I

The Context of Lactation and Breastfeeding

PRELUDE: INFLUENCE OF THE POLITICAL AND SOCIAL LANDSCAPE ON BREASTFEEDING

Breastfeeding and the provision of human milk define a relatively short window of opportunity to provide the foundation for a person's lifelong health. Increasing the rate of breastfeeding in the United States has been a public health priority for more than a century. For three decades, the U.S. Department of Health and Human Services (HHS) has promulgated breastfeeding goals for the nation through the Healthy People initiative, which provides science-based, 10-year national objectives for improving the health of all Americans. The breastfeeding objectives for 2020 include improving the breastfeeding initiation and duration rates, raising the exclusive breastfeeding rates, increasing the number of employers who have worksite lactation support programs, reducing the proportion of newborns who receive formula supplementation in the hospital, and increasing the number of infants born in hospitals that provide optimal lactation care (HHS, 2010).

Currently, 79.2% of mothers in the United States initiate breastfeeding, with 40.7% exclusively breastfeeding at 3 months (Centers for Disease Control and Prevention [CDC], 2014). A great deal of progress has been made in the political and social environment surrounding breastfeeding (**Box I-1**). Contributing to the progress seen in breastfeeding support over the last 25 years has been the increase in

Box I-1 Timeline of Breastfeeding Progress, 1996–2015

1996	The Loving Support campaign from Food and Nutrition Service is formed to increase the number of breastfeeding mothers in the WIC program.
1997	The American Academy of Pediatrics releases its first policy statement on breastfeeding.
1998	The U.S. Breastfeeding Committee is formed.
1999	The Right to Breastfeed Act (H.R. 1848) by Representative Carolyn Maloney (D-NY) is passed, ensuring a woman's right to breastfeed on all federal property.
2000	The *Healthy People 2010* guidelines for the nation are released with breastfeeding objectives.
2003	The National Breastfeeding Awareness Campaign is conducted, aimed at increasing breastfeeding among first-time parents. Of note, it used a risk-based format and was significantly watered down by interference from the infant formula industry.
2005	• The *Healthy People 2010* mid-course review adds exclusive breastfeeding targets. • HHS issues *Blueprint for Action on Breastfeeding*, which positions breastfeeding as a public health issue, not just an individual choice.
2007	The CDC conducts the first Maternity Practices in Infant Nutrition and Care (mPINC) survey, which highlights hospital practices related to breastfeeding. The results demonstrated how poorly many hospitals were supporting breastfeeding and has led many hospitals to improve their practices.
2008	With the creation of the Business Case for Breastfeeding program, the Maternal and Child Health Bureau and Health Resources and Services Administration involve employers in supporting breastfeeding mothers by providing a package of information on how best to provide lactation accommodations in the worksite.
2009	WIC food packages are revised to better promote breastfeeding.
2010	• The Joint Commission establishes the Perinatal Core Measure Set, which measures, among other things, the number of infants exclusively fed breastmilk upon discharge from the hospital. • The Patient Protection and Affordable Care Act of 2010 (P.L.111-148, Sec. 4207 [2010]) introduces specific worksite protections for breastfeeding employees on a national level. • A presidential memorandum orders the creation of appropriate workplace accommodations for nursing mothers who are federal civilian employees. • The *Healthy People 2020* goals add three more breastfeeding objectives, (1) increase the proportion of employers that have worksite lactation support programs, (2) reduce the proportion of breastfed newborns who receive formula supplementation within the first 2 days of life, and (3) increase the proportion of births that occur in facilities that provide recommended care for lactating mothers and their babies.
2011	• The Surgeon General issues *The Call to Action to Support Breastfeeding*. • The Internal Revenue Service allows breastfeeding equipment to be reimbursed from flexible health spending accounts.

- The Patient Protection and Affordable Care Act states that health insurers will be required to pay for a range of preventive care services specifically aimed at women, including "comprehensive lactation support and counseling, by a trained provider during pregnancy and/or in the postpartum period, and costs for renting breastfeeding equipment."

2012
- The Joint Commission mandates that all birthing hospitals with more than 1,100 deliveries per year must participate in its Perinatal Care Core Measure Set to remain accredited.
- Rhode Island and Massachusetts are the first and second states to achieve the elimination of formula discharge bag distribution in all of their birthing hospitals.
- A CDC grant to the National Institute for Children's Healthcare Quality is made to facilitate 90 hospitals achieving the Baby-Friendly designation.

2014 The TRICARE Moms Improvement Act is signed into law. The law makes breastfeeding supplies, services, and counseling available to military family members covered under the federal Tricare health insurance program.

2015
- Births in Baby-Friendly designated facilities exceed the *Healthy People 2020* goal. More than 17% of births occur in Baby-Friendly designated facilities; the *Healthy People 2020* goal is 8.1%.
- A revised meal pattern is proposed related to the Healthy, Hunger-Free Kids Act of 2010. As an incentive for encouraging breastfeeding and to better align program rules, this proposed rule would allow reimbursement for meals served to infants younger than 6 months of age when the mother directly breastfeeds her child at the childcare facility. Meals containing breastmilk or iron-fortified infant formula supplied by the parent or the facility are already eligible for CACFP reimbursement.

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employers who provide time and space to express milk at work, the increase in state legislation mandating worksite support for breastfeeding employees and laws protecting the right to breastfeed in public, the expansion in breastfeeding education and training opportunities for healthcare providers, the increase in the interest and number of hospitals obtaining the Baby-Friendly designation, the availability of advanced lactation support and services from international board certified lactation consultants (IBCLCs), and increased research on breastfeeding and human lactation.

