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Mucosal Immunity and Liver Metabolism in the Complex Condition of Lactation Insufficiency

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Abstract

Lactation insufficiency is variously defined and includes the inability to produce milk, not producing enough milk to exclusively meet infant growth requirements, and pathological interruption of lactation (e.g., mastitis). Of women with intent-to-breastfeed, lactation insufficiency has been estimated to affect 38%–44% of newly postpartum women, likely contributing to the nearly 60% of infants that are not breastfed according to the World Health Organization's guidelines. To date, research and clinical practice aimed at improving feeding outcomes have focused on hospital lactation support and education, with laudable results. However, researchers' reports of recent rodent studies concerning fundamental lactation biology have suggested that the underlying pathologies of lactation insufficiency may be more nuanced than is currently appreciated. In this article, we identify mucosal biology of the breast and lactation-specific liver biology as two under-researched aspects of lactation physiology. Specifically, we argue that further scientific inquiry into reproductive state-dependent regulation of immunity in the human breast will reveal insights into novel immune based requirements for healthy lactation. Additionally, our synthesis of the literature supports the hypothesis that the liver is an essential player in lactation—highlighting the potential that pathologies of the liver may also be associated with lactation insufficiency. More research into these biologic underpinnings of lactation is anticipated to provide new avenues to understand and treat lactation insufficiency.

Keywords

breastfeeding, breast immunology, insufficient milk, lactation, lactation disorders, liver metabolism, mastitis, maternal physiology

Background

Exclusive breastfeeding for the first 6 months of life for all infants has been recommended by the World Health Organization (WHO) since at least 2001, as breastfeeding strongly associates with reduced infant morbidity and mortality (Edmond et al., 2006; Lamberti et al., 2011, 2013; World Health Organization & UNICEF, 2019). However, as of 2019, only an estimated 41% of infants, globally, were breastfed according to these guidelines (Sultana et al., 2013). There are many barriers to exclusive breastfeeding (EBF), including lactation insufficiency (Sultana et al., 2013). Lactation insufficiency is variously defined, and ranges from the inability to produce milk, not producing enough milk to meet infant growth requirements (Figure 1A), and unintended pathological interruption of lactation, for example, mastitis (Sultana et al., 2013). Determining the extent of lactation insufficiency is difficult given that the human mammary gland is one of the only organs without a diagnostic test to measure function (Hartmann & Cregan, 2001; Hurst, 2007). Mammary glands are unique in that full glandular development requires the hormones of pregnancy for

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maturation. Lactogenesis occurs in two stages. The first stage is secretory differentiation, which takes place during pregnancy when the initiation of glandular capacity for milk synthesis begins. The second is secretory activation, which occurs after delivery of the placenta and the associated progesterone withdrawal, and results in the onset of copious milk production (Neville & Morton, 2001). Secretory activation is an endocrine-driven process which should occur regardless of milk removal, while the ongoing ability to produce milk is driven by autocrine control. If milk is not removed regularly, local feedback inhibition will lead to mammary gland involution and weaning (Neville & Morton, 2001). The secretory activation phase is also characterized in humans and rodents by increasing lactose concentrations in the milk, putatively via galactose synthesis by mammary epithelial cells (Mohammad et al., 2012). Interference of any of these processes, from insufficient glandular tissue to altered hormonal concentrations to ineffective milk removal, could result in lactation insufficiency.

While many primiparous women struggle with delayed lactogenesis (estimates range from 38% to 44% in recent studies; Nommsen-Rivers et al., 2012), true failure of the onset of lactation remains rare, with only 1.7% of women not experiencing the onset of lactation within the first week following parturition (Nommsen-Rivers et al., 2010). Clinical estimates have found that, for women intending to breastfeed, between 5% and 15% experience either delayed or failed lactogenesis (Hurst, 2007). However, considering the reported disparities between intent to lactate and meeting lactation goals, the actual incidence of lactation insufficiency could be higher.

