produce milk, and lactogenic hormones change LALB expression only in particular species. For example, in humans, prolactin increases LALB mRNA, but in rabbits it decreases it, and in other species it does not produce any change [20, 24]. Also, it has been proposed that hexokinase and the different enzymes involved in glucose transformation to UDP-galactose are important for lactose synthesis [7].

As it has been described, the limiting stage in lactose synthesis is the availability of glucose in the Golgi [27], but a combination of lactogenic hormones failed to induce their expression in bovine mammary explants [22, 30]. There are not changes in GLUT8 or GLUT12 expression in response to insulin, leptin, growth hormone, or glucose, but estradiol and progesterone increase GLUT1 in MEC [15, 17, 19, 38]. GLUT1 was redistributed to an intracellular compartment, presumably the Golgi, in response to prolactin and hydrocortisone, associated with phosphatidylinositol-3-kinase (PI3K) and protein kinase C (PKC) pathways and STAT5 binding to its promotor [39–41]. The upregulation of GLUT1 in pregnancy and lactation also has been associated with an increase in serotonin via 5'adenosine monophosphate-activated protein kinase (AMPK) and hypoxia via HIF1α [22, 28]. On the other hand, serotonin increased GLUT8 in the mammary gland and hypoxia and lipopolysaccharide decrease it [10]. GLUT8 is internalized in response to insulin in trophodermic cells and changed its expression in insulin-sensitive tissues, such as the liver and kidney, but failed to produce effects in adipocytes and

neuroblast cells [42–44]. Some carbohydrates also produce changes in location and expression of GLUT8, i.e., glucose induces GLUT8 trafficking from the Golgi to the reticulum of hippocampal cells in rats and upregulates it in 3T3-L1 adipocytes [42, 45]. On the other hand, fructose downregulates GLUT8 expression in colon tissue and CaCo-2 colon carcinoma cells but increases its expression in hepatocytes, where it is located in the plasma membrane [46–48]. GLUT8 promoter has a binding sequence to transcription factor NF1, which has been associated with the response of GLUT4 to insulin and cyclic adenosyl monophosphate (cAMP) [42, 49].

4. Conclusion

Mammals rely exclusively on milk supply from the mammary gland to survive at an early age. The proliferation of mammary epithelial cells and mammary establishment depend on glucose supply to the gland, whereas lactose synthesis depends directly on glucose entry into the Golgi of MEC, which is conjugated with UDP-galactose by lactose synthase to produce the disaccharide lactose. MEC presents polarized expression of GLUT1, SGLT1, and GLUT12 in its plasma membrane and also expresses GLUT1, GLUT8, and SGLT1 in the Golgi. Hormones and oxygen tension regulate the expression of these transporters; however, further studies are necessary to explore the effects of light/dark cycles and suckling in their expression, since these are factors involved in milk production. Additionally, the kinetics of transporters involved in glucose uptake in the Golgi or cytoplasm of MEC also needs to be explored. Understanding the regulation and function of glucose transporters will be useful to improve efficiency of milk yield in both, humans and cattle.

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Conflict of interest

The authors declare no conflict of interest.

Nomenclature

MEC	mammary epithelial cell
GLUT	facilitative glucose transporter
SGLT	sodium-glucose transporter
SWEET	sugars will eventually be exported transporter
LALB	lactalbumin
LS	lactose synthase
SREBP	sterol regulatory element-binding protein
K_m	Michaelis-Menten kinetic constant
β-COP	110-kDa coatomer-associated protein
AMPK	5’ adenosine monophosphate-activated protein kinase

PI3K	phosphatidylinositol-3-kinase
cAMP	cyclic adenosyl monophosphate
NF1	nuclear factor 1
STAT5	signal transducer and activator of transcription 5
PKC	protein kinase C

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