While steady progress has been made, there remain many challenges and gaps in care that prevent mothers from meeting their breastfeeding goals. The prevalence of breastfeeding among African American mothers is consistently lower than that among mothers of other races and ethnicities (CDC, 2013). This persistent gap in breastfeeding rates between black women and women of other races and ethnicities might indicate that black women are more likely to encounter unsupportive cultural norms, perceptions that breastfeeding is inferior to formula feeding, lack of partner support, lack of self-efficacy, inadequate care from healthcare providers, social media influence, and an unsupportive work environment (Johnson, Kirk, Rosenblum, & Muzik, 2015).

The Surgeon General's Call to Action to Support Breastfeeding outlines 20 steps that can be taken to remove some of the obstacles faced by women who wish to breastfeed their infants (HHS, 2011) (**Box I-2**).

BOX I-2 Action Items from *The Surgeon General's Call to Action to Support Breastfeeding*

Actions for Mothers and Their Families

1. Give mothers the support they need to breastfeed their babies.
2. Develop programs to educate fathers and grandmothers about breastfeeding.

Actions for Communities

3. Strengthen programs that provide mother-to-mother support and peer counseling.
4. Use community-based organizations to promote and support breastfeeding.
5. Create a national campaign to promote breastfeeding.
6. Ensure that the marketing of infant formula is conducted in a way that minimizes its negative impacts on exclusive breastfeeding.

Actions for Health Care

7. Ensure that maternity care practices around the United States are fully supportive of breastfeeding.
8. Develop systems to guarantee continuity of skilled support for lactation between hospitals and healthcare settings in the community.
9. Provide education and training in breastfeeding for all health professionals who care for women and children.
10. Include basic support for breastfeeding as a standard of care for midwives, obstetricians, family physicians, nurse practitioners, and pediatricians.
11. Ensure access to services provided by IBCLCs.
12. Identify and address obstacles to greater availability of safe banked donor milk for fragile infants.

Actions for Employment

13. Work toward establishing paid maternity leave for all employed mothers.
14. Ensure that employers establish and maintain comprehensive, high-quality lactation support programs for their employees.
15. Expand the use of programs in the workplace that allow lactating mothers to have direct access to their babies.
16. Ensure that all child care providers accommodate the needs of breastfeeding mothers and infants.

Actions for Research and Surveillance

17. Increase funding of high-quality research on breastfeeding.
18. Strengthen existing capacity and develop future capacity for conducting research on breastfeeding.
19. Develop a national monitoring system to improve the tracking of breastfeeding rates as well as the policies and environmental factors that affect breastfeeding.

Action for Public Health Infrastructure

20. Improve national leadership on the promotion and support of breastfeeding.

Each step includes implementation strategies and places responsibility for breastfeeding improvement on all stakeholders.

Social attitudes toward breastfeeding contribute to shaping and influencing a mother's view on breastfeeding. The HealthStyles survey has been conducted since 1995, asking adults 18 years and older questions about their health orientations and practices (CDC, 2010). Progress has not been made in some areas; for example, in the 2010 survey, 32% believed that it is embarrassing to breastfeed in front of others compared with 29% in the 2000 survey. However, progress can be seen in other areas; for example, 59% in 2010 believed that women should have the right to breastfeed in public places compared with 43% who agreed with this statement in 2001. It is disappointing to see that certain misperceptions have become more prevalent; for example, in 2000, 44% thought that mothers had to give up too many lifestyle habits like favorite foods, cigarette smoking, and drinking alcohol, and in 2010, over 48% still thought that mothers had to give up personal preferences or change their lives in order to breastfeed. This attitude, plus other societal constraints such as lack of paid maternity leave, uncooperative employers, being asked to leave public places while breastfeeding, and a lack of understanding regarding the outcomes of not breastfeeding, place barriers in front of mothers that clinicians must address if a mother is to meet her breastfeeding goals.

Some of these barriers are being addressed through state and federal legislation. All states in the United States have at least one breastfeeding law on the books. The National Conference of State Legislatures (2015) catalogs and summarizes all of the state breastfeeding laws. The first state breastfeeding law was passed in New York in 1984, exempting breastfeeding from public indecency offenses. Laws vary from state to state, with some laws encouraging or requiring employer accommodations for breastfeeding mothers, permitting mothers to breastfeed in public, exempting breastfeeding from public indecency laws, allowing breastfeeding mothers to postpone or be excused from jury duty, or outlining some other special or unique requirements. One study showed that the most robust laws associated with increased infant breastfeeding at 6 months were an enforcement provision for workplace pumping laws (odds ratio [OR], 2.0; 95% confidence interval [CI], 1.6–2.6) and a jury duty exemption for breastfeeding mothers (OR, 1.7; 95% CI, 1.3–2.1). Having a private area in the workplace to express breastmilk (OR, 1.3; 95% CI, 1.1–1.7) and having break time to breastfeed or pump (OR, 1.2; 95% CI, 1.0–1.5) were also important for infant breastfeeding at 6 months (Smith-Gagen, Hollen, Tashiro, Cook, & Yang, 2014). When scrutinizing these laws relative to African American mothers, however, it appears that the laws were significantly less helpful to African American mothers compared with Hispanic and white mothers (Smith-Gagen, Hollen, Walker, Cook, & Yang, 2014). For example, most laws that mandate break-time provisions for expressing breastmilk require that it be unpaid break time. Many African American mothers may not be able to afford the income lost during unpaid breaks.