Psychosocial factors (e.g., stress, misaligned expectations about nursing an infant, lack of significant other support, and lack of adequate lactation education) have all been reported as causes of lactation insufficiency (Lau, 2001; Lau & Simpson, 2004). However, there are numerous physiologic risk factors for lactation insufficiency including maternal age, diabetes, polycystic ovarian syndrome, preterm birth, and inadequate breast development during puberty and pregnancy (Neifert et al., 1985). Additionally, in the clinic, lack of change in breast size during pregnancy is viewed as a risk factor for lactation insufficiency (Hurst, 2007). The hypothesis is that insufficient or stunted glandular development during pregnancy may result in too few mammary epithelial cells to produce an adequate milk supply. The same could be true of stunted mammary development during puberty. However, very little is known about normal mammary gland development on the tissue or cellular level specifically pertaining to milk production, and there is likely natural variation from person to person. As a result, there is scant scientific evidence to either support or reject this "mammary gland development" hypothesis.

Mastitis, another commonly recognized cause of lactation insufficiency, can result in gland damage and/or activation of unplanned involution, both of which would lead to the removal of milk producing cells, thereby reducing milk production. Specifically, bacterial sensing during mastitis via the circulating factor lipopolysaccharide binding protein (LBP) culminates in STAT-3 activation, a known trigger of involution (de Andrés et al., 2018; Jena et al., 2019; Stein et al., 2004; Zeng et al., 2009). Mastitis caused by overt bacterial infection is a well-studied issue among lactating women with effective treatment options, and has been covered in other excellent reviews (Barbosa-Cesnik et al., 2003; Jahanfar et al., 2013).

Another clinically recognized risk factor for lactation insufficiency is obesity. Women with overweight/obesity who intended EBF have lower rates of EBF 6 weeks to 6 months after birth compared to their peers of normal weight (Marshall et al., 2019, 2020). The exact biological mechanism(s) are unknown but may involve obesity-associated systemic metabolic dysregulation that influences the ability to make milk. The study of obesity as a cause of lactation insufficiency is widely recognized but remains an emerging field; it is not the focus of this article.

Considering the exclusions we outline above, this paper is not a systematic review, but rather seeks to synthesize potential biologic mediators not commonly connected to lactation. Recently, researchers conducting rodent studies concerning fundamental lactation biology have suggested that the underlying pathologies of lactation insufficiency may be more nuanced than is currently appreciated. Specifically, new evidence suggested that active mucosal immune programs within the breast, and enhanced function from the liver, might contribute to healthy lactation. The primary literature exploring the role of liver function and immunity in the breast is modest but

Key Messages

- Lactation insufficiency has been estimated to negatively influence 38%–44% of newly postpartum women. However, the physiological underpinnings of lactation insufficiency in humans are still under investigation.
- Results from preclinical models strongly support the hypothesis that active immune programs in the breast during lactation support healthy lactation—indicating autoimmunity as a potential factor in lactation insufficiency for some women.
- Likewise, researchers have reported results from both rodent and human studies supporting a functional role for the liver in milk production—suggesting that metabolic dysregulation and pathologies of the liver may contribute to lactation insufficiency.
- Roles for mammary gland immune function and liver function in healthy lactation and lactation insufficiency are worthy of additional study, with the hope of positively influencing lactation insufficiency management.

