Biological underpinnings of breastfeeding challenges: the role of genetics, diet, and environment on lactation physiology

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Lee S, Kelleher SL. Biological underpinnings of breastfeeding challenges: the role of genetics, diet, and environment on lactation physiology. Am J Physiol Endocrinol Metab 311: E405–E422, 2016. First published June 28, 2016; doi:10.1152/ajpendo.00495.2015.—Lactation is a dynamic process that has evolved to produce a complex biological fluid that provides nutritive and nonnutritive factors to the nursing offspring. It has long been assumed that once lactation is successfully initiated, the primary factor regulating milk production is infant demand. Thus, most interventions have focused on improving breastfeeding education and early lactation support. However, in addition to infant demand, increasing evidence from studies conducted in experimental animal models, production animals, and breastfeeding women suggests that a diverse array of maternal factors may also affect milk production and composition. In this review, we provide an overview of our current understanding of the role of maternal genetics and modifiable factors, such as diet and environmental exposures, on reproductive endocrinology, lactation physiology, and the ability to successfully produce milk. To identify factors that may affect lactation in women, we highlight some information gleaned from studies in experimental animal models and production animals. Finally, we highlight the gaps in current knowledge and provide commentary on future research opportunities aimed at improving lactation outcomes in breastfeeding women to improve the health of mothers and their infants.

lactation; mammary gland; genetics; diet; environment

Breast milk is a complex biological fluid that provides for optimal infant growth and development, but only if lactation functions properly to produce an adequate volume of milk that contains optimal amounts of nutritive and nonnutritive factors. Successful lactation requires extensive breast tissue expansion and differentiation during pregnancy, followed by the ability to produce sufficient amounts of milk after birth. Although ~75% of new mothers intend to breastfeed, not all women are able to breastfeed their infants exclusively for the first 6 mo of life, as recommended by the American Academy of Pediatrics and the World Health Organization. In fact, it has been estimated that the prevalence of women who overtly fail to produce enough milk may be as high as ~10–15% (15, 64, 206) and can quickly lead to hypernatremia (28, 231, 293, 311), nutritional deficiencies (16, 139, 287), or failure to thrive (141, 164, 207). Moreover, the prevalence of lactation “insufficiency” may be much higher, as ~40–50% of women in the US (1, 162) and 60–90% of women internationally (76, 99, 113, 135, 266) cite “not producing enough milk” or “baby not satisfied with breast milk” as the primary reasons for weaning prior to 6 mo. The etiology of lactation insufficiency is clearly multifactorial and complex. Breast hypoplasia or other abnormal breast conditions and previous breast surgeries (206) are certainly factors that contribute to lactation insufficiency. In addition, numerous social, psychological, and behavioral factors associated with early breastfeeding cessation have been described, and the reader is referred to excellent reviews on these topics (81, 90, 192, 280). What is much less appreciated and poorly understood is the role that maternal genetics and modifiable factors such as energy balance, diet, and environmental exposures may have on reproductive endocrinology, lactation physiology, and the ability to successfully breastfeed. The major unsolved questions regarding these maternal factors have been outlined in a recent review (209). In this review, we provide a broad overview on our current understanding of the molecular etiology behind these factors that play a critical role in lactation physiology and the ability to optimally nourish the nursing infant. Because studies in breastfeeding women are profoundly lacking, some aspects and comparisons will be drawn from experimental animal models and studies in production animals where relevant; however, it is important to note that lactation physiology between humans and other mammals can vary, and where appropriate these differences will be discussed in context.

Mammary Gland Biology and the Production of Milk

The mammary gland is a highly complex exocrine gland that functions to produce, secrete, and deliver milk to the infant. Mammary glands are unique in that most development occurs...
postnatal. From birth to puberty, the rudimentary branches elongate through the mammary fat pad and form terminal end buds in preparation for functional activation during pregnancy and lactation. Successful lactation requires coordination of mechanisms responsible for nutrient transport, milk production, and secretion from the mammary gland and is driven by molecular, biochemical, and cellular events that are regulated largely by reproductive hormones, which has been described thoroughly by Anderson et al. (7). During early pregnancy, primary hormones, including estrogen, progesterone, prolactin, and placental lactogen, induce the physiological transition of the mammary gland from a nonsecreting branched tissue into a highly active secreting organ comprised of a vast network of ducts and alveoli that are grouped into seven to 10 lobes in humans (243). Following this morphological change, the alveolar cells increase the expression of lactogenic genes, which differentiate them into secretory mammary epithelial cells (MECs) or lactocytes, and this process is termed secretory differentiation (225). In response to progesterone and estrogen withdrawal, concomitant with prolactin release following parturition, the differentiated epithelium gains a remarkable capacity to finely coordinate the synthesis and transport of various milk constituents for the onset of milk secretion, termed secretory activation (225), which usually occurs after full-term birth in humans (210). During this time, tight junctions between lactocytes close and limit the passage of ions (e.g., sodium and chloride) and small molecules, which is important for the establishment of lactation and motivating milk secretion (147, 272). Milk ejection is regulated by oxytocin, which acts on myoepithelial cells to generate contractile force required for milk ejection during lactation (103). In contrast, milk secretion from lactocytes is regulated by a complex hormonal milieu that includes numerous reproductive hormones (e.g., prolactin) and metabolic hormones [e.g., glucocorticoids, insulin, insulin-like growth factor I (IGF-I), growth hormone, and thyroid hormone]. These hormones can act directly on the lactocyte or indirectly by altering endocrine response and nutrient delivery to the mammary gland for milk production (34). The main stimulus for the initiation of lactation is infant suckling and milk removal, which activates the milk ejection reflex through the release of oxytocin from the posterior pituitary gland, leading to the contraction of the myoepithelial cells around the alveoli and ducts, ultimately forcing milk into the alveolar lumen and out the nipple, providing milk to the developing infant (225). Once lactation has been established, prolactin secretion from the pituitary gland decreases, but milk removal from the mammary gland promotes continuous milk production or galactopoiesis (5, 19, 86, 138, 221). However, as discussed below, both genetic and modifiable factors may play important roles in the ability of the breast to respond to infant demand and provide optimal nutrition for growth and development.