While these laws protect breastfeeding mothers to varying degrees, most lack any penalties for their violation, and large numbers of mothers are not protected by comprehensive laws. Laws that protect all breastfeeding mothers are extremely variable in their coverage and are made less effective by lack of knowledge of their existence and the absence of penalties (Nguyen & Hawkins, 2012). Informing mothers of their breastfeeding rights within their state may help them address various public challenges they encounter. For example, the Massachusetts Breastfeeding Coalition has a "license to breastfeed," which is a two-sided card that states the law regarding breastfeeding in public and where to file a grievance if a mother is harassed for breastfeeding in public (**Figure I-1**). Mothers carry these cards with them and

Figure I-1 "License to breastfeed" to help mothers know their rights.

Courtesy of the Massachusetts Breastfeeding Coalition, http://massbreastfeeding.org. Retrieved from http://massbreastfeeding.org/2011/06/21/get-your-license-to-breastfeed-2/

present the card to anyone who harasses them for breastfeeding in public. These cards can be distributed to breastfeeding mothers by healthcare providers or downloaded from the coalition's website. Laws cannot protect mothers if mothers are unaware of their rights.

Set against the landscape of variable legal protections for breastfeeding mothers came section 4207 of the Patient Protection and Affordable Care Act of 2010 (PL 111-148). This act was the second piece of federal (not state) legislation that offered legal protection for an aspect of breastfeeding (Murtagh & Moulton, 2011). The first piece of federal legislation was section 647 of the Treasury and General Government Appropriations Act (1999), which affirmed that a woman may breastfeed her child at any federal building or federal location where she is authorized to be (Public Law no. 106-058). The Affordable Care Act requires all employers to provide reasonable break time to express milk for a child up to 1 year of age in a private location other than a bathroom. Employers of less than 50 employees who can demonstrate hardship may be exempted from the law. This law applies only to employees who work for hourly wages

and does not apply to salaried workers and certain other classes of employees such as administrative employees, school teachers, and many agricultural workers. If a state has a stronger worksite protection law, it takes precedence over the federal law. While this law covers only a portion of employed breastfeeding mothers, it has proven to be a start toward eliminating or reducing employment-related barriers to breastfeeding.

Also under the Affordable Care Act, health insurers will be required to pay for a range of preventive care services specifically aimed at women. These services include "comprehensive lactation support and counseling, by a trained provider during pregnancy and/or in the postpartum period, and costs for renting breastfeeding equipment." While this provision is well intended, the HHS did not provide implementation guidelines for insurers, leaving them to determine for themselves how to interpret the law. This has resulted in some mothers being provided with inappropriate breast pumps and inadequate lactation care and services. See the Resources section for samples of best practices for insurers regarding the Affordable Care Act's breastfeeding provisions. Clinicians are of great importance as a source for informing mothers and employers of the laws and providing help in securing the services to which mothers are entitled.

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RESOURCES

Sample best practices for insurers

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Chapter 1

Influence of the Biospecificity of Human Milk

INTRODUCTION

Effective breastfeeding management requires a general understanding of the structure and function of human milk itself. Many of the recommendations for successful breastfeeding and optimal infant health outcomes are based on using what the clinician knows about the components of human milk, what they do, and how they work. This chapter and Appendix 1-1 provide an overview of the components of breastmilk and of breastfeeding management based on milk function and composition.

Human milk is a highly complex and unique fluid that is strikingly different from the milks of other species, including the cow. Aggressive marketing of infant formula has blurred the public's perception of the differences between human milk and infant formula. Data from the HealthStyles survey, an annual national mail survey to U.S. adults, were examined to understand changes in public attitudes toward breastfeeding. The 1999 and 2003 HealthStyles surveys (Li, Rock, & Grummer-Strawn, 2007) included four breastfeeding items related to public attitudes toward breastfeeding in public and toward differences between infant formula and breastmilk. The percentage of respondents in agreement with the statement, "Infant formula is as good as breastmilk," increased significantly, from 14.3% in 1999 to 25.7% in 2003 (Li et al., 2007). In 2010, 20% of respondents still agreed that infant formula is as good as breastmilk, and over 30% neither agreed nor disagreed (Centers for Disease Control and Prevention, 2010). This figure has improved over time, with 15.52% currently agreeing that infant formula is as good as breastmilk and 26.28% neither agreeing nor disagreeing (Centers for Disease Control and Prevention, 2014a). This finding probably suggests that many people may still be sufficiently confused regarding the similarity or difference between infant formula and breastmilk that they cannot form an opinion. Lack of clarity regarding the difference between formula and breastmilk can be caused by clever marketing of infant formula, by contradictory Internet resources, and by social media postings that leave mothers vulnerable to marketing claims and peer opinions. Many of these interwoven resources are typically not evidence based and prey on vulnerabilities of new mothers, resulting in mothers who may be less likely to initiate or sustain breastfeeding. Hundreds of human milk components interact synergistically to fulfill the dual function of breastmilk, nourishing and protecting infants and young children who are breastfed or who receive human milk. The addition of ingredients into infant formula derived from nonhuman sources

and pre- and probiotics cannot duplicate the health, cognitive, and developmental outcomes seen in infants fed human milk, no matter what formula advertising might claim.