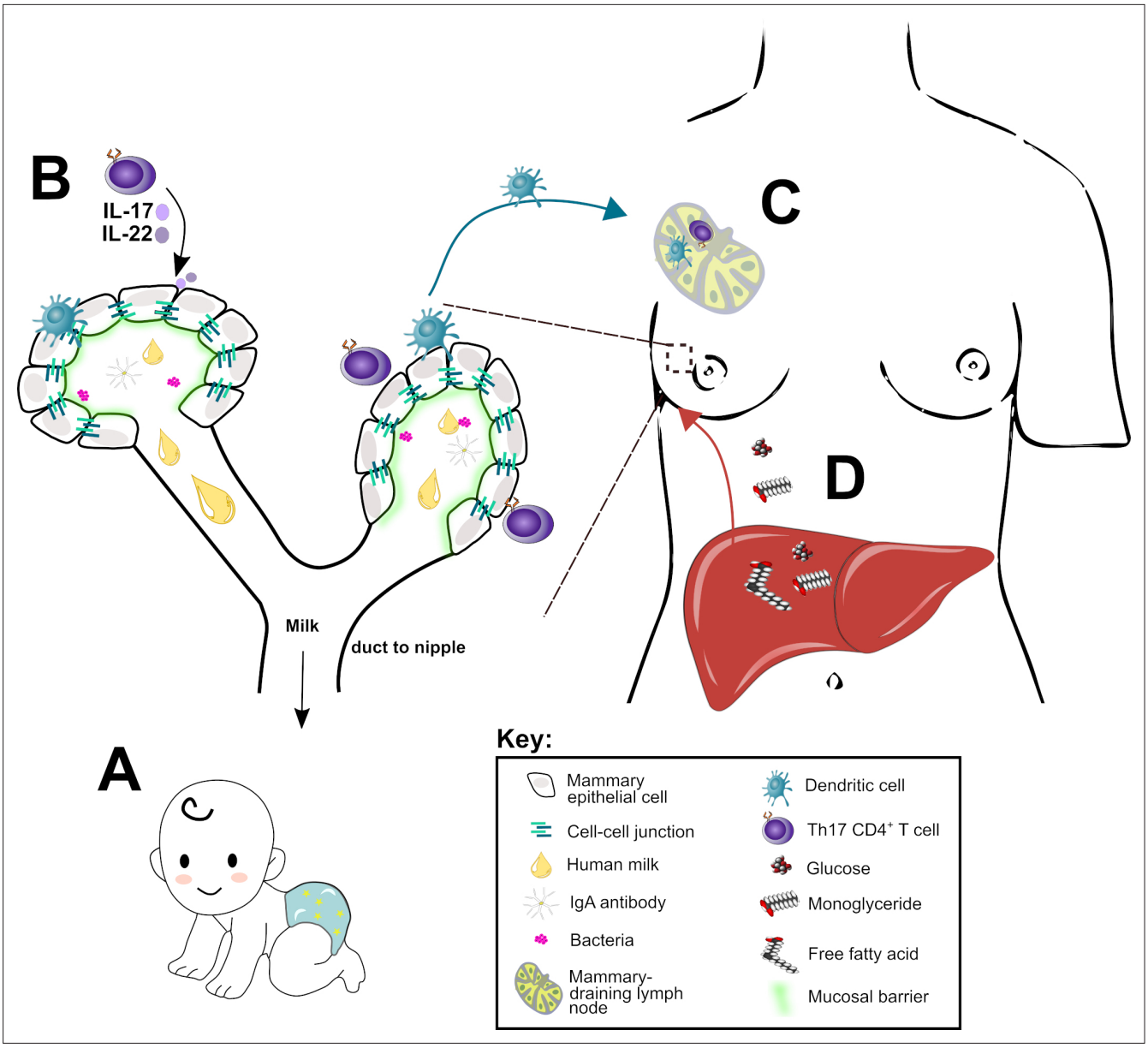

Figure 1. Mucosal Immunity in the Mammary Gland and Liver Metabolism as Potential Contributors to Healthy Lactation.
Note. A: Successful lactation is measured indirectly via infant growth and development. B: Mucosal immunity in the lactating mammary gland including barrier function and tolerogenic dendritic cells. C: Mammary draining lymph node regulates naïve T cell priming during lactation. D: Liver-mammary gland link during lactation for metabolic coordination and exchange of milk raw materials.

intriguing. Here, we present data supportive of the hypothesis that lactation is influenced by liver function and immunity, with the goal of illuminating future avenues for quantitatively assessing lactation function.

