Lactose

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Introduction

Milk provides an essential and complete diet for the newborn, which initially is unable to collect, chew, or digest solid food. Milk consists of a large number of both major and minor components. Lactose is one of the major components of milk, ranging in concentration from as little as $1\,g\,l^{-1}$ in fur seals to more than $70\,g\,l^{-1}$ in humans and donkeys (**Table 1**). In ruminants, the concentration of lactose in milk appears to be between 40 and $50\,g\,l^{-1}$. In fact, of all milk components, lactose content is the least variable, and, as will be discussed in greater detail later, it is the major osmole in milk that determines to a large extent milk volume or the amount of water in the milk. A lower content of lactose in milk tends to be associated with less water in the milk or a higher content of milk solids. The advantages of this to dairy manufacturers in terms of lower storage and transportation costs are obvious. This is the reason why, despite it being one of the major milk components, not many countries consider the value of lactose in their milk pricing system. Nevertheless, from a biological point of view, lactose is an essential milk component.

Biosynthesis and Secretion

Lactose is a disaccharide consisting of two six-carbon carbohydrates: a glucose and a galactose molecule. Since the latter is also derived from glucose, glucose is the sole precursor of lactose in both ruminant and nonruminant species. The mammary gland itself does not produce glucose, because it lacks the crucial glucose-6-phosphatase (EC 3.1.3.9) enzyme, and all glucose must be supplied via the mammary arterial blood supply. During full lactation in dairy species, as much as 85% of the circulating blood glucose supply may be extracted by the mammary gland.

There is a distinct difference in glucose metabolism between ruminants and nonruminants. In the latter, food carbohydrates are enzymatically broken down in the digestive tract into simple sugars, and glucose is one of the major breakdown products readily absorbed from the small intestine into the bloodstream. In ruminants, on the other hand, the vast majority of food carbohydrates are broken down in the rumen by bacteria and protozoa to satisfy their own energy demand. They in turn produce volatile fatty acids such as butyrate, acetate, and propionate, which are absorbed through the rumen wall into the blood. In the liver, propionate is converted to glucose via gluconeogenesis. This means that ruminants are in a state of continuous gluconeogenesis and that blood glucose levels in ruminants are less than those in nonruminants. However, in both, glucose is essential for the maintenance of critical body functions, which means that the large drain of glucose by the lactating mammary gland potentially has greater repercussions on the ruminant. Potentially, ruminants have adopted a number of 'glucose-sparing' strategies. Instead of glucose, they can also use ruminally produced acetate as an energy source. Furthermore, in contrast to nonruminants, in which glucose is the major precursor for fatty acid synthesis in the mammary gland, in ruminants the required acetyl CoA carboxylase comes instead from acetate, thus leaving more of the precious glucose available for lactose synthesis.

The fact that all glucose, as the sole precursor of lactose, has to come from the blood raises the following question: How glucose gets into mammary secretory cells? High glucose concentrations in the blood and very low intracellular glucose levels imply a large concentration gradient. Formerly, it was generally believed that glucose entered the cell from the blood via a passive process of diffusion. However, it is now clear that transmembrane glucose transport is facilitated by at least two active transport systems. A family of facilitated glucose transporters (GLUTs) is expressed in the bovine mammary gland, with GLUT1 being the key transporter; several other members of the GLUT family are also expressed in the bovine mammary gland. Moreover, expression of the sodium/glucose cotransporters (SGLT1 and SGLT2) has been demonstrated in bovine mammary tissue, and the SGLT1 protein has been detected in the plasma membrane fraction. Significant upregulation of both GLUT and SGLT at the onset of lactation, compared with the nonlactating gland during gestation, clearly demonstrates a significant role of these transport systems in the uptake of glucose by the mammary epithelium.

Once in the cytosol, glucose has to move into the Golgi apparatus where the actual synthesis of lactose occurs. *In vitro* studies in the early 1980s with purified Golgi membrane vesicles from rat mammary cells suggest that glucose can freely enter the Golgi from the cytosol, but

Table 1 Gross composition of milks of various species

		Percentage by weight				
Species		*Water*	*Fat*	*Protein*	*Lactose*	*Ash*
Aardvark	*Orycteropus afer*	68.5	12.1	14.3	4.6	1.4
Black bear	*Ursus americanus*	55.5	24.5	14.5	0.4	1.8
Camel	*Camelus dromedarius*	86.5	4	3.6	5	0.8
Cow	*Bos taurus*	87.3	3.9	3.2	4.6	0.7
Dog	*Canis familiaris*	76.4	10.7	7.4	3.3	1.2
Dolphin	*Tursiops truncatus*	58.3	33	6.8	1.1	0.7
Donkey	*Equus asinus*	88.3	1.4	2	7.4	0.5
Fringed bat	*Myotis thysanodes*	59.5	17.9	12.1	3.4	1.6
Fur seal	*Callorhinus ursinus*	34.6	53.3	8.9	0.1	0.5
Goat	*Capra hircus*	86.7	4.5	3.2	4.3	0.8
Gray squirrel	*Sciurus carolinensis*	60.4	24.7	7.4	3.7	1
Guinea pig	*Cavia porcellus*	83.6	3.9	8.1	3	0.8
Hedgehog	*Erinaceus europaeus*	79.4	10.1	7.2	2	2.3
Horse	*Equus caballus*	88.8	1.9	2.5	6.2	0.5
Human	*Homo sapiens*	87.1	4.5	1	7.1	0.2
Indian elephant	*Elephas maximus*	78.1	11.6	4.9	4.7	0.7
Manatee	*Trichechus manatus*	87	6.9	6.3	0.3	1
Opossum	*Didelphis virginiana*	76.8	11.3	8.4	1.6	1.7
Pig	*Sus scrofa*	81.2	6.8	4.8	5.5	1
Rabbit	*Oryctolagus cuniculus*	67.2	15.3	13.8	2.1	1.8
Rat	*Rattus norvegicus*	72.4	9.3	8.2	3.7	1.4
Red kangaroo	*Macropus rufus*	80	3.4	4.6	6.7	1.4
Reindeer	*Rangifer tarandus*	66.7	18	10.1	2.8	1.5
Sheep	*Ovis aries*	82	7.2	4.6	4.8	0.9
Sloth	*Bradypus variegatus*	83.1	2.7	6.5	2.8	0.9
Tree shrew	*Tupaia belangeri*	59.6	25.6	10.4	1.5	
Water buffalo	*Bubalus bubalis*	82.8	7.4	3.8	4.8	0.8
Yak	*Bos grunniens*	82.7	6.5	5.8	4.6	0.9
Zebu	*Bos indicus*	86.5	4.7	3.2	4.7	0.7

Reproduced from Jenness R (1986) Lactational performance of various mammalian species. *Journal of Dairy Science* 69: 869–885.

work on mammary GLUT1 expression in the mouse mammary gland demonstrates targeting of this GLUT to the Golgi membrane, which obviously contradicts earlier observations of unrestricted entry of glucose into the Golgi. The mode of entry of glucose into the Golgi has not yet been resolved, but the presence of GLUT1 strongly suggests an active transport mechanism for glucose.

The synthesis of lactose from glucose requires a number of enzyme-mediated steps (**Table 2**). The initial steps occur in the cytosol, whereas the final step takes place inside the Golgi. The fact that UDP-galactose is formed in the cytosol means that it too needs to be transported into the Golgi and, as for glucose, there appears to be an active transport mechanism for UDP-galactose in the Golgi membrane. The final step of combining glucose and galactose takes place inside the Golgi, and the reaction is catalyzed by the enzyme lactose synthetase (EC 2.4.1.22). This critical enzyme consists of two proteins, the enzyme galactosyltransferase (EC 2.4.1.22) and α-lactalbumin, one of the major whey proteins in milk that is produced in the rough endoplasmic reticulum. Despite galactosyltransferase being bound to the inside of the Golgi membrane, the enzyme is not specific to

Table 2 Enzyme-mediated steps in lactose synthesis

1	Glucose + ATP	(1) →	Glucose-6-P + ATP
2	Glucose-6-P	(2) →	Glucose-1-P
3	Glucose-1-P + UTP	(3) →	UDP-glucose + PP_i
4	UDP-glucose	(4) →	UDP-galactose
5	UDP-galactose + glucose	(5) →	Lactose + UDP

(1) hexokinase (EC 2.7.1.1), (2) phosphoglucomutase (EC 5.4.2.2; formerly EC 2.7.5.1), (3) UDP-glucose pyrophosphorylase (EC 2.7.7.9), (4) UDP-galactose-4-epimerase (EC 5.1.3.2), (5) lactose synthetase (EC 2.4.1.22); UDP, uridine diphosphate; UTP, uridine triphosphate.

mammary cells. Only after forming a complex with α-lactalbumin, which increases the affinity of the enzyme for glucose, is it able to facilitate the formation of lactose, a process that is unique to the mammary gland. Although both galactosyltransferase and α-lactalbumin are essential components of lactose synthetase, the enzyme complex can be activated only in the presence of bivalent cations, probably manganese, zinc, and/or calcium.

Once synthesized, lactose cannot 'escape' from the Golgi organeller other than in vesicles budding from the inner Golgi membrane. These vesicles, called secretory vesicles, also contain milk proteins and move with the help of the microtubular component of the cytoskeleton through the cytosol toward the apical membrane of the cell. Here they fuse with the apical membrane and release their contents, including lactose, into the milk pool inside the alveolar lumen. However, because lactose makes the inside of the vesicles hypertonic with respect to the cytosol, water is drawn from the cytosol into the secretory vesicles until they are in equilibrium with the cytosol. Hence, an increase in lactose synthesis and thus higher concentrations inside the secretory vesicles result in more water being drawn in. This explains why the lactose content of milk varies very little within species, but it also means higher milk production. In other words, lactose determines to a large extent milk volume or the amount of water in the milk. Although ions and other small solutes will also contribute to the hypertonic environment within the secretory vesicles, lactose is by far the major osmotic constituent.

Reducing Milk Lactose Content

Lactose obviously plays a major role in determining milk volume, which would be of great benefit to the farmer if it were not for the fact that it does so solely by increasing water content and thus increasing milk transportation and handling costs. For this reason, many have considered lowering the lactose content of milk in an attempt to produce more concentrated milk, or even producing milk without lactose. The latter would make milk and dairy products, in general, more acceptable to people who are lactose intolerant. However, the biology of milk synthesis and secretion is such that this is impossible, atleast until the advent of modern biotechnology. This issue can be addressed through transgenics and gene knockout technology, albeit in laboratory animals.

Gene knockout experiments in mice not only demonstrate irrefutably the absolute requirement for α-lactalbumin for lactose synthesis, but also show that reducing or eliminating lactose synthesis will lead to a significantly lower, but more concentrated milk, yield. Moreover, milk production can be restored by introducing the gene for human α-lactalbumin (**Table 3**). These experiments not only show that α-lactalbumin is not species specific, but also demonstrate that it is not possible to produce lactose-free milk. Despite the fact that mice had both lactose alleles knocked out, they produced viable offspring, but the pups could not survive if left with the mother, presumably because the 'milk' was too concentrated to be removed from the mammary glands.

It should probably be pointed out that currently dairy processors would have to struggle to deal with milks of different levels of viscosity, because factories are set up to handle large amounts of milk with a consistent solids content of approximately 12%. A more realistic scenario, considered in some countries where milk has to be transported over long distances, is to set up regional substations where milk could be condensed to a desired solids level before it is transported. This would, however, require drastic changes in milk reception areas of dairy factories.

Table 3 The effect of α-lactalbumin gene knockout and gene replacement on milk yield and composition in mice

	Genotype			
Parameter	α-*lac*^{m}/α-*lac*^{m}	α-*lac*^{m}/α-*lac*$^-$	α-*lac*$^-$/α-*lac*$^-$	α-*lac*^{h}/α-*lac*^{h}
Fat (%) (vol/vol)	28.23 ± 1.65	29.6 ± 1.3	45.25 ± 2.15***	21.2 ± 0.23*
Protein (mg ml^{-1})	87.52 ± 5.82	95.81 ± 9.5	164.63 ± 13.92***	77.7 ± 1.05
Lactose (mmol l^{-1})	62.44 ± 9.27	42.7 ± 4.2	0.7 ± 0.34**	56.85 ± 3.8
Milk yield (g day^{-1})	7.51 ± 0.44	6.7 ± 0.38	1.37 ± 0.48***	9.94 ± 0.65*

Reproduced with permission from Stacy A, Schnieke A, Kerr M, *et al*. (1995) Lactation is disrupted by α-lactalbumin deficiency and can be restored by human α-lactalbumin gene replacement in mice. *Proceedings of the National Academy of Science of the United States of America* 92: 2835–2839.
Values given are the mean ± SE; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ by unpaired *t* test. α-lac^m/α-lac^m = wild-type mice, with both α-lactalbumin alleles intact; α-lac^m/α-lac$^-$ = mice deficient for one α-lactalbumin allele; α-lac$^-$/α-lac$^-$ = mice deficient for both α-lactalbumin alleles; α-lac^h/α-lac^h = mice carrying two human α-lactalbumin alleles.

Factors Affecting Milk Lactose Synthesis

Diet and Environmental Effects

A more practical and less dramatic approach to manipulating milk lactose content would be through environmental and/or dietary measures. However, one must keep in mind that due to its role as the major determinant of milk volume, the lactose concentration in milk is very stable. Lactose yield, on the other hand, may fluctuate considerably, but this is a direct result of corresponding changes in milk yield. Nutritional factors, such as feeding frequency and level and order of concentrate and forage feeding, can significantly increase or reduce lactose yield by affecting arterial glucose supply to the mammary gland (i.e., changes in blood glucose concentration and/or mammary blood flow), changes in mammary uptake of glucose, and changes in the metabolic rate within the mammary gland. None of these factors, however, will have much of an impact on milk lactose content.

Environmental stress factors can similarly affect lactose yield. Temperatures on either side of the thermoneutral zone and high relative humidity have been shown to lower milk lactose yield. These observations are consistent with the results from experiments on the effects of providing shade during hot weather, which had shown that shade can prevent the adverse effects of high ambient temperatures on lactose production. In addition to these physical stress factors, it is well known that psychological stressors, such as noise, transport, novel environments, and fear, can adversely affect milk yield and thus lactose yield. None of these factors are likely to have any major direct effect on lactose synthesis in the mammary gland, but they act indirectly through reduced substrate supply due to directing away of glucose from the mammary gland to supply energy for other more acute body functions to maintain body temperature (e.g., shivering, cooling). Under psychological stress, critical tissues such as brain, heart, and skeletal muscle have an increased energy demand and levels of epinephrine, increase. These ensure that glycogenolysis increases in the liver and skeletal muscles, and glucose supply to the mammary gland is reduced, to favor of adequate supply to other critical tissues.

One interesting difference between nutritional factors and environmental factors in terms of their effect on lactose is that with the former, milk lactose content remains constant, whereas stress, both physical and psychological, results in lower concentrations of lactose in the milk. This is an interesting phenomenon given that lactose is the major osmotic factor in milk. The only way by which lactose content can decrease is by 'leaking' from the milk pool between adjacent alveolar cells into the bloodstream. Normally, this so-called paracellular pathway is blocked by tight junctions, the 'gasket-like' structures surrounding each secretory cell in the mammary gland, but there is now good evidence that stress can temporarily impair these tight junctions, resulting in elevated levels of milk lactose in the blood. Once in the blood, lactose is not metabolized, but it is cleared rapidly by the kidneys. The elimination half-life of lactose is approximately 40 min in cows.

Milking Frequency

Milk lactose content may also fluctuate as a result of milking frequency. Compared to twice-daily milking, the concentration of lactose in milk may be higher with thrice-daily milking. In contrast, there is ample evidence that once-daily milking results in lower levels of lactose in the milk. Given that milk cells cannot secrete lactose through the basolateral membrane, the only way the concentration of lactose in milk secreted into the alveolar lumen can decrease is by 'leaking' between secretory cells via open tight junctions into, the interstitial fluid and blood, as pointed out in the previous section. In fact, the measurement of lactose in plasma provides a good *in vivo* indicator of tight junction patency.

Once-daily milking decreases milk yield by 15–20%, and one of the processes occurring during the first 24 h, after switching from twice-daily milking to once-daily milking, is the opening of tight junctions after approximately 18 h of milk accumulation. This is demonstrated by a large increase in the level of plasma lactose (**Figure 1**). It has been shown that tight junctions are closely connected to the cytoskeleton of the cell, and that components of the tight junction are directly involved in gene expression through interaction with transcription factors. Thus, disruption of tight junctions, via disruption of their interaction with the cytoskeleton, may restrict the movement of secretory vesicles from the Golgi to the apical membrane, which would explain the decrease in milk yield with once-daily milking. Alternatively, with thrice-daily milking, tight junctions appear to be tighter than with twice-daily milking, and milk yield is increased correspondingly.

Milking frequency may also have a direct effect on lactose synthesis. A putative feedback inhibitory protein isolated from milk decreases lactose synthesis *in vitro* and decreases milk yield *in vivo* when administered via the teat to goats. It is proposed that with once-daily milking, this protein may act to decrease lactose synthesis and thus milk yield, whereas with more frequent milking the feedback inhibitory activity of the protein is reduced or prevented, allowing for increased milk yield. However, the exact mode of action of this protein and its sequence is yet to be established.

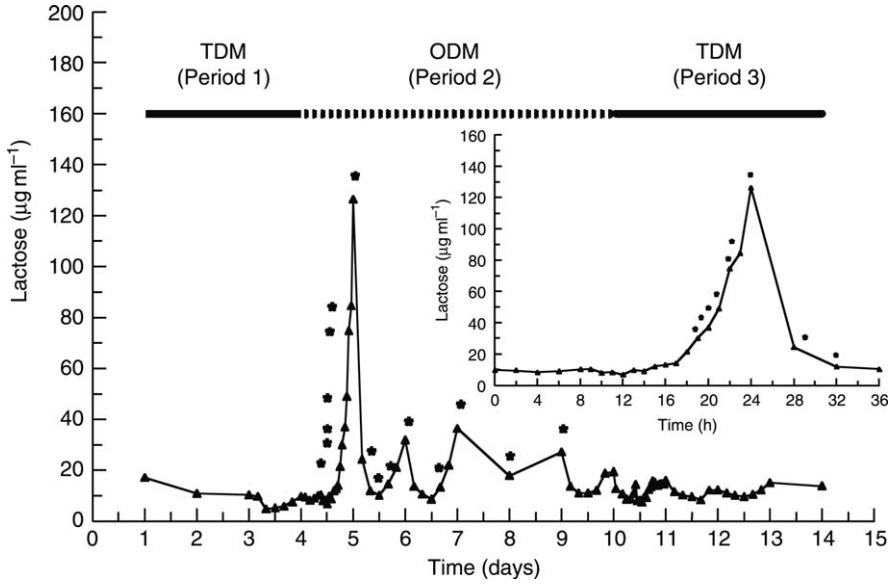

Figure 1 Effect of twice-daily (TDM) and once-daily (ODM) milking of Jersey cows on the concentration of lactose in blood plasma. Plasma lactose levels start to increase after approximately 18 h of milk accumulation, indicating that tight junctions between adjacent mammary secretory cells open. *Significantly increased compared to baseline levels ($P < 0.05$). Reproduced with permission from Stelwagen K, Farr VC, McFadden HA, Prosser CG, and Davis SR (1997) Time course of milk accumulation-induced opening of mammary tight junctions and blood clearance of milk components. *American Journal of Physiology – Regulatory, Integrative and Comparative Physiology* 273: R379–R386.

In conclusion, there are a number of different mechanisms operating in the mammary gland that regulate milk yield in response to changes in milking frequency. Ultimately, the effects of these mechanisms appear to be mediated by lactose synthesis.

Uses of Lactose

Although lactose plays a crucial role in determining milk volume, not many countries include lactose in their milk payout scheme. Lactose, however, does hold value as a milk component in its own right. Globally, milk lactose is being used as a fermentation precursor to produce ethanol. This use of lactose may become increasingly important as demand for ethanol as an alternative to fossil fuel continues to increase. Traditionally, lactose has also been valued by the pharmaceutical industry as a filler compound in many common drugs. Finally, lactose is also the precursor of lactulose, an inert sugar molecule that is used as a prebiotic to stimulate gastrointestinal function and also as a treatment for constipation.

Health Considerations

Ketosis or acetonemia is a metabolic disorder that is caused by a sudden excessive influx of fatty acids into the liver as a result of lipolysis. There are several factors that may induce ketosis, but they are all related to a situation in which energy output exceeds energy intake. In high-producing cows, especially during early lactation, the sharp increase in mammary glucose uptake from the blood to facilitate lactose biosynthesis may cause the animal to become hypoglycemic and develop ketosis. Intravenously administered glucose and/or propylene glycol solutions may be used to treat hypoglycemia and ketosis. Glucocorticoids may also be an effective treatment. In ruminants, glucocorticoids reduce mammary utilization of glucose, thereby maintain higher systemic levels, whereas in nonruminants, glucocorticoids appear to enhance glucose production.

In certain human populations, in particular those of Asian and African origin, lactose intolerance is a common problem. Although this problem is not directly related to lactose biosynthesis in the mammary gland as such, it is an important consideration when milk-derived products are used for human consumption. People affected by lactose intolerance lack the enzyme lactase (EC 3.2.1.108). Lactase, produced by the small intestine, breaks down lactose into glucose and galactose, which can then be readily absorbed through the intestinal wall. In the case of lactase deficiency, lactose will not be broken down and absorbed, but instead it provides an excellent nutrient for bacteria residing in the gastrointestinal tract, resulting in increased carbon dioxide production, which leads to excessive flatulence. Excess lactose may also upset the osmotic balance in the intestine and interfere with water absorption from the intestine, causing, among other things, diarrhea. These properties,

however, may also be used to our advantage, for example, by using lactulose, for which lactose is the precursor, as a stool softener to alleviate constipation in humans. Although the effects of lactose intolerance are not life-threatening, they can cause significant discomfort. However, it should be pointed out, that lactose intolerance is not a disorder. It could even be considered a normal developmental phenomenon. In many people, the production of lactase starts to decline after 2 years of age, when the diet changes from a milk-based to a nonmilk-based diet. In fact, humans are the only mammals that rely on milk-based foods as a major part of their adult diet. Therefore, perhaps lactose-tolerant people are the odd ones out, in a biological sense.

Conclusions

Glucose is the sole precursor for the biosynthesis of lactose in the mammary gland, and it is converted into lactose via a number of enzymatic steps. The final step, linking glucose and galactose, is mediated by lactose synthetase, which requires galactosyltransferase to form a complex with α-lactalbumin in order to increase the affinity for glucose. Because lactose is the predominant osmoregulatory component in the secretory vesicles produced within the secretory cells, it draws in water from the surrounding cytosol and, as such, determines milk volume when the vesicles fuse with the apical membrane and release their contents into the alveolar lumen. Management factors and environmental factors can affect lactose synthesis and, as a result, milk volume. However, most of these effects are mediated indirectly by affecting substrate (i.e., glucose) availability for mammary uptake.

See also: **Lactose and Oligosaccharides**: Lactose: Chemistry, Properties; Lactose: Crystallization; Lactose: Derivatives; Lactose: Galacto-Oligosaccharides; Lactose Intolerance; Lactose: Production, Applications; Indigenous Oligosaccharides in Milk; Maillard Reaction.

Further Reading

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