Lactation is an ancient process that is thought to predate placental gestation and mammals themselves. It appears to have evolved in incremental steps as part of the innate immune system and over time acquired its nutritional function. The mammary gland is thought to have first developed as a mucous skin gland that secreted antimicrobial substances to protect the surface of the egg and skin of the newborn (**Figure 1-1**). Oftedal (2002) suggests that these glands evolved from the role of providing primarily moisture and antimicrobials to parchment-shelled eggs to the role of supplying nutrients for offspring. Fossil evidence indicates that some of the therapsids (mammal-like reptiles) and the mammaliaformes ("mammal-shaped," a branch of life that contains the mammals and their closest extinct relatives), which were present during the Triassic period more than 200 million years ago, produced a nutrient-rich milk-like secretion. Much later, due to gene sharing and gene duplication events, two antimicrobial enzymes (lysozyme and xanthine oxidoreductase) evolved new functions within the mammary epithelium, which allowed the secretion of fat, whey protein, sugar, and water, resulting in the unique and complex fluid we call milk (Vorbach, Capecchi, & Penninger, 2006).

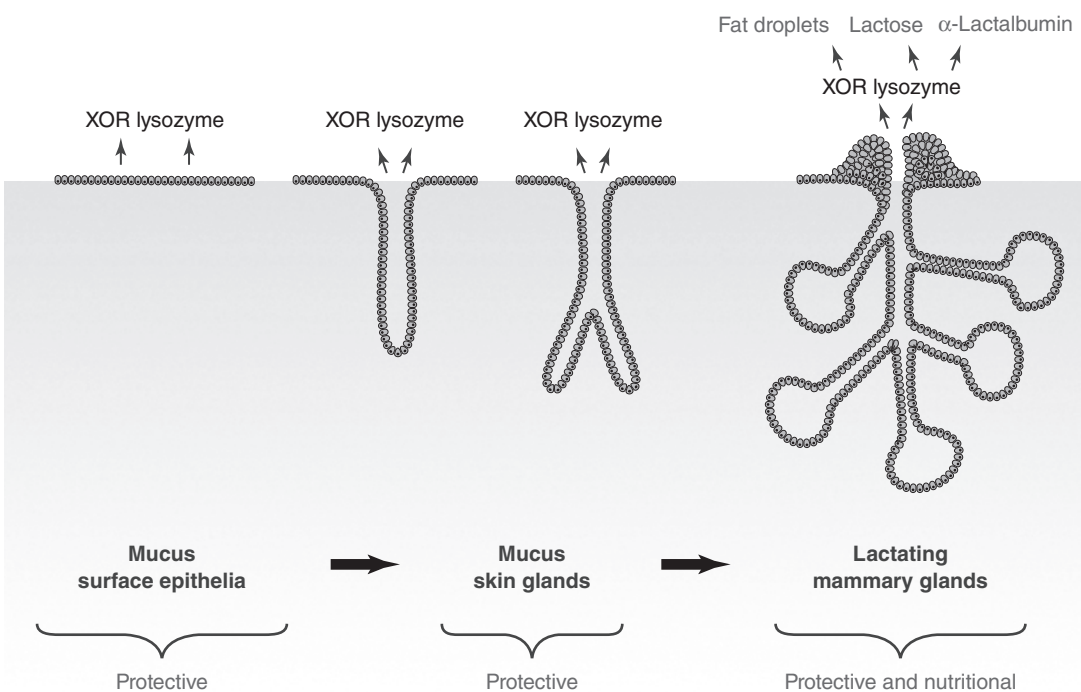

Figure 1-1 Proposed evolution of the mammary gland from a mucus-secreting epithelial gland.

Vorbach, C., Capecchi, M. R., & Penninger, J. M. (2006). Evolution of the mammary gland from the innate immune system? *BioEssays, 28*, 606–616. BioEssays by International Council of Scientific Unions; Company of Biologists. Reproduced with permission of John Wiley & Sons Ltd.

Milk composition and the length of lactation have been modified and adapted to meet the needs of each particular species. Generally, the protein content of milk varies with the rate of growth of the offspring. In many species, including humans, low-solute milk with relatively low concentrations of protein is related to a pattern of frequent feedings. Researchers often refer to species that manifest or practice this concept as "continuous contact" species. Calorie-dense milk with a high fat concentration can be associated with both the size of the species and low environmental temperatures. For example, marine mammals have fat concentrations of 50% or more in their milk to enable their young to lay down a thick insulating layer of fat. Each species has features (e.g., an organ, a behavior, a body system) that serve as major focal points for determining the type, variety, and interactions of the milk components fed to the young. In humans, these focal points include the brain, the immune system, and the acquisition of affiliative behavior.

Human milk composition is not static or uniform like infant formula. Breastmilk is a living dynamic fluid that represents an elegant interplay between the needs and vulnerabilities of the infant and the rapid adaptability of the mother's body to provide milk components to meet those needs and support those vulnerabilities:

- Colostrum (1–5 days) evolves through transitional milk (6–13 days) to mature milk (14 days and beyond).
- During early lactation, a few hours can show a significant change in milk composition. Lactoferrin, for example, decreases significantly over the first 3 days of lactation.
- Milk composition changes during each feeding as the breast drains and the fat content rises.
- Milk composition changes during each day and over the course of the entire lactation.
- Milk of preterm mothers differs from that of mothers delivering at term.
- More than 200 components have been identified in human milk, with some having still unknown roles.
- Hundreds of thousands of immune cells in breastmilk are ingested by the breastfed infant every day.
- Human milk contains stem cells that are involved in the regulation of mammary gland development and tumorigenesis (Thomas, Zeps, Cregan, Hartmann, & Martin, 2011). These stem cells can migrate to different organs to provide active immunity and boost infant development in early life (Hassiotou & Hartmann, 2014).
- Infant formula is an inert nutritional medium with no growth factors, hormones, or live cells like those found in breastmilk.
- Human milk is a biological mediator, carrying a rich variety of bioactive substances intended to grow a brain, construct an immune system, and facilitate affiliative behavior.