The Study of Mastitis Reveals Insight Into Mammary Gland Mucosal Immunity

Mucosal immunity is classically defined in organs with exposure to the outside world (e.g., the lungs and gastrointestinal tract). Modulation of mucosal immunity functions by reducing tissue-damaging responses due to persistent foreign antigen (Gill et al., 2010). Within the field of mammary gland biology, the mammary gland is not widely regarded as a mucosal organ that is exposed to persistent foreign antigen. However, the complex condition of clinical mastitis offers some insights into mucosal-like biological processes within the breast that may ensure lactation success. Mastitis occurs in 10%–27% of breastfeeding women (Ingman et al., 2014; Sordillo & Streicher, 2002); it is

commonly associated with a form of lactation insufficiency driven by interrupted lactation (Wöckel et al., 2008). The etiology of mastitis is thought to be largely due to overt bacterial infections (Boakes et al., 2018). Notably, skin-resident *staphylococcus epidermidis* is the most common bacterial species found in milk cultures of women with clinical symptoms of mastitis, suggesting entrance of externally-located bacteria into the mammary gland (Marín et al., 2017). Also, animal models of bacterial mastitis commonly rely upon the administration of bacteria into the teat canal (Glynn et al., 2014; Ingman et al., 2015). Taken together, these results suggest that translocation of bacteria from the skin into the mammary duct, and then into the mammary tissue can result in pathogenic mastitis in animal models, and thus may account for mastitis in women. However, bacteria also are present in healthy mammary tissue and in the milk of healthy women (Figure 1B), which suggests that bacteria can be a normal component of the mammary gland environment (Ballard & Morrow, 2013; Kvist et al., 2008; Urbaniak et al., 2014). Interestingly, researchers have shown that the breast microbiome is complex and can be influenced by diet, indicating that the mere presence of bacteria in the breast alone is not sufficient to explain the etiology of mastitis (Fernández et al., 2016; Jiménez et al., 2008; Shively et al., 2018).

During lactation, the normal mammary gland may be protected from bacterial translocation into underlying breast tissue by a highly impermeable epithelial barrier that is actively immune monitored (Figure 1B, cell-cell junctions; Betts et al., 2018; Nguyen et al., 2001; Owens et al., 2013; Stelwagen et al., 1999). Evidence that this protection is mediated in part by immunological programs is suggested by extensive studies in the gut, which show that highly orchestrated chemical and cellular barriers are essential for staving off infection from exogenous bacteria (Moens & Veldhoen, 2012; Okumura & Takeda, 2017). Based on these gut studies, we propose one origin of breast mastitis is through loss of epithelial cell barrier function and immune surveillance, resulting in aberrant bacterial translocation into the breast. However, the presence of a mucosal immunologic barrier has not been systematically investigated or demonstrated in the human breast. One could argue for the existence of a barrier function in the healthy lactating breast given the relative infrequency of mastitis compared to the number of nursing events that could introduce aberrant bacteria to the breast. Further, overt bacterial infection does not account for all cases of mastitis (Boakes et al., 2018; Kvist et al., 2008); thus, a full understanding of both microbe-dependent and independent inflammatory processes during lactation is required to fully understand mastitis.

Betts et al. (2018) is the first study of its kind to systematically assess the mammary gland for mucosal immunological hallmarks. This research team found that the adult murine mammary gland exhibits numerous mucosal hallmarks that are highly enriched during lactation, including a predominance of Th17 $CD4^+$ T cells (Figure 1B), which play important roles in mucosal barrier function (Betts et al., 2018). The role for Th17 $CD4^+$ T cells has been exquisitely described in the gut mucosa, where these cells produce IL-17, a cytokine that directly regulates epithelium impermeability by enhancing inter-cellular tight junctions (Kinugasa et al., 2000; Lee et al., 2015; Reynolds et al., 2012). Specifically, in models of intestinal injury IL-17 positively regulates the localization of the tight junction protein occludin, leading to decreased inter-cellular permeability, as measured by increased electrical resistance and protection from pathological bacterial colonization (Reynolds et al., 2012). Similar findings have corroborated the role of IL-17 cytokines in gut epithelial cell tight junction integrity in both homeostatic and acute injury contexts (Kinugasa et al., 2000; Reynolds et al., 2012). Further, IL-17 and IL-22 produced by Th17 $CD4^+$ T cells in the gut stimulate receptors on mucosal epithelial cells, leading to enhanced secretion of the antimicrobial β-defensin 2 (Huang et al., 2007; Kao et al., 2004). In addition, the Th17 $CD4^+$ T cell produces IL-22 (Figure 1B), which acts on intestinal epithelial cells to induce the production of the Reg family of antimicrobial proteins, including RegIIIγ (Zheng et al., 2008). Enhanced mRNA expression of antimicrobial mucins 1, 3, and 10 during lactation suggest that similar Th17 mucosal function is active in the lactational mammary gland (Betts et al., 2018).

Another component of mucosal immune programs that may be active in the mammary gland is immune tolerance. Immune tolerance is enhanced in the mammary gland during lactation in comparison to other reproductive states (Betts et al., 2018). Mucosal tissues are at risk of over-reacting to foreign antigens leading to pathologic inflammation, a risk mitigated by active immune tolerance programs (Steele et al., 2012). To address whether the mammary gland displays immune tolerance, a novel DO11.10 mouse model of naïve T cell priming was utilized. It was found that naïve systemic $CD4^+$ T cells became activated in the mammary gland draining lymph node when their specific antigen was introduced into the mammary gland of the nulliparous host (Betts et al., 2018). Conversely, T cell activation was not observed when antigen was injected into the lactating mammary gland. This relative lack of naïve T cell activation during lactation associated with increased numbers of tolerogenic antigen presenting dendritic cells (Figure 1B and C), specifically dendritic cells with reduced MHC-II and co-stimulatory molecule expression (CD80, CD86; Betts et al., 2018). Also, mammary gland dendritic cells during lactation showed reduced antigen presentation capability when assessed functionally. Altogether, this body of work is consistent with mucosal immune tolerance being active in the lactating gland, supporting the idea that mucosal immune tolerance plays a role in lactation success.

If mucosal immune tolerance is essential for lactation, then disruption of this tolerance mechanism is anticipated to contribute to lactation insufficiency. Recently, researchers have provided support for this nascent hypothesis. In

particular, a form of non-pathogen mastitis called idiopathic granulomatous has sparked interest in the immune requirements for successful lactation. In women, idiopathic granulomatous mastitis is a local inflammatory reaction that does not respond to antibiotics but does respond to steroids (Kim et al., 2003). This has led to the postulation of idiopathic granulomatous mastitis as an autoimmune disease of the lactating breast (Azlina et al., 2003; Katz et al., 2007). To investigate the possibility of autoimmunity in the mammary gland leading to lactation insufficiency, Kesaraju and colleagues (2012) utilized a unique rodent model that elicited immunity to a lactation-specific milk protein. The expected outcome was that autoimmunity to a milk protein would result in autoimmune inflammation, damage to secretory mammary epithelium, and reduced milk production. In this model, mature SWR/J female mice were immunized prior to mating with the milk protein alpha lactalbumin, a protein necessary for lactose production. Following immunization, females were randomized to a lactating or nulliparous study arm. Mammary tissue inflammation consistent with autoimmunity, that is Th1 skewed T cells that produced the inflammatory cytokine IFNγ, was only observed in the lactation group. Mammary tissue inflammation was not observed in non-immunized or immunized but nulliparous mice. Further, the immunized lactation group mice failed to sustain their pups, leading to pup alopecia and runting (Kesaraju et al., 2012). These outcomes supported an important conclusion: Autoimmunity to a lactation specific antigen is possible and leads to lactation insufficiency. This evidence suggested that mucosal immune tolerance, to functionally oppose potential autoimmunity, is a critical component of lactation success in rodents. The presence and requirement of mucosal immune programs in rodent models of lactation provide impetus to investigate the role of breast mucosal biology in supporting lactation in women.

Evidence of a Liver–Mammary Gland Functional Unit During Lactation and Weaning

To meet the nutritional needs of a nursing infant it is understood that the liver and the mammary gland both support lactation by increasing metabolic output (Tigas et al., 2002). As a result, it has been hypothesized that during lactation the mammary gland and the liver contribute to lactation success by working as a functional unit. Therefore, understanding the role of the liver in milk production, including through the normal regulatory pathways of liver glucose production (Tigas et al., 2002), may yield insight into lactation insufficiency of unknown origin. It is well accepted that mammary epithelial cells are the major producers of mature milk, including lipids, in part through the up-regulation of gene expression in the mammary gland related to fatty acid synthesis (Rudolph et al., 2007). Nonetheless, it is also known that the majority of fatty acids needed for milk production are obtained through diet via absorption, bile emulsification, and the break down to free fatty acids and monoglycerides, which occurs in the small intestine (Rudolph et al., 2007). In this capacity, the liver, which has a role in lipid homeostasis through adsorption of circulating lipids, has been viewed as an intermediate storehouse for fatty acids prior to transit to the mammary gland. However, recent evidence has suggested an even more important role for the liver in milk production, one that requires the liver to undergo a reproductive state change that has not previously been described.

Early evidence for reproductive state alterations in the liver included the demonstration of increased biosynthetic capability during lactation. For example, in sows, on the day prior to birth, higher circulating urea and creatinine concentrations—both made by the liver—positively correlated with colostrum yield, the first product of lactation (Loisel et al., 2014). Additionally, both fatty acid oxidation and glucose production in the rodent liver significantly increase during lactation, ostensibly to meet the elevated glucose needs of milk production (Goddard et al., 2017; Rawson et al., 2012). The liver produces glucose through glycogenolysis and gluconeogenesis (Tigas et al., 2002). Intriguingly, gluconeogenesis pathways are more active in the liver than in the mammary gland during lactation (Figure 1D), suggesting that the liver is a primary source of glucose for milk production (Rudolph et al., 2007). Consistent with this finding, protein and lactose concentrations in milk correlate strongly with hepatic glucose metabolism in dairy cows (Grum et al., 2002; Weber et al., 2013). In further support of the liver playing an active role in lactation, researchers recently have reported the lactating rodent liver is metabolically distinct from pre-pregnant and post-weaning stages, with increasing anabolic metabolism (Goddard et al., 2017). These data support the hypothesis that during lactation the liver plays a key role in the production of milk, alongside the mammary gland, with the liver as the predominate site of glucose synthesis. How glucose production in the liver is regulated during lactation remains an open question. One potential explanation is that the liver increases nutrient output in response to low nutrient blood levels, which are lowered due to increased uptake by the lactating mammary gland. However, in multiple small studies with lactating women, researchers reported neither lactation nor feeding at the breast to result in hypoglycemia (Bentley-Lewis et al., 2007; Colatrella et al., 2012), raising an intriguing alternative hypothesis to account for the increased metabolic output during lactation. Specifically, the liver may increase metabolic output in response to developmentally regulated cues from the mammary gland.

Additionally, similar architectural and physical changes occur in the mammary gland and liver during lactation, which could be interpreted as evidence for coordinated lactational programs in these two organs. For example, quantitative extracellular matrix proteomics demonstrates that the liver changes its matrix composition during lactation, as

does the mammary gland (Goddard et al., 2016). In addition, the physical size of the mammary gland and liver are regulated in tandem during pregnancy and lactation in the rodent (Dai et al., 2011; Hollister et al., 1987). Furthermore, in rodents, liver size decreases to its pre-pregnant size concurrent with weaning rather than at parturition, demonstrating that liver size is dissociated from the overall body weight of the dam at the pregnancy to lactation switch (Goddard et al., 2017). Additional data that are consistent with the regulated coordination of the liver and the mammary gland have been observed upon weaning. The process that results in the reduction in liver size post-wean shares all the hallmarks of weaning-induced mammary gland involution, including programmed cell death of the hepatocytes, immune cell influx, extracellular matrix remodeling, and catabolic metabolism consistent with tissue loss. In further support of a liver–mammary gland functional link, a recent study by Hyatt et al. (2019) found evidence of a causal relationship between lactation and liver size increase. These authors found that parous rats which were permitted to lactate had significantly greater liver mass compared to parous rats not permitted to lactate (Hyatt et al., 2019). We argue that these data and the synchronous growth during pregnancy, maintenance during lactation, and weaning-induced involution processes in the mammary gland and liver provide strong support for a mammary gland–liver functional unit that is established to meet the metabolic demands of lactation. However, whether this functional link is regulated in response to the developmental programs of pregnancy and lactation, or indirectly regulated due to increased metabolic demands of lactation, remains to be determined.

Summary and Future Directions

Lactation insufficiency is a serious clinical problem that will likely require interdisciplinary research to fully delineate. Here, we identify mucosal biology of the breast and liver biology as two under-researched aspects of lactation that may provide new avenues to understand the etiology of lactation insufficiency—especially for cases not corrected with education, support, or antibiotics.

We argue that further scientific inquiry into reproductive state-dependent regulation of immunity in the breast will likely reveal immunological requirements for healthy lactation. Specifically, autoimmunity to milk proteins might be one mechanism underlying lactation insufficiency. However, active immune regulation in the lactating mammary gland has so far only been demonstrated in animal models, including evidence of autoimmunity influencing milk production (Kesaraju et al., 2012; Schwartz & Strauchen, 1990). Research into mucosal breast biology in healthy women may yield insight into the initiation of autoimmune disorders within the breast and lead to new strategies for the prevention of some types of lactation insufficiency. Moreover, the demonstration of immune suppression in the breast as a requisite for healthy lactation in women could contribute to the development of new standards of care in the detection and treatment of lactation insufficiency. Specifically, the development of clinical diagnostic tests to detect auto-antibodies to milk components in lactating women would broaden the tool kit available to clinicians treating mastitis of unknown origin.

Additionally, the hypothesis that the liver is an essential player in lactation because of its enhanced glucose production, metabolic processing of lipids, and shuttling of these vital milk components to the mammary gland, has an evidence-base. An outstanding question is whether various liver pathologies may impede successful lactation, including fatty liver disease, hepatitis C, and cirrhosis. One untested hypothesis is that fatty liver disease of any etiology may reduce the liver's capacity to support lactation, limiting lipid or glucose transport to the mammary gland, and reducing milk supply. These questions could be addressed by epidemiological studies of reproductive-age women with liver conditions, as well as prospective studies in lactating women.

Altogether, this commentary highlights the potential influence that investigating the physiological underpinnings of healthy lactation could have on improving lactation success, and thus infant health. While lactation insufficiency has obvious implications for the infant, there may be unappreciated consequences for maternal health. For example, the "reset hypothesis" proposes that lactation is critical for the metabolic health of a new mother by decreasing insulin resistance, a potentially powerful biology in reducing the development of diabetes (Stuebe & Rich-Edwards, 2009). Also, a lactation duration of 6 months or longer has been associated with reduced incidence of non-alcoholic fatty liver disease and breast cancer (Ajmera et al., 2019; Palmer et al., 2014). Therefore, lactation represents a significant opportunity to potentially make inroads in the treatment of multiple health problems that disproportionately affect women, including breast cancer, liver disease, autoimmune disorders and, of course, lactation insufficiency. Studying the role of immunity and the liver in supporting lactation may lead to important discoveries in all these fields.

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