Breast milk is considered the gold standard for infant feeding, as it provides a perfect combination of nutrient and nonnutritive factors that are essential for infant growth and development. Breast milk contains a diverse array of proteins, lipids, and carbohydrates that provide energy, bioactive proteins (e.g., bile salt stimulated lipase, lactoferrin, lysozyme, secretory IgA, and α-lactalbumin), and both group I (vitamins A, E, B1, B2, B6, B12, and D as well as choline, selenium, and iodine) and group II (calcium, iron, copper, zinc, and folate) micronutrients (6). In addition, breast milk contains human milk oligosaccharides (reviewed in Ref. 269) and numerous growth factors, hormones, chemokines, and cytokines (reviewed in Ref. 259) as well as DNA, RNA, microRNAs, white blood cells, and live bacteria (reviewed in Ref. 153) and stem cells (reviewed in Ref. 111). Breast milk composition changes over the course of lactation. In women, secretory activation occurs after parturition (30–40 h after birth), and the first fluid produced is colostrum (225). Only a small volume (~30 ml/24 h) of colostrum is available for the first few days, but contains high concentrations of immunological components such as secretory IgA, lactoferrin, and leukocytes as well as developmental factors such as epidermal growth factor (49, 147, 225). The concentration of lactose, the most osmotically active component in breast milk, is low in colostrum, thus explaining the low volume of colostrum that is secreted and also indicating that the primary function of colostrum is immunological rather than nutritional (17). Compared with mature milk, colostrum contains higher levels of sodium, chloride, and magnesium and lower levels of potassium and calcium (17, 147, 225). As the mammary gland transitions from pregnancy to lactation, tight junctions between MECs close, and consequently the sodium/potassium ratio declines and the lactose concentration increases (147). This reflects secretory activation and the synthesis of transitional milk, which marks the period of copious milk production and the secretion of nutritive and nonnutritive factors to support nutritional and developmental needs of the growing infant. After the first month, breast milk is considered fully mature and contains ~0.9–1.2 g protein/dl, 3.2–3.6 g fat/dl, and 6.7–7.8 g lactose/dl (17). Although there is a dramatic shift in composition during the first month, the concentration of macronutrients in breast milk remains relatively constant over the course of lactation, with gradual increases in milk fat content over the course of one feeding (17). However, it is important to note that milk composition, as well as mammary gland number, location, and architecture, is different between humans and animals, which is a limitation to extrapolating information on human lactation from animal studies (2). For example, cattle have a total of four inguinal mammary glands producing milk that is higher in protein compared with humans, and mice have four thoracic, two abdominal, and four inguinal mammary glands producing milk that is higher in protein, fat, and energy compared with humans (2, 128).

As a result of this complex mixture of nutritive and nonnutritive factors, numerous studies have shown that breastfed infants have a lower risk for adverse health consequences, including gastroenteritis, pneumonia, childhood obesity, leukemia, and sudden infant death syndrome (124). Therefore, the American Academy of Pediatrics recommends exclusive breastfeeding for the first 6 mo of life. Although breastfeeding initiation rates have increased to ~75% in the US, the duration of exclusive breastfeeding continues to fall below national goals; only 47% of infants are breastfed at all by 6 mo of age, and of those, only 14% are exclusively breastfed as recommended (89). For the most part, it is assumed that once lactation is initiated, a breastfeeding woman can produce enough breast milk that meets the nutritional needs of her infant, unless she is severely malnourished. Yet, as noted above, ~50% of mothers cite concerns about insufficient milk volume or poor milk quality as motivation to wean their infants.
ROLE OF GENETICS, DIET, AND ENVIRONMENT ON LACTATION

Shp2 and PTP1B (8), and NHERF1/EBP50, which forms a multimeric complex with PRLR (200) all compromise differential and lactogenesis or lead to suboptimal lactation outcomes in experimental animal models. The complexity of this key lactogenic pathway provides numerous opportunities for (de)activation resulting from genetic variation in the genes that encode these molecules in humans.

Surprisingly, little is currently known about the genes that govern lactation physiology in humans. Recent studies using transcriptomic analysis of isolated milk fat globules (199) and milk fat (159) have begun to reveal changes in the patterns of gene expression that occur during secretory activation and across the course of lactation, respectively. In addition, Daniels et al. (70), Lemay et al. (160), and Rijnkels et al. (250) have shown that epigenetic regulation is important in lactation physiology, using the mouse as a model. Further transcriptomic and epigenetic studies will be critical to identify and understand the regulation of genes that are vital to human lactation. Studies in production animals suggest that genetic variation may also play an important role in modifying lactation success in humans. The role of genetics as a key modifier of milk production in the dairy industry has been recognized for many years (93), as the execution of large genome-wide association studies (GWAS) and linkage studies has been driven by the economics of the dairy industry (59, 112, 182, 246). Over the years, genomic evaluations of dairy cattle have discovered more than 43,000 single nucleotide polymorphisms (SNPs), which have profoundly improved breeding decisions and milk production traits (51) and have led to the development of SNP genotyping chips to predict lactation efficiency (302). Several different approaches can be used to identify genes involved in lactation physiology in women. One approach is to conduct GWAS and select the most abundant or significantly different genes between two different phenotypes (e.g., low-volume producers vs. high-volume producers) and then determine the functional consequences on mammary gland biology and lactation performance (60). Another approach is to use targeted exome sequencing to select genes that are involved in specific biological processes and pathways that are important for mammary development and/or milk production and determine whether variation in these genes is associated with milk production or composition (246). A third approach is to use quantitative trait locus analysis to identify polymorphisms at a specific chromosomal locus (e.g., chromosome 22) that is correlated with phenotype variation (e.g., milk fatty acid composition) (88).

Using a variety of these approaches, recent advancements have identified numerous genetic variants associated with milk production traits in production animals. A recent study using GWAS data collected from 16,812 Holstein and Jersey dairy cattle identified SNPs in key pathways that are critical for mammary gland development, prolactin signaling, and involution that explained variation in milk production and milk composition (246). For example, ~50% of SNPs found in genes critical for prolactin signaling, including SOCS2, STAT3, STAT5A, STAT5B, PRLR, and β-casein, were associated with three or more milk production traits (246). Additionally, a large number of SNPs in genes that are critical for mammary gland involution, including ATF4, IGFBP4, IFR1, LIFR, OSMR, PTK2, and STAT3, were also associated with milk production traits (246). It is important to note that there are limited data in production animals that actually address the effect of genetic variation on mammary gland function or lactation physiology; these data have been used strictly for genetic association studies and breeding for production traits, and therefore, further research is required to better understand the physiological implications.
We propose that similar variation may govern lactation physiology, milk production, and composition in breastfeeding women; however, to date, few studies have explored this possibility. As proof of concept, several mutations in prolactin and PRLR have been identified in humans (155, 215). In addition, several studies have found that SNPs in PRLR are associated with elevated serum prolactin levels (rs26355518, rs10941235, rs1610218, rs34024951, and rs9292575) and increased risk for breast cancer (rs249537, rs7718468 and rs13436213) and gestational diabetes (rs10068521 and rs9292578). Collectively, this suggests that SNPs in PRLR may affect breast function; however, to our knowledge, the effects of SNPs in PRLR on lactation outcomes have not been explored. Recently, we identified a woman with a “gain-of-function” variant in PRLR (I170L, also known as I146L) who had elevated milk zinc concentration (2.82 mg/l; normal = 1 mg/l) (4). Subsequent analysis of her milk composition determined that the ratio of sodium to potassium was also elevated (~0.9; Kelleher SL, unpublished data), also suggesting subclinical breast dysfunction (263). Moreover, a “loss-of-function” variant in PRLR (H138R) results in secondary hyperprolactinemia and is associated with persistent galactorrhea (212). Genetic variation in downstream molecules that elicit prolactin signaling may also be important candidates. Gain-of-function mutations in Jak2 (V617F) and STAT5B have been identified in humans (18, 239). Constitutive activation of Jak2 through expression of V617F in mice elevates STAT5 signaling and promotes alveologenesis, although lactation outcomes in women expressing V617F have not been investigated. Clearly, a profound lack of understanding exists regarding the consequences of genetic variation in the prolactin-Jak2/STAT5 signaling pathway on lactation outcomes. Collectively, these observations suggest that alterations in prolactin signaling pathways may affect breast function and lactation outcomes and warrant further investigation. The fact that recombinant prolactin has successfully been used to increase milk volume and lactose, calcium, and oligosaccharide concentrations in some women, but with limited success in others (235), suggests that pharmacogenomics targeted at this pathway may be an approach to improve lactation outcomes in some women in the future.

Other cell signaling pathways. Apart from prolactin signaling, the mammary gland possesses a network of signaling pathways that detect metabolic status, regulate gene transcription and milk protein synthesis, and maintain many cellular processes during lactation. One such pathway is the mammalian target of rapamycin (mTOR) pathway, which has a well-established role in regulating protein synthesis by phosphorylating eukaryotic translation factor 4E binding protein 1 (4EBP1), thereby releasing eukaryotic translation initiation factor 4E (eIF4E) to regulate translation (296). The mTOR pathway is a well-known therapeutic target for breast cancer (154), and emerging studies also illustrate the importance of mTOR in modulating lactation outcomes. Inhibition of mTOR signaling using RAD001 during lactation markedly impairs lactation (87). Moreover, a role for mTOR signaling in milk protein synthesis has been demonstrated recently (10, 11, 38, 39, 294, 313), as synthesis of proteins like β-lactoglobulin in bovine mammary glands (201) or α-s1 casein in bovine mammary cells (309) is upregulated through the phosphorylation of 4E-BP1. The mTOR pathway has also been shown to be the missing link between factors involved in milk synthesis and MEC proliferation, such as glycogen synthase kinase 3 (GSK-3) and leucyl-tRNA synthetase (LeuRS) (294, 313). In contrast, AMP-activated protein kinase (AMPK) activation by energy depletion in bovine MECs inhibits mTOR signaling and decreases milk protein synthesis rate (39). Numerous mutations in genes encoding mTOR components have been identified, and the reader is referred to an excellent review by Saxena and Sampson (260) on this topic. Another potential target is the peroxisome proliferator-activated receptor-γ (PPARγ) pathway, which is critical for the maintenance of the secreting mammary epithelium (9) and milk quality by suppressing the production of inflammatory lipids (292). Moreover, PPARγ regulates triglycerol synthesis and secretion in cultured breast cells (284) and in dairy goats (265). Although SNPs in PPARγ have been identified, are associated with enhanced breast density, and are modified by diet (157), no clear relationship with lactation outcomes (226) has yet been identified. Going forward, it will be important to differentiate between problems resulting from inadequate mammary gland development during puberty or inadequate secretory development in pregnancy from those that impact secretory activation or secretion of milk components.

Other hormones and endocrine factors/inhibitors. In addition to prolactin, a complex combination of hormones works together to maintain the differentiated epithelium and milk secretion during lactation, including insulin, glucocorticoids, growth hormone, oxytocin, and thyroid hormone. Secretory activation and milk ejection require insulin and glucocorticoids to synergistically regulate the formation of tight junctions in the mammary gland (223, 312), stimulate mammary differentiation (48), and induce milk protein expression (73). Insulin levels rapidly decrease during early lactation and steadily increase over time (301). Recent work shows that insulin in the presence of prolactin and hydrocortisone plays a pivotal role in regulating milk protein synthesis in mammary explants from animals by increasing the expression of transcription factors Elfs and STAT5 (193, 194). Studies in dairy cows show conflicting effects of exogenous insulin on milk fat levels, where long-lasting insulin treatment tends to increase milk fat content and milk yield (305), whereas hyperglycemic insulin clamp reduces milk fat and milk yield (61, 173). Similarly, women with gestational diabetes who are on insulin therapy have a delay in the onset of secretory activation (179), which suggests that insulin treatment may have adverse effects on milk production or composition in humans.

In contrast, growth hormone deficiency compromises lactation outcomes in rats, as it reduces milk yield by ~24% (83). Moreover, artificial growth hormone or recombinant bovine somatotropin administration to ewes (56, 256) and dairy cows (91, 188, 230) increases milk protein yield and milk volume, and growth hormone has been used as a pharmacological agent to augment milk production in women (100, 198). It is speculated that this occurs through the activation of cell proliferation (174) and through increased STAT5 expression (33). Genetic variation in growth hormone receptors has recently been associated with milk production traits in dairy cattle (51, 240), sheep (175), and water buffalo (264). Although numerous SNPs in growth hormone, its cognate receptor, placental lactogen, and placental growth hormone have been identified in humans, to our knowledge, genetic variation in this pathway...
has yet to be explored as a modulator of lactation outcomes in women (3).

Several studies have shown that SNPs in the oxytocin gene are associated with shorter breastfeeding duration, which may be due to impaired myoepithelial contraction and milk ejection (131, 233). Finally, thyroid hormones are also galactopoietic and help to establish the metabolic priority of the mammary gland during lactation (43). Hypothyroidism has deleterious effects on milk composition (108) and milk synthesis and ejection in rats (107). However, there is little information on the effects of hypo- or hyperthyroidism on lactation outcomes in women.

In contrast to the factors that promote lactation, feedback inhibitor of lactation (FIL) is a polypeptide that controls lactation by reversibly blocking milk synthesis and secretion when the breast is full (229, 249, 303). Specifically, FIL is endogenously synthesized in lactocytes and secreted into the milk as whey protein, which allows for local regulation of milk secretion through an autocrine feedback inhibition (229, 303), and is critical for matching milk supply to infant demand (304). However, there is very little understanding of the precise mechanisms through which FIL may exert its actions. Finally, serotonin has been proposed to be an additional feedback inhibitor of lactation that reduces milk synthesis and milk yield (117). However, recent studies demonstrate that serotonin is also critical for numerous cellular pathways and biological processes regulating lactation (151). Further studies are needed to understand how factors secreted into milk assist in regulating milk volume and the onset of mammary gland remodeling during involution.

### Milk Composition and Potential Genetic Modifiers

The discovery of variants in specific milk proteins dates back to 1955 by Aschaffenburg and Drewry (12), in which two variants of β-lactoglobulin were detected in bovine milk. In this section, we will review our current understanding of the role of genetic variation of milk components on milk composition. Many studies have identified genetic variation in the major milk proteins, including α-lactalbumin (27, 57, 178), α-caseins [α-S1 and α-S2, (45, 46)], β-casein (134, 189), and κ-casein (140, 238). Variation in specific milk proteins appears to affect mammary gland function, as they are associated with differences in milk production and milk composition. A study in Holstein dairy cows found that α-S1 and β-casein variants were associated with higher milk volume, milk fat, and protein yield (213). Consequently, such cows with variants in caseins, as well as α-lactalbumin and β-lactoglobulin, are economically favored for selective breeding (29, 30, 119). α-Lactalbumin-deficient mice produce abnormally viscous milk and fail to expel milk to sucking pups (274); however, it is important to note that the concentration of α-lactalbumin is quite high in human milk and is likely not a limiting factor. Although one might speculate that since in humans genetic variation in α-lactalbumin is quite common (57), perhaps variation in α-lactalbumin may alter lactose production and thus affect milk quality and milk ejection in women. Because these major milk proteins also have biological functions beyond providing energy (195), genetic variation may have an effect on the growth and development of offspring; however, to our knowledge there is currently no information in this regard. In addition, the contribution of several candidate genes on lactation outcomes has been explored directly. For example, diacylglycerol O-acyltransferase 1 (DGAT1), which catalyzes the formation of triglycerides, is the most prominent gene affecting milk fat composition in cows (172, 261), and a K<sup>252</sup>A substitution very likely represents the causal mutation (282). DGAT1 is an example of a quantitative trait locus candidate gene in the centromeric region of chromosome 14 that was demonstrated to have a major effect on the fat content of cow milk associated specifically with higher saturated fat (i.e., C14:0) compared with unsaturated fat (i.e., C18:1) (261, 282). Studies of DGAT1-deficient mice demonstrate that these defects in lipid metabolism impair mammary development (47). Moreover, several variants of fibroblast growth factor 2 (FGF2) in Holstein cattle are associated with fat yield and milk quality, as measured by somatic cell scores (295). FGF2 is important for mammary gland development and also regulates the expression of interferon-tau, a key factor in STAT activation for milk production (295), which suggests that variation in FGF2 could be an additional potential candidate as a modifier of milk production in women.

Genetic variation in a few nutrient-specific genes has been associated with alterations in milk composition in humans. Polymorphisms in the vitamin D receptor are common and are associated with altered breast function and breast cancer in women (102, 241). Moreover, Bezerra et al. (21) found that SNPs in vitamin D receptor are associated with milk calcium concentration. Recently, there has been much interest in better understanding the mechanisms through which zinc is secreted into milk, as there are numerous reports in the literature of exclusively breastfed infants being diagnosed with severe zinc deficiency. We were the first to identify a mutation in the gene SLC30A2 that encodes the zinc transporter ZnT2 (58). This mutation leads to an amino acid substitution in the NH<sub>2</sub> terminus of the protein and the secretion of ~75% less zinc into breast milk than normal, and since then, several other mutations in SLC30A2 that lead to substitutions in ZnT2 (G<sup>87</sup>R, W<sup>152</sup>R, S<sup>296</sup>L, R<sup>340</sup>C) have subsequently been associated with this disorder (125, 152, 196). More recent studies indicate that defects in ZnT2 may have much broader implications for mammary gland biology, as ZnT2-null mice have severe defects in lactation outcomes, including profound impairments in mammary gland differentiation, milk production, composition, and secretion (158). Moreover, studies in mice and cultured lactocytes indicate that ZnT2 imports zinc into lysosomes and plays a critical role in activating cell death and mammary gland involution (114). This may be of importance to subpopulations of women because ~35% of otherwise healthy breastfeeding women had nonsynonymous genetic variation in ZnT2 (4), which was associated with both low and high milk zinc concentrations. In addition, two particular variants (D<sup>103</sup>E and T<sup>288</sup>S) were found with high frequency (~12%) and were associated with high milk sodium levels, a known hallmark of breast dysfunction (4). Exciting opportunities exist to better understand the role that nutrigenetics plays in modulating lactation physiology and breast milk composition so that personalized strategies to optimize lactation outcomes can be developed.
Role of Energy Balance

The nutritional requirements during lactation increase considerably to maintain maximal mammary gland secretory capacity and milk production and also to maintain maternal energy balance for optimal milk production (234). Energy requirements increase during lactation (40), and the energy cost of lactation is determined by the amount of milk production and secretion, milk energy content, and the efficiency of converting dietary energy to milk energy (41), which usually increases over time in women who breastfeed exclusively (234). As a result, early investigations have focused on the role of diet in maintaining energy balance and milk production and composition during lactation (reviewed in Ref. 92). Because of the epidemic of obesity in Western countries, more recently the consequences of dietary excess on mammary gland function have garnered much interest. In this section, we will focus on recent advances in understanding the consequences of obesity and physical activity on mammary gland function and lactation outcomes.

Maternal metabolism and obesity. The World Health Organization has declared that obesity is one of the most significant human health problems, with the expectation that 80% of women will be overweight (BMI of 25–29) or obese (BMI >30) by 2020 (297). This could have a profound consequence on the number of women who breastfeed successfully, and thus the health of infants and the US population at large, because mothers who are overweight or obese are less likely to initiate lactation, have delayed secretory activation, and are prone to early breastfeeding cessation (15, 130, 161, 244, 245). Biological complications include prolactin resistance and reduced STAT5 activation (37), which is linked to delayed initiation and premature cessation of lactation (245), and decreased insulin sensitivity (50), which may result in insufficient glandular development and reduced milk volume (202, 279). Recently, many researchers have used dietary-induced obese animal models to further investigate the effects of obesity on the lactating mammary gland and milk composition that in turn affect lactation outcomes. As described by multiple studies, the consequences of obesity in rodents include increased adipocyte size (115, 133) and disturbances in the mammary gland microenvironment and impaired development during pubertal stages (133). In addition, the growth hormone/IGF-I axis, somatostatin/cortistatin, and ghrelin systems are increased in the mammary fat pads of diet-induced obese mice (288), which may be involved in the pathophysiology of the mammary gland (77, 148). Although growth hormone is known to improve milk volume (197, 316), obese mice also have increased plasma leptin (288), which inhibits the effect of oxytocin on myoepithelial contraction for milk ejection (20). Moreover, during pregnancy and lactation, obese rodents have significant defects in alveolar development (84, 115), abnormal collagen deposition, and incomplete myoepithelial lining around ductal structures (133). During lactation, the mammary glands of obese mice accumulate lipid in the MECs, consistent with secretory inactivation (84, 156). Growing evidence suggests that in addition to systemic inflammation, obesity is also associated with an inflammatory microenvironment in the mammary gland (22, 36, 277), which has recently been associated with premature involution in murine models (115). A few studies provide insight into the possible mechanism underlying obesity-induced inflammation in the mammary gland. Inflammation in the mammary gland is marked by elevated levels of proinflammatory factors like TNFα, IL-1β, and Cox2 (277), increased macrophage infiltration (163), perturbed zinc metabolism (115), and increased NF-κB signaling (42). Moreover, there is some suggestion that obesity increases local estrogen production (36), which in turn may function to downregulate prolactin signaling, and suppress lactation (reviewed in Ref. 218). In addition, lipid synthesis is impaired, which can result from l interference with dietary lipid transport into and out of the mammary gland and 2) suppression of de novo lipogenesis in the mammary gland, resulting in lipid accumulation and low milk triglycerides (254, 291). Fundamentally, the altered mammary gland microenvironment that occurs in obesity can lead to failed secretory activation or suboptimal lactation, whereby the mammary gland is incapable of secreting copious milk to nourish the newborn. Therefore, understanding the lactogenic mechanisms that are compromised by obesity will assist clinicians in designing strategies to better support these mothers.

Physical activity and fasting. A major concern of women postpartum is the desire to lose excess weight gained during pregnancy. The American College of Obstetricians and Gynecologists recommends that women resume physical activity after delivery as soon as it is physically and medically safe. However, intensity and duration appear to have important implications on the effects of exercise on lactation. Moderate levels of exercise do not affect milk production, milk composition, or infant growth (68, 166, 167, 276); rather, exercise improves the mother’s overall health and sense of well-being (72, 168, 236). Moreover, short-term weight loss has no effect on lactation performance, milk volume, or milk protein concentration (187). However, several other reports examining the effects of more intense exercise and starvation (i.e., religious fasting) have observed significant changes in specific milk proteins and other essential factors in milk, such as immunoglobulins (94), lactose (315), and micronutrients (zinc, magnesium, and potassium) (242). This is consistent with studies in rats, where Matsuno et al. (181) found that chronic low-intensity exercise during lactation decreases milk lactose, increases milk protein and milk fat, and compromises offspring growth. However, it is important to note that lactation is much more energy demanding in rodents than humans. For example, rats lactate at their maximum physiological capacity and require ≥100–300% of their energy to produce milk (237), and therefore, changes in maternal diet and metabolism are more robustly reflected in their milk composition. However, this concept does not apply to breastfeeding women, who need only ~20% of their total energy (237). For women, intense exercise or “exhaustive exercise” may have short-term effects on milk composition, such as increased lactic acid and decreased secretory IgA concentration; however, this appears to return to normal levels within 1 h (44, 94). It is important to note that increased milk lactic acid secretion after moderate- or high-intensity exercise is common and does not impede infant acceptance of breast milk (307). Likewise, short-term starvation decreases amino acid supply to the mammary gland and lowers free amino acid concentration in the milk of fasted rats (289) and also decreases expression and activity of essential enzymes such as fatty acid synthase, glucose-6-dehydrogenase, and lipoprotein lipase, all of which are critical for fatty acid production (95, 129). However, these effects are easily re-
versed with refeeding or resumption of normal activity. An additional concern is that with severe calorie restriction, fat diets and rapid weight loss, fat-soluble environmental contaminants, and toxins, including polychlorinated biphenyls (PCBs) and pesticides that are stored in body fat, can be secreted into milk (149). Moreover, it is hard to make generalized recommendations regarding diet and exercise during lactation due to differences in individual genetics and the changes in gene expression influenced by modulation of energy balance (224). Nowadays, much interest in understanding the effects of physical activity on breastfeeding has shifted toward studies on the effects of physical activity on breast cancer progression (205). Multiple studies have explored the effects of exercise on the normal mammary gland in an attempt to identify tumor-associated/inhibiting markers. Hopefully, these studies will indirectly provide information regarding the effects of exercise on breast development (298) and function (205) that impact lactation outcomes.

Role of Diet

Nutrient intake: deficiency and supplementation. In recent years, increasing evidence shows not only that adequate nutrient intake and appropriate nutrient homeostasis are important for maintaining maternal energy balance but that suboptimal nutrition has significant effects on breast physiology and milk production, secretion, and composition. Nutrient deficiencies can result in failed secretory activation from several perspectives, such as insufficient hormone responsiveness, and defects in cellular processes involved in morphogenesis and secretory pathways. Moreover, energy/nutrient imbalance may cause more pernicious effects on immune response and increased risk of mastitis (127, 203, 204, 216). This next section highlights our limited understanding of the role of diet and specific nutrients and dietary factors on lactation physiology. Because the ethics of manipulating maternal diet in humans preclude randomized clinical studies, much of our current understanding comes from studies conducted in animal models.

Vitamins. Vitamin A deficiency remains a major worldwide health problem that leads to blindness, growth retardation, and death, particularly in developing countries. Its prevalence is estimated at ~29%, with Sub-Saharan African and Southeast Asian countries most affected (273), and affects largely preschool children, pregnant and lactating mothers, and the rural poor (101). Whereas vitamin A intake is positively correlated to milk vitamin A concentration (97), vitamin A deficiency did not appear to have major effects on mammary gland biology in a rat model (257). However, vitamin A deficiency in rodent models decreases expression of mammary gland iron transporters and milk iron levels (136), alters zinc transporter expression (137), and leads to pathological damage to the mammary gland after intramammary challenge with staphylococcus (54). Similarly, vitamin D deficiency is presumed to be very common in pregnant women (177). Studies in rodent models found that vitamin D deficiency significantly reduces milk protein, calcium, and lactose levels and impairs milk protein synthesis, suggesting a role in hormone-induced functional differentiation of the mammary gland (23). However, studies on the effects of vitamin D deficiency on mammary gland function have not been conducted since the 1980s and necessitate more robust molecular investigation. Finally, B vitamins like thiamin (B₁), riboflavin (B₂), and niacin (B₃) are critical for energy metabolism, and folate and B₁₂ are essential for DNA synthesis. Although intake of B vitamins (particularly B₁, folate, and B₁₂) varies widely, few studies exploring the effects of B vitamin deficiency on mammary gland biology have been described. Dangat et al. (69) found that B₁₂ deficiency may reduce milk volume; however, no studies toward understanding the molecular underpinnings have since been described. Given the importance of B vitamins in energy metabolism, DNA synthesis, and cell proliferation, understanding the effects of suboptimal B vitamin nutrition on lactation outcomes warrants further consideration.

Minerals. Minerals play diverse roles in biology ranging from cofactors for protein stability or activity to the transfer of electrons in numerous biological pathways. The mammary gland has a remarkable capacity to homeostatically regulate milk concentrations of minerals such as iron and zinc in times of maternal deficiency in these minerals (165). Iron deficiency is one of the most common nutrient deficiencies in the world. Iron bound to transferrin in serum is taken into the mammary gland by transferrin receptors (267) and may be imported into the endoplasmic reticulum by ferroportin for incorporation of iron into proteins such as lactoferrin in humans and mice or transferrin in rats (165). However, there remains an incomplete understanding of the molecular regulation of iron transport in the mammary gland for secretion into milk. However, due to the role of iron in energy generation (306), it is reasonable to assume that iron deficiency may have an impact on mammary gland biology and lactation outcomes. Although Grill et al. (96) found that iron deficiency has minimal effects on pubertal mammary expansion, to our knowledge, no studies have explored effects of iron deficiency on mammary gland function during lactation. In contrast, the potential impact of zinc deficiency on lactation outcomes has been better documented. Zinc is a catalytic, structural, or regulatory cofactor for >10% of the proteome (184). Marginal zinc deficiency is common in women of reproductive age (262). Studies in mice show that marginal zinc deficiency leads to a toxic mammary gland microenvironment that impairs mammary development and subsequently affects lactation outcomes, including mammary gland expansion, milk composition, and milk volume (32). Most studies have not found a relationship between maternal zinc status and milk zinc levels (55, 145). However, a recent report in Thai mother-infant pairs found that exclusively breast-fed infants had the highest prevalence of zinc deficiency and that maternal zinc status was indeed positively correlated to milk zinc concentration (75). Our current understanding in lactocytes is that a complex network of zinc transporters may regulate zinc uptake and export into milk (184). It is not yet understood how the lactocyte takes up zinc from the systemic circulation or how this process is regulated; however, we previously proposed roles for ZIP5, ZIP8, and ZIP10 in this process. Much more is known about how zinc is secreted into milk. The important role of ZnT2 in zinc secretion was discussed above. In addition to ZnT2, several studies have shown that ZnT4 may also play a role in zinc secretion into milk; however, there is far less understanding of its relevance. We have shown previously that ZnT4 transports zinc into the trans-Golgi apparatus and across the apical cell surface (185). Presumably, insufficient zinc in the trans-Golgi apparatus impairing galactosyltransferase activity and the production of...
lactose may underlie the phenotype of reduced milk secretion and milk zinc levels in ZnT4-null mice (186). However, a much more impressive phenotype in ZnT4-null mice is the dramatic loss in alveolar structure and activation of precocious involution and early lactation failure (186); thus more research is required to understand the role of ZnT4 in lactation physiology and how the lactocyte regulates zinc transport to meet its unique physiological needs. Given the role of genetic variation in ZnT2 (and perhaps other zinc transporters) in modifying milk zinc levels, further studies are needed to determine the contribution of maternal genetics to regulating zinc homeostasis during times of zinc deficiency or excess. Finally, the other mineral that is of global concern is iodine. In the mammary gland, iodide transport is mediated by the sodium/iodide symporter (NIS) (251, 281), and the concentration of milk iodine varies widely, ranging from 5.4 to 2,170 μg/L reflecting the lack of homeostasis (74). This not only affects infant iodine nutrition but can also have profound effects on breast physiology, resulting in mammary dysplasia and atypia to atrophy and necrosis in the alveolar cells (78, 275), most likely through alterations in the production, secretion, and activity of thyroid hormones. As noted above, further studies are needed to better understand effects of hypo- and hyperthyroidism on lactation outcomes and milk composition. In contrast to iodine, maternal intake of calcium does not directly affect calcium levels in breast milk (123) because a large amount of calcium is drawn from the bone (144) to transfer ~300–400 mg calcium/day into milk (142). Therefore, dietary calcium intake prior to pregnancy may be most critical for providing calcium for milk secretion during lactation and has been reviewed recently by Kovacs (143). This large amount of calcium is secreted by forming a complex with citrate, casein, or phosphates that are packaged into secretory vesicles for exocytosis (208) or by direct transport across the apical membrane through calcium ATPase type 2 (248). For an excellent review on calcium transport in the breast during lactation, the reader is referred to Cross et al. (65).

**Protein and amino acids.** In addition to vitamins and minerals, protein deficiencies have been reported to reduce milk production (14). Rats fed an arginine-deficient diet had impaired mammary gland growth (228). Moreover, excess protein intake in lactating mice decreases mammary gland mass, reduces expression of major milk constituents, and lowers milk lactose concentration (146). Milk lactose and milk fat are essential components in milk that reflect the capacity for secretory activation, milk synthesis, and milk quality; therefore, when dietary glucose (a precursor for lactose synthesis) or fatty acids are restricted, substantial impacts on milk composition (protein, fat, and glucose) and mammary gland growth occur in lactating rat dams (150). However, it is not clear whether dietary glucose restriction will affect lactation in women, because studies in developing countries of women with poor diets have shown no effect on milk output (62).

**Fatty acids.** Milk fat is a major source of energy and fat-soluble vitamins as well as essential fatty acids (31, 82). Fatty acid composition in milk is species specific and is extremely sensitive to maternal nutrition and dietary alterations (82, 227). In particular, long-chain polyunsaturated fatty acids (LCPUFAs) such as docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and arachidonic acid in breast milk are obtained from maternal diet and are implicated in neurological development and susceptibility to allergies during infancy (169, 283, 286). Maternal fatty acid intake not only affects the fatty acid profile of human milk but has also been shown to be an important dietary determinant of mammary gland function. For example, MECs of rats fed a LCPUFA-deficient diet are unresponsive to prolactin stimulation and have impaired secretion resulting from low phospholipid levels in the mammary gland and accumulation of secretory vesicles in the cytoplasm (219). This suggests that low intake of LCPUFAs may have profound effects on milk volume and composition. Moreover, medium-chain fatty acids (C:8–C14:0) are found in breast milk, and the concentration increases over the duration of lactation (13, 82, 104). Medium-chain fatty acids are synthesized in the mammary gland through de novo fatty acid synthesis, which is catalyzed by the enzyme fatty acid synthase (FASN) (278, 314). In addition to reduced milk fatty acids, FASN-deficient mice showed defects in mammary development and underwent precocious involution (278). Recent studies reveal genomic variability and functional expression of the FASN gene in buffalo and found the highest expression of FASN1 in mammary gland during lactation when fat turnover is the greatest (214), which implicates its importance in the active stage of milk production (190). Genetic variations in other genes that govern fatty acid metabolism have been shown to have a profound impact on milk composition and infant health outcomes. A T347S variant of apolipoprotein A4, which is involved in dietary fat absorption, is present in about one-third of the US population, and women with this variant have a greater level of DHA in their breast milk (98, 222). Moreover, 20% of the population carries one or two copies of the E4 variant of the apolipoprotein E gene, which regulates circulating fat metabolism, and women with the E4 variant have significantly less milk fat than women without this variant (132, 268). The most well-studied genetic variants are SNPs in fatty acid desaturase 1 and 2 (FADS1 and FADS2), which encode the enzymes Δ5 and Δ6 desaturases, respectively, and are rate-limiting enzymes in the synthesis of polyunsaturated ω-3 and ω-6 fatty acids (arachidonic acid, eicosapentaenoic acid, and DHA). Several SNPs in FADS1 and FADS2 in Canadian Holstein cows (121) and breastfeeding women (308) are associated with alterations in milk fatty acid composition. Importantly, these alterations have critical implications for infant health and development, as an interaction between exclusive breastfeeding and asthma modified by FADS genotypes in children has been noted (271). Furthermore, breastfeeding, FADS genotype, and fish intake are important determinants of DHA status in late infancy (110). This may be particularly important given the role of EPA and DHA in neurological development during infancy, which has been elegantly reviewed by Innis (122).

**Role of Environmental Factors on Mammary Gland Physiology**

In addition to genetics and diet, environmental factors may contribute to suboptimal lactation (26, 35, 79, 211). Many of these environmental chemicals have become a part of our daily diet and exposures, especially since they are often found at high concentrations in fat-containing food such as red meat and dairy products (191). Constant exposure to various chemicals in the environment can put a toxic burden upon the body, and
many such chemicals are stored in the mother’s adipose tissue and then found in breast milk, which is then transferred to the nursing infant (191). This suggests that analysis of milk fat from breastfeeding women might be useful in assessing environmental contaminants in populations. For a more in-depth review of the transfer of toxins into breast milk and effects on child health, the reader is referred to several recent reviews (171, 285). However, in this section we will review the evidence that exposure to environmental toxins may also have important effects on mammary gland physiology. In particular, several studies have shown that exposure to chemicals or endocrine-disrupting compounds (EDCs) during gestation can

Fig. 1. Maternal factors that can lead to lactation insufficiency. Lactation insufficiency is a condition in which lactation is insufficient or unsuccessful due to inadequate milk production. A and B: images of hematoxylin and eosin-stained mouse mammary gland tissues represent a functional mammary gland undergoing successful lactation (A) and a mammary gland with impaired mammary differentiation and milk secretion that results in lactation insufficiency (B). C: list summarizing genetic, dietary, and environmental factors that have been shown to affect lactation outcomes. PRLR, prolactin receptor; Jak2, Janus kinase 2; SOCS2, suppressor of cytokine signaling 2; STAT, signal transducer and activator of transcription; ATF4, activating transcription factor 4; IGFBP-4, insulin-like growth factor-binding protein 4; IRF1, interferon regulatory factor 1; LIFR, leukemia inhibitory factor receptor; OSMR, oncostatin M receptor; PTK2, protein tyrosine kinase 2; mTOR, mammalian target of rapamycin; Gsk3, glycogen synthase kinase 3; LeuRS, leucyl-tRNA synthetase; PPARγ, peroxisome proliferator-activated receptor-γ; GH, growth hormone; OXTR, oxytocin receptor; FGF2, fibroblast growth factor 2; VDR, vitamin D receptor; ZnT2, zinc transporter 2; DGAT1, diacylglycerol O-acyltransferase 1; apoE, apolipoprotein E; FADS, fatty acid desaturase; LCPUFA, long-chain polyunsaturated fatty acids; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; MCFA, medium-chain fatty acid; AA, arachidonic Acid; BPA, bisphenol A; PBDE, polychlorinated dibenzofurans; PDD, polychlorinated dibenzo-p-dioxins; PCDD, polychlorinated dibenzofurans; PCB, polychlorinated biphenyls; DDE, dichlorodiphenyl dichloroethene.
cause adverse effects on mammary gland development and lactation (80). Numerous chemicals such as atrazine and dioxin (in herbicides), bisphenol A (BPA), and dibutylphthalate (in plastics), nonylphenol (in laundry and dish detergent), polybrominated diphenyl ethers (a flame retardant), and perfluorooctanoic acid (PFOA; in cleaning products and pesticides) have been observed to disrupt mammary gland function. Dioxins or dioxin-like compounds are comprised of members of three structurally related families of chemicals: polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans, and polychlorinated biphenyls (PCBs). Dioxins activate the aryl hydrocarbon receptor, which increases expression of numerous genes, including ABCG2, which is the main xenobiotic transporter in the mammary gland (106). PFOA is a known agonist of PPARα, which profoundly impairs normal differentiation of lobular-alveolar units during lactation (310). Studies in rodents show that gestational exposure to dioxins (290), PFOA (299, 300), atrazine (247), and BPA (180) leads to changes in mammary gland development that include mammary hypoplasia, reduced apoptosis in terminal end buds, altered gene or protein expression, and accelerated alveolar differentiation. In addition, mice exposed to dioxins during gestation have activated aryl hydrocarbon receptor, severe defects in mammary gland differentiation during lactation, and reduced milk protein expression that leads to offspring death within 24 h postpartum (290). PFOA exposure in mice during early gestation or lactation permanently impairs mammary gland differentiation, reduces milk protein expression, and decreases pup survival over the lactation period (300). Finally, exposure to BPA during pregnancy in mice leads to lactation insufficiency and offspring death (180). Thus far, only a few studies in women have shown an association between toxins like PCBs and dichlorodiphenyl dichloroethene and lactation defects such that exposure to these toxins is associated with shorter breastfeeding duration (67, 252). Given the burden of these often hidden environmental toxins in daily life, understanding the consequences of these environmental toxins on mammary gland physiology in humans is an important area for future research.

The toxins mentioned above are man-made environmental contaminants, but even natural components such as heavy metals exposure can affect the mammary gland during lactation. Some heavy metals (copper, zinc, and manganese) are biologically essential; however, the most pollutant heavy metals are lead, cadmium, and mercury that bioaccumulate following absorption, causing adverse health effects. Human, ruminant, and rodent studies have all demonstrated that cadmium levels remain low in breast milk, suggesting that the mammary gland is capable of protecting the growing infant from heavy metal exposure (24, 25, 85, 105, 170, 232, 270). As a result, cadmium sequestration in the mammary glands of lactating rodents has been observed (232), which disturbs alveoli morphology and reduces β-casein production and secretory activation (217). Although these studies illustrate profound lactation defects resulting from these environmental factors, there remains conflicting information regarding the mechanistic explanation(s) (180, 290). Hence, the mechanism of action(s) of heavy metals on breast development and lactation outcomes still remains to be explored. More importantly, because exposures to heavy metals as well as environmental toxins have become inevitably common, it is of much greater significance to investigate potential ways to reduce transfer into milk or to buffer these harmful factors in infants (191).

Conclusion

Breast milk clearly provides optimal nutrition for the growing infant as long as the mother is capable of producing and secreting an adequate volume of high-quality milk. Epidemiological studies suggest that many breastfeeding women may suffer from suboptimal lactation, and increasing evidence implicates maternal genetics, diet, and environmental toxins as modifiers of reproductive endocrinology, mammary gland physiology, lactation, and milk composition (Fig. 1). Although infant formula meets the nutritional needs of infants, there remain clear differences in short- and long-term health outcomes between breast-fed and formula-fed infants. Clearly, understanding factors that impact lactation and developing methods to accurately assess lactation outcomes before a breast-fed infant becomes ill will directly inform the development of therapeutic strategies to improve poor lactation performance. Importantly, these advancements will have a major impact on increasing the number of exclusively breastfed infants and decreasing the burden of noncommunicable disease. Moreover, understanding these factors and improving lactation success will be important to develop novel nutrigenomic or pharmacogenomic approaches for reducing breast disease and cancer.

REFERENCES

15. Balasubramanian S.


17. Boensch M, Oberthuer A, Eifinger F, Roth B.

18. Bezerra FF, Cabello GM, Mendonca LM, Donangelo CM.


42. Cao Y, Karin M.

43. Casey TM, Boecker A, Chiu JF, Plaut K.


55. Chiarelli R, Saccomandi D, Vigi V. Dietary supplements for the lactating mother: influence on the trace element content of milk article-title/H11022


kappa-casein gene of Indian native cattle (Bos indicus) and buffalo (Bubalus bubalis). *Meta Gene* 2: 769–784, 2014.


172. McCormick NH, Kelleher SL. ZnT4 provides zinc to zinc-dependent proteins in the trans-Golgi network critical for cell function and Zn


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290. Xie L, Innis SM. Genetic variants of the FADS1 FADS2 gene cluster are associated with altered (n-6) and (n-3) essential fatty acids in plasma and erythrocyte phospholipids in women during pregnancy and in breast milk during lactation. *J Nutr* 138: 2222–2228, 2008.