COLOSTRUM

Colostrum, the first milk, is present in the breasts from about 12–16 weeks into the pregnancy onward. This thick fluid's yellowish color comes from beta-carotene. It differs from mature milk both in the nature of its components and in their relative proportions. Colostrum has a mean energy value of 67 kcal/dL (18.76 kcal/oz), compared with mature milk's mean energy value of 75 kcal/dL (21 kcal/oz). The volume of colostrum per feeding during the first 3 days ranges from 2 to 20 mL and sometimes more. Colostrum is higher in protein, sodium, chloride, potassium, and fat-soluble vitamins such as vitamin A (3 times

higher on day 3 than in mature milk), vitamin E (3 times higher than in mature milk), and carotenoids (10 times higher than in mature milk). It is lower in carbohydrates, lipids (2%), potassium, and lactose.

During the early days following delivery, the tight junctions between the mammary epithelial cells are relatively open and allow the transport of many bioactive immune substances from the mother's circulation into her colostrum (Kelleher & Lonnerdal, 2001). This enrichment of the early milk helps compensate for the relatively naïve neonatal immune system. Colostrum is rich in antioxidants, antibodies, and immunoglobulins, especially secretory immunoglobulin A (sIgA). Colostrum contains a high concentration of sIgA, approximately 10 g/L compared with approximately 1 g/L in mature milk. It contains interferon, which has strong antiviral activity, and fibronectin, which makes certain phagocytes more aggressive so that they ingest microbes even when not tagged by an antibody. Colostrum contains pancreatic secretory trypsin inhibitor (PSTI), a peptide found in the pancreas that protects it from damage by the digestive enzymes that it produces. PSTI is also found in mature breastmilk, but it is seven times more concentrated in colostrum. Marchbank, Weaver, Nilsen-Hamilton, and Playford (2009) found that PSTI stimulated cell migration and proliferation by threefold and reduced apoptosis (cell death) in damaged intestinal cells by 70–80%. PSTI both protects and repairs the delicate intestines of the newborn, readying the organ for processing future foods. Feeding infants colostrum establishes and maintains gut integrity, an important advantage over infant formula, because PSTI is not found in artificial milks. The newborn infant is deficient in CD14, part of a complex that can activate the innate immune system and that is important for protection against pathogen invasion. CD14 is present in human milk, with the highest concentration being present in colostrum. Colostrum's potent cocktail of components also includes specific oligosaccharides that change in concentration over the first 3 days to meet the physiological demands of the infant (Asakuma et al., 2007). They serve as a decoy to inhibit the attachment of pathogenic microorganisms, helping to protect newborns during an especially vulnerable time. Not only is colostrum replete with anti-infective properties, but the colostrum of mothers delivering preterm is more highly enriched with potent disease protectors than the colostrum of mothers delivering at term.

Preterm infants consuming their own mother's colostrum can benefit from ingestion of up to twice as many macrophages, lymphocytes, and total cells compared with those which are present in term colostrum (Mathur, Dwarkadas, Sharma, Saha, & Jain, 1990). They also receive more IgA, lysozymes, lactoferrin, and neutrophils than if they were receiving term colostrum. However, the colostrum of mothers delivering very preterm infants has lower concentrations of secretory IgA and several cytokines than the colostrum of mothers delivering after 30 weeks of gestation (Castellote et al., 2011). The degree of prematurity may affect the immunological composition of breastmilk, with earlier colostrum and milk showing reduced concentrations of some anti-infective factors. Infant formula, however, contains none of these formidable fighters of infection, leaving infants who are not fed colostrum or human milk much more vulnerable to infections and diseases prevented or reduced by breastfeeding or the provision of expressed colostrum and milk.

Colostrum of diabetic mothers is subject to biochemical and immunological alterations that affect the levels of some of its components. The protein expression involved in immunity and nutrition differs between the colostrum of mothers with gestational diabetes and that of mothers without gestational diabetes (Grapov et al., 2015). The colostrum of diabetic mothers is higher in glucose, lower in secretory IgA and secretory IgG, lower in C3 protein, lower in amylase, and higher in lipase (Morceli et al., 2011).

Colostrum of mothers who smoke has a significantly lower antioxidant capacity than the colostrum of mothers who do not smoke (Zagierski et al., 2011). This impairs the colostrum's ability to protect the infant from free radicals that contribute to conditions related to oxidative stress to which preterm infants are so vulnerable, such as necrotizing enterocolitis (NEC) and retinopathy of prematurity.

Maternal smoking alters the colostrum levels of a number of cytokines, which in turn increases the susceptibility of the newborn to infections (Piskin, Karavar, Arasli, & Ermis, 2012). In addition, the mode of delivery affects the antioxidant capacity of colostrum. The colostrum of mothers who deliver by cesarean section is lower in its antioxidative status than the colostrum of mothers who deliver vaginally (Simsek, Karabiyik, Polat, Duran, & Polat, 2014), potentially impeding the ability of colostrum to protect the infant from cellular damage caused by oxidative stress. Cesarean delivery can reduce the volume and prolactin concentration of colostrum as well as decrease the fatty acid levels.

Colostrum contributes to the establishment of bifidus flora in the digestive tract. The composition and volume of colostrum are in keeping with the needs and stores of a newborn human baby. Its primary function is anti-infective, but its biochemical composition has a laxative effect on meconium. It also provides a concentrated dose of certain nutrients such as zinc.

Genetic and environmental features may contribute to the compositional diversity seen in the colostrum of mothers worldwide. Musumeci and Musumeci (2013) reported the compositional differences between the colostrum of mothers living in Sicily and those living in Burkina Faso, one of the poorest countries of the African sub-Saharan area. The colostrum of the African mothers was richer in growth factors (IGF-I) that favor intestinal maturation; endorphins and S100B, which protect the brain from the consequences of asphyxia under difficult childbirth conditions; and chitotriosidase (an enzyme produced by activated macrophages), which is protective against gut pathogens, *Candida albicans*, and nematodes. It is thought that these components are present in higher quantities in African mothers' colostrum due to the precarious conditions of living in Africa, which exert a selective pressure to preserve the newborn.

Given the potential stressors on the composition of colostrum, it would seem prudent to assure maximum intake of colostrum for infants who are born by cesarean section, who experienced a difficult or precarious delivery, whose mothers smoke or have been exposed to secondhand smoke, whose mothers are diabetic, or who are born preterm.

CLINICAL IMPLICATIONS: ALLERGY AND DISEASE

It has long been thought that the gut (gastrointestinal [GI] tract) of a term fetus is sterile and that the bacterial colonization of the newborn gut occurs only following transit through the birth canal, where maternal vaginal and fecal bacteria become the first residents of the neonate's gut. More recent research, however, has shown that infants could develop their original gut microbiome well before birth. Researchers have reported that the meconium of term infants is not sterile, revealing that gut colonization actually starts prior to delivery (Jimenez et al., 2008). Bacteria have been isolated from amniotic fluid without any clinical or histological evidence of infection or inflammation in either the mother or the infant. Given that the fetus continuously swallows amniotic fluid in utero, bacteria present in that amniotic fluid from the maternal digestive tract may be the origin of the first infant gut colonizers. This suggests that the bacterial composition of the maternal gut could affect the bacterial content seen in infant meconium and serve as the pioneer bacteria colonizing the fetal gut.

Further influences and additions to the infant's gut microbiome occur during and after delivery through several mechanisms and routes:

- Method of delivery: During a vaginal delivery, bacteria from the maternal vaginal and intestinal microbiota colonize the infant gut. In a cesarean delivery, infants avoid contact with the maternal vaginal microbiota, leading to a deficiency of strict anaerobes such as *E. coli, Bacteroides*, and *Bifidobacterium* and a higher presence of facultative anaerobes such as *Clostridium* species, compared with vaginally born infants (Adlerberth & Wold, 2009). The cesarean-born infant's initial bacterial exposure is more likely to be from environmental microbes in the air, other infants, and the nursing staff, all of which serve as vectors for transfer. Infants born by cesarean section prior to the rupture of the amnion membrane are not exposed to the maternal flora in the birth canal. These infants are also subject to longer separations from their mother, longer hospital stays, and a shorter duration of breastfeeding—all of which increase the likelihood of significant alterations in the colonization of the infant's intestine.
- Gestational age: The pattern of gut colonization in preterm infants differs from that in healthy term infants. The aberration in colonization is due to a number of factors, including the use of sterile infant formula and the common administration of antibiotics, which could also contribute to feeding intolerance and the development of NEC (Neu & Walker, 2011). Preterm infants are also often born by cesarean section, are colonized with fewer bacteria, are separated from their mother, and are exposed to pathogenic institutional organisms.
- Feeding modality: Newborns receive gut-colonizing bacteria from their mother's milk. Breastmilk is thought to be one of the most important postpartum elements modulating the metabolic and immunological programming of a child's health (Aaltonen et al., 2011). Breastmilk is not sterile, nor is it meant to be. In fact, researchers have identified more than 700 bacterial species in human milk that vary from mother to mother depending on the mode of delivery and the obesity status of the mother. Colostrum has an even higher diversity of bacterial species than does transitional or mature milk (Cabrera-Rubio et al., 2012). The conditions of maternal overweight and obesity have been associated with an inflammation-prone aberrant gut microbiota that can be transferred to the infant, provoking unfavorable metabolic development in the baby (Collado, Isolauri, Laitinen, & Salminen, 2010). Divergence or deviation from breastmilk-directed microbial colonization during the early weeks and months of life interferes with many functions in the gut. This departure from the norm provokes a slower postnatal maturation of epithelial cell barrier functions, which alters the permeability of the gut and facilitates invasion of pathogens and foreign or harmful antigens (Perrier & Corthesy, 2011). The perinatal period, therefore, is a critical window of time where "set points" are imprinted in the neonatal gut. The nature of the microbiota acquired during the perinatal period is crucial in determining the intestinal immune response and tolerance. Alterations of the gut environment are directly responsible for mucosal inflammation and disease, autoimmunity conditions, and allergic disorders in childhood and adulthood (Gronlund, Arvilommi, Kero, Lehtonen, & Isolauri, 2000). The lower the percentage of breastmilk intake (less than 88%), the greater the risk of gut inflammation (Moodley-Govender, Mulol, Stauber, Manary, & Coutsoudis, 2015).

The bacterial composition of breastmilk also exerts an influence on the health of the maternal breast itself. The composition of the bacterial communities in the breastmilk are unique to each mother and could influence whether a woman develops mastitis or recurrent mastitis, or never develops mastitis at all (Hunt et al., 2011). It is thought that bacterial competition for nutrients or production of bacteriocins (toxins produced by bacteria that inhibit the growth of similar or closely related bacterial strains) might reduce or eliminate potential pathogens and prevent or remove subsequent signs and symptoms of mastitis (Heikella & Saris, 2003).

The effects of the composition of the first bacterial colonizers of the newborn gut are not confined to the newborn period, but rather endure well into adulthood. If intestinal flora develop on an alternate trajectory as caused by a cesarean delivery and/or feeding with infant formula, the development of the immune system might also be different, leaving it vulnerable to a number of diseases and conditions, including autoimmune disorders. For example, atopic diseases appear more often in infants who have experienced a cesarean delivery compared with those delivered vaginally. One meta-analysis found a 20% increase in the subsequent risk of asthma in children who had been delivered by cesarean section (Thavagnanam, Fleming, Bromley, Shields, & Cardwell, 2008). Cardwell et al. (2008) showed a 20% increase in the risk of childhood type 1 diabetes after cesarean delivery. There is also an increased risk for children born by cesarean delivery to acquire celiac disease (Decker et al., 2010). Cesarean delivery may cause a shift in the gut to a more inflammation-prone environment and an increase in intestinal permeability leading to a higher risk for diseases and conditions caused by inflammatory conditions and pathogenic microorganisms (Decker, Hornef, & Stockinger, 2011). Chronic immune disorders such as asthma, systemic connective tissue disorders, juvenile arthritis, inflammatory bowel diseases, immune deficiencies, and leukemia have all been found to be significantly increased in children delivered by cesarean section (Sevelsted, Stokholm, Bennelykke, & Bisgaard, 2015). The primary gut flora in cesarean-born infants may be disturbed for as long as 6 months after birth (Gronlund, Lehtonen, Eerola, & Kero, 1999). Coinciding with cesarean deliveries is the delayed onset of lactogenesis II (Dewey, 2003; Evans, Evans, Royal, Esterman, & James, 2003; Scott, Binns, & Oddy, 2007), leaving these infants without the early support of breastmilk for the colonization and physiological development of their intestinal flora. Infants at highest risk of colonization by undesirable microbes, or when transfer from maternal sources cannot occur, are cesarean-delivered babies, preterm infants, full-term infants requiring intensive care, or infants separated from their mothers. Infants requiring intensive care acquire intestinal organisms slowly and the establishment of bifidobacterial flora is retarded. Such a delayed bacterial colonization of the gut with a limited number of bacterial species tends to be virulent.

Control and manipulation of the neonatal gut with human milk can be used as a strategy to prevent and treat intestinal diseases (Dai & Walker, 1999). Major ecological disturbances are observed in newborn infants treated with antimicrobial agents. If several infants in a hospital nursery are treated with antibiotics, the intestinal colonization pattern of other infants in the same nursery may be disturbed, with the intestinal microflora returning to normal after several weeks (Tullus & Burman, 1989). One way of minimizing ecological disturbances in the neonatal intensive care unit (NICU) is to provide these infants with fresh breastmilk (Zetterstrom, Bennet, & Nord, 1994). Infants treated with a broad-spectrum antibiotic during the first 4 days of life show reduced colonization of the gut with *Bifidobacterium* and unusual colonization of *Enterococcus* in the first week compared with infants who have not

been treated with antibiotics. Overgrowth of enterococci and arrested growth of *Bifidobacterium* occurred in antibiotic-treated infants (Tanaka, Kobayashi, et al., 2009). At 1 month of age, infants treated with antibiotics had a higher intestinal population of Enterobacteriaceae than untreated infants. Infants of mothers who had received a broad-spectrum antibiotic prior to cesarean delivery showed weaker but similar gut alterations.

Breastfed and formula-fed infants have different gut flora (Mountzouris, McCartney, & Gibson, 2002). Breastfed infants have a lower gut pH (acidic environment) of approximately 5.1–5.4 throughout the first 6 weeks, which is dominated by *Bifidobacterium* with reduced pathogenic (disease-causing) microbes such as *Escherichia coli*, *Bacteroides*, *Clostridia*, and streptococci. Flora with a diet-dependent pattern are present from the 4th day of life, with breastmilk-fed guts showing a 47% *Bifidobacterium* level and formula-fed guts showing a 15% level. In comparison, enterococci prevail in formula-fed infants (Rubaltelli, Biadaioli, Pecile, & Nicoletti, 1998). Infants fed formula have a high gut pH of approximately 5.9–7.3 characterized by a variety of putrefactive bacterial species. In infants fed breastmilk and formula supplements, the mean pH is approximately 5.7–6.0 during the first 4 weeks after birth, falling to 5.45 by the 6th week. Supplementation with formula induces a rapid shift in the bacterial pattern of a breastfed infant. The dominance of bifidobacteria during exclusive breastfeeding decreases when infant formula is added to the diet (Favier, Vaughan, De Vos, & Akkermans, 2002). When formula supplements are given to breastfed infants during the first 7 days of life, the production of a strongly acidic environment is delayed and its full potential may never be reached. Breastfed infants who receive formula supplements develop gut flora and behavior like those of formula-fed infants. This effect can be seen well beyond the early days. The infant intestinal microbiome at 6 weeks of age is significantly associated with both delivery mode and feeding method. The supplementation of breastfed infants with infant formula is associated with a gut microbiome composition at 6 weeks, which resembles that of infants who are exclusively formula-fed (Madan et al., 2016). This immediately increases the risk of gut inflammation and disease during a very vulnerable period of time. Another bacterial group found in breastfed infants that is almost as widespread as bifidobacteria is the genus *Ruminococcus* (Morelli, 2008). *Ruminococcus* has a protective function because it produces ruminococcin, which inhibits the development of many of the pathological species of *Clostridium* (Dabard et al., 2001). One notable difference between the microflora of breastfed and formula-fed infants is the low presence of clostridia in breastfed infants as compared with formula-fed infants. New molecular biology techniques have detected the presence of the genus *Desulfovibrio* mainly in formula-fed infants (Hopkins, Macfarlane, Furrie, Fite, & Macfarlane, 2005; Stewart, Chadwick, & Murray, 2006). These organisms have been linked with the development of inflammatory bowel disease.

Free fatty acids created during the digestion of infant formula (but not breastmilk) have been shown to cause cellular death that may contribute to NEC in preterm infants. NEC is much more likely to develop in preterm infants who are fed formula. Penn et al. (2012) "digested" infant formulas and breastmilk in vitro and tested for free fatty acids and whether these fatty acids killed off three types of cells involved in NEC: epithelial cells that line the intestine, endothelial cells that line blood vessels, and neutrophils that respond to inflammation. The digestion of formula lead to cell death in less than 5 minutes in some cases, while breastmilk did not. Digestion of infant formula caused death in 47% to 99% of neutrophils while only 6% of them died as a result of breastmilk digestion. This overwhelming cytotoxicity of infant

formula should signal clinicians that every effort should be made for breastmilk to be fed to all infants, but especially preterm infants.

Breastmilk can contain up to 10^9 microbes/L in healthy mothers (West, Hewitt, & Murphy, 1979). Breastmilk from overweight mothers or those who put on more weight than recommended during pregnancy has been found to contain fewer species of bacteria, and mothers who had a planned cesarean delivery have been noted to have fewer species of bacteria in their breastmilk than those who had a vaginal birth (Cabrera-Rubio et al., 2012). The diversity of bacteria in a mother's milk can thus be affected by a number of factors that influence the initial colonization of the infant's gut. *Weisella*, *Leuconostoc*, *Staphylococcus*, *Streptococcus*, and *Lactococcus* were predominant in colostrum samples in the Cabrera-Rubio study, whereas in 1- and 6-month milk samples, the typical inhabitants of the oral cavity (e.g., *Veillonella*, *Leptotrichia*, and *Prevotella*) increased significantly. Frequently encountered bacterial groups in human milk also include staphylococci, streptococci, corynebacteria, lactobacilli, micrococci, propionibacteria, and bifidobacteria. These bacteria can originate from the maternal nipple and areola, the surrounding skin, as well as perhaps the milk ducts within the breast. The mother's nipple, areola, and surrounding skin and the infant's oral cavity represent their own ecological niche, with breastmilk being a relevant source of lactobacilli for the newborn. Allergic mothers have significantly lower amounts of bifidobacteria in their breastmilk compared with nonallergic mothers, which can alter the infant's intestinal microbiota in an infant already at a higher risk for the development of allergies (Gronlund et al., 2007).

Differences in intestinal microbiota may precede the development of overweight and obesity, as data are accumulating that implicate systemic low-grade inflammation and local gut microbiota as contributing factors to overnutrition (Backhed et al., 2004; Fantuzzi, 2005). High levels of *Bacteroides* in the gut microbiota in animal models were shown to predispose toward increased energy storage and obesity (Backhed et al., 2004; Ley et al., 2005). Alteration of gut microbiota in infants during a critical developmental window has been linked to a number of inflammatory conditions (Kalliomaki et al., 2001), creating an environment ripe with the potential for the acquisition of health challenges that have inflammatory origins. Kalliomaki, Collado, Salminen, and Isolauri (2008) demonstrated that bifidobacterial numbers were higher in infancy and *Staphylococcus* aureus was lower in infancy for children remaining at normal weight at 7 years than in children developing overweight. This finding implies that high numbers of bifidobacteria and low numbers of *S. aureus* during infancy as seen in breastfed infants may confer a degree of protection against overweight and obesity. Because adiposity is characterized by low-grade inflammation, the provision of breastmilk with its control of inflammatory pathways contributes to the protection of infants from the development of overweight and obesity. Infant formula has a different effect on the architecture, hydrolysis, and absorption functions in the intestine compared with breastmilk. Infant formula has a trophic or accelerated growth effect on the intestine with gut hypertrophy and acceleration of hydrolytic capacities. This may happen as a result of an adaptive reaction of the intestine to match the nutrient composition of formulas with their high protein content. The result appears as a higher absorption rate of nutrients in formula-fed infants compared to those fed breastmilk for the same food intake (Le Huerou-Luron, Blat, & Boudry, 2010). It could be speculated that this effect also could prime the body for overweight or obesity.

Food intolerances during infancy are common and thought to be related to the failure of adequately developed tolerance to antigens (Field, 2005). The incidence of cow's milk allergy in early childhood is

approximately 2–3% in developed countries. Clinical reactions to cow's milk protein in breastmilk have been reported in 0.5% of breastfed infants. Tolerance contributes to reduced incidences of food-related allergies in breastfed infants (van Odijk et al., 2003) as a result of an active process whereby dietary antigens present in breastmilk combine with immunosuppressive cytokines to induce tolerance to dietary and microflora antigens (Brandtzaeg, 2003). Breastmilk contains components that significantly affect the efficiency of the induction of immune tolerance. For example, transforming growth factor beta (TGF-β) is a growth factor that helps inhibit inflammation and promotes T-cell tolerance. Neonates have low levels of TGF-β in the intestine; the high amounts of this factor in breastmilk compensate for this temporary deficiency. The amount of TGF-β in breastmilk is inversely correlated with the risk of allergy development in breastfed children. Specific deviations of the gut flora such as atypical composition and decreased numbers of bifidobacteria (Kalliomaki et al., 2001) can predispose infants to allergic disease (Salminen, Gueimonde, & Isolauri, 2005), inflammatory gut disease, and rotavirus diarrhea (Lee & Puong, 2002).

Immune physiology has been shown to differ between breastