The Evolution and Genomic Aspects of Milk

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1 Introduction

Lactation can be defined as a process by which milk is secreted in large amounts by mammary glands in order to nourish the organism's offspring. It plays a key role in the reproductive strategy of mammals, so much so that the Linnaean classification that created the class Mammalia in 1758 did so on the basis of the ability to lactate, rather than on other features of anatomy shared by the group. Choosing lactation as the basis to categorize the group is reflective of the great significance lactation plays in nourishing the young of all mammalian species, including humans. Indeed, lactation is said to have created a unique nutritional environment in this group of organisms [1].

According to the fossil record and molecular evolutionary studies, the earliest mammals evolved during the Triassic era (252–201 million years ago (Mya)), from Synapsid animals which had diverged from Sauropsids within the Permian era (299–252 Mya). A comparison of genomes undertaken recently has proven that the molecules which make lactation possible have existed for a very long time. The beginnings of lactation are seen in the therapsids, developing over the course of the Triassic era. Lactation is likely to have been present in the earliest common ancestor of all current mammalian species, as well as in the mammaliaformes, by the late Triassic. Lactation appears to have evolved in cynodonts at the same times as the other features associated with the mammals as a group, such as the integumentary system, ability to generate their own body heat and growth of hair/fur [1, 2].

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Thus, mammalian lactation has been evolving for over 200 million years, which has helped to generate the radical differences in reproductive strategy seen within the group. Nowhere is this more apparent than in the lactational differences, which may affect either an entire lineage or a single species. Within mammals, there are three subgroups, which diverged around 220 Mya: the Prototheria (known nowadays as the monotremes), the Metatheria (containing the marsupials), and the Eutheria (the placental mammals). There are only two families extant from the monotremes, both in Australasia, namely the platypus (*Ornithorhynchus anatinus*) and the echidnas (belonging to the genus *Tachyglossus or Zaglosus*). The monotremes lay eggs and possess a more primitive system for lactation than that found in the other two groups of mammals. Molecular-based research using genomic and transcriptomic techniques has permitted comparison between the prototherian, metatherian, and eutherian lactational systems [2–5].

There is a great deal of complexity in the way lactation occurs within different mammalian species and the process involves more than simply meeting the nutritional requirements of the offspring. Milk itself exerts a regulatory role on the mammary gland and the growth of the offspring. The complexity of the processes involved is emerging through studies of highly specialized lactational systems, such as those possessed by fur seals or marsupials [6].

2 The Evolution of Mammals and how Lactation Originated

The exact evolutionary mechanism which must have operated on the mammary gland and lactation over the course of time in the synapsid branch is much debated since direct evidence in the form of extant species showing intermediate stages or fossils indicating parental care and the changing morphology of the mammary gland are not found. In current mammalian species, lactation is a sophisticated process that exhibits many different features in terms of morphology, physiology, biochemistry, ecology, and ethology. It is improbable that such diversity could appear all at once, and a more primitive system must clearly have once existed, from which the adaptations arose. There is almost no evidence for such a primitive system in any living species. So, striking is this absence that it has even been cited as a reason why evolutionary theory may be flawed. After all, what evolutionary advantage would accrue from a system providing small quantities of low-quality secretion? This argument even goes back to the time of Charles Darwin, whose 1872 second edition of “On the Origin of Species” put forward the hypothesis that the evolutionary precursor of the mammary gland was the intrauterine brood patch identified in certain sea-dwelling animals, such as sharks and certain fish. This structure guards the eggs and provides nutrition after hatching. Darwin’s hypothesis arose from the conviction that the pouch possessed by metatherian and prototherian species had evolved from the uterus. Currently, however, the consensus is that the mammary glands are specialized adaptations of the glands within the integument, and had already evolved within the therapsids [1, 7].

3 Genomic Factors Affecting Milk

Breast milk varies in its composition. There are a number of causes for this variety, such as the lactational stage, how many previous pregnancies the mother has had, the stage of gestation, mother's nutritional intake, the time feeding starts, and how long it has been continuing for [8–12]. Much of the variability in milk composition arises for normal physiological reasons and is tailored to the nutritional requirements of the child, such as the variation within a feeding session. However, there may be other, harmful causes for variation, such as when the mother's diet is unbalanced. This type of variability impairs the nutritional benefits of breast milk, and may cause malnutrition in the infant, either an insufficiency or a surfeit of calories [8].

It is overly simplistic to see breast milk as supplying only the energy requirements of the child or as providing a supply of raw materials to fuel growth. Some breast milk constituents also influence gene activity [5]. For example, aliphatic acids in breast milk not only serve as energetic metabolic substrates but also may be incorporated into plasma membranes, be modified to become immunoregulatory signaling molecules or control the expression of specific gene products [6–13]. There are now two new branches of nutritional science that attempt to understand this complexity. Nutrigenomics examines the direct or indirect mechanisms through which genetic expression is regulated by specific nutrients, while nutrigenetics examines how the genes individuals inherit determine the response to a particular nutrient [9].These new disciplines may eventually explain why the nutritional requirements differ so greatly between individuals and explain how specific individuals are likely to respond to nutritional interventions [5].

This chapter accordingly focuses on the way a lactating mother's consumption of fatty acids in the diet affects the amount of milk lipids she expresses, both through the nutrigenomic effects of the fatty acids on maternal genetic expression and the nutrigenetic effects of the mother's genetic composition on the resulting breast milk she excretes. The initial discussion covers what role fatty acids in milk play and where they originate, before discussing how the breast milk composition is influenced by maternal nutrigenomic and nutrigenetic factors.

4 Anatomy of the Breast and Fatty Acids Contained in Breast Milk

Production of milk occurs within the breast itself. The breast is made up of a variety of tissue types, namely glandular (containing the lactocytes), connective, adipose, and a stroma with its own blood supply. Within the glands, the epithelium contains two layers of different cell types, i.e., secretory cells facing the lumen and myoepithelial cells constituting the base layer. The myoepithelial cells form a branched network enveloping the alveoli and small-caliber ducts. Myoepithelial cells possess

longitudinal striations [10]. When the myoepithelium contracts, milk is ejected out of the alveoli, through the smaller ducts and into the principal lactiferous duct, which terminates at the nipple [11].

The mammary glands begin to assume their adult form at puberty. When a woman becomes pregnant, the breast keeps on developing up to the point when lactation commences. The breast goes through four phases of development, i.e., mammogenesis, lactogenesis, galactopoiesis, and involution [12]. There are steady alterations in the composition of breast milk as the period of breast feeding continues, both in terms of macro- and micronutrient levels. Three specific types of breast milk have been identified, namely colostrum, transitional, and mature types. These types are different in several ways from each other [8, 14].

5 Roles Played by Aliphatic Acids in Milk

Lipids comprise the second most abundant constituent of human breast milk. They are key to the development of the infant, both as a metabolic substrate to generate energy and to supply essential fats [13, 15]. The principal lipids within milk are aliphatic acids bound to glycerol as triglycerides. Some 98% of the milk lipids are in this form [16]. These fatty acids can be categorized as saturated (containing only single bonds) or unsaturated (with one or more double bonds). The role milk lipids play as a substrate for energy generation and to dissolve lipophilic vitamins, as well as to support the child's development has been well-described elsewhere [7, 14, 17]. The aliphatic acids in milk also play a role in regulating the hepatic synthesis of lipoproteins at the transcription level [18], which ensures that infants are competent to synthesize the lipoproteins they need. Furthermore, n-3 long-chain PUFAs are essential for the development of the central nervous system (CNS) and retina [19–21]. If they are lacking, the plasticity of the CNS is impaired and neurological function is affected when the child grows into an adult [22]. The results from a number of studies support the hypothesis that mothers who consume omega-3 long-chain PUFAs while pregnant produce infants with improved cognitive function and focus than the infants of those who do not [23, 24].

6 Nutrigenomic Aspects of Aliphatic Acids in Milk

Lipids have recently been shown, in research involving rodents, cattle, and humans, to possess the ability to regulate hepatic and mammary gland expression of particular genes, so as to ensure sufficient local levels of saturated fatty acids and mono- and poly-unsaturated fatty acids [22, 23]. Fats in the diet potentially regulate the synthesis of lipids through interactions with certain transcription factors, notably the peroxisome proliferator-activated receptor (PPAR) and sterol regulatory element binding protein (SREBP), both found in the cell nucleus [23, 24]. These two

receptors regulate the expression of the gene FADS1, which encodes delta-5 desaturase and FADS2, the product of which is delta-6 desaturase. They also regulate the genes which encode the elongase enzymes, i.e., ELOV-2 and 5. The PPAR superfamily of proteins includes alpha, gamma, and beta/delta variants. When the gamma and beta/delta variants are activated, the genetic expression of molecules involved in de novo manufacture of fatty acids increases. The activated alpha variant, by contrast, increases the genetic expression of molecules involved in oxidizing fatty acids [25]. SREBP denotes a group of related transcription factors, three of which have been discovered, namely SREBP1a, 1c, and 2. They have a proven role in maintaining cholesterol levels within cells and also help to control the manufacture of fatty acids and their uptake. Despite the fact that SREBP-1c and 2 share similarities in their structure, there are many differences in their hepatic function in response to endocrine signals, nutrient levels, and according to the stage in infant development [25, 26]. SREBP2 increases the manufacture of cholesterol, whereas SREBP-1a and -1c upregulate the production of fatty acids through their control of specific genes, in particular lipoprotein lipase, acetyl-CoA carboxylase α, FAS, SCD, and FADS1 and 2, as well as FA ELOVL-2 and ELOVL-5 [26, 27]. The synthesis of SREBP-1c involves an initially larger gene product that adheres to the endoplasmic reticulum. This protein is then truncated, the N-terminal region moving into the nucleoplasm, where it becomes bound to the sterol regulatory element. In this position, it can regulate its target genes [25].

7 Nutrigenetic Factors Related to Aliphatic Acids in Milk

The extent to which genetics affects the fatty acids present in milk is an ongoing research topic in cattle and other ruminants. Candidate genes for more detailed research have been identified through quantitative trait locus analysis, which linked particular patterns of fatty acids with specific regions within the genome [28, 29]. The existence of single nucleotide polymorphisms has established that genetic variability in these regions is indeed related to which fatty acids are present in milk [30–36]. These studies were driven by the commercial need to breed cattle or other ruminants that produce milk with the desired fatty acid content. Such milk offers better health benefits to human consumers or fits with the need for particular dairy products, such as butter that can be spread more easily. Once the genes were identified, the animals could be selectively bred and given a tailored diet to produce the maximum yield of milk. Currently, the dairy industry wants to produce milk that contains more C18 monounsaturated fatty acids and polyunsaturated fatty acids, especially eicosapentaenoic acid, docosahexaenoic acid, and conjugated linoleic acid [37, 38].

By contrast, there has been relatively little research on nutrigenetic factors affecting the fatty acid composition of human breast milk. This topic appears to have become of interest once it became known that specific SNPs correlated with the amounts of some fatty acids (especially LC-PUFAs) in the circulation and within

the tissues. Frequently noted SNPs occurring in the region where FADS is encoded were shown by Schaeffer et al. [39] to be related to the level of LC-PUFA seen in phospholipids within the cell membrane. This finding has since been replicated [40–43]. Simultaneously, it was shown by the work of Rodriguez-Cruz *et al.* that rats express fatty acid desaturases in their mammary glands [26]. This prompted researchers to investigate whether the same enzymes might be present in human mammary glands and involved in manufacturing LC-PUFA for packaging in breast milk. Other researchers, notably Xie and Innis [44] and Moltó-Puigmartí and colleagues [45], taking this as the basis for their inquiry, found that SNPs in the FADS region had effects beyond the one shown by Schaeffer et al., viz regulation of LC-PUFA content [39].

Dietary PUFA actually serves to regulate the level in breast milk through its effects on the SREBP, which regulates the genetic expression of FADS1 and 2 and ELOVL-2 and 5. Unfortunately, maternal dietary intake of artificially produced trans-fatty acids (TFAs) has a detrimental effect on the fatty acid composition of breast milk. This is because these TFAs alter lipid metabolism.

However, the way the mother responds to dietary intake of fatty acids may alter depending on her genetics, as the association of variability within the FADS and ELOVL coding regions has been established. Specifically, mothers with two copies of the minor allele secreted breast milk with lower levels of arachidonic acid and docosahexaenoic acid (DHA) than women with at least one copy of the major allele. The levels in the maternal circulation were also lower. Furthermore, SNP mutations in the FADS encoding region were also noted by Moltó-Puigmartí et al. [45] to have an association with the level of DHA in breast milk. Mothers with the major allele secreted milk richer in DHA in response to increased dietary consumption in the form of fish, but increasing dietary consumption of DHA did not produce the same effect in women with two copies of the minor allele, within the limits of the observations. This result may have important consequences for public health since LC-PUFAs are essential for the normal development of the nervous system. Breastfed infants of mothers with two copies of the minor allele potentially face a risk of abnormal neurodevelopment. Since this initial research was published, findings connecting SNPs in the FADS encoding region with the fatty acid content of breast milk have been replicated on three occasions [45, 46]. Furthermore, the PUFA content of breast milk has now also been connected to SNPs in the regions containing the ELOVL-2 and -5 genes, which encode elongases [46]. It is reasonable to suppose that, as understanding of the metabolism of aliphatic acids moves forward and more genetic analyses are undertaken, there will emerge new associations between genetic variants and the fatty acid content of human milk. It does not appear currently that any group has studied the effect of dietary manipulation on breast milk composition in groups with different genetics, other than the study previously mentioned [45]. A knowledge of the effect that genetics plays in concert with dietary modifications to alter the composition of breast milk lays open the exciting possibility that breast milk quality can be improved as needed by nutrigenetic techniques [45].

8 Conclusion

The ideal way to feed an infant is by breastfeeding since breast milk delivers the full range of nutritional requirements and other molecules with beneficial biological activity needed for the infant to develop normally, including the nervous system. It has been shown several times that dietary modification in the mother while pregnant and lactating influences the quality of breast milk and hence affects how the child grows. This chapter has touched on both the nutrigenomics and nutrigenetic aspects of this problem, in other words, both how the mother's diet influences her expression of genes and how her genes affect the processing of nutrients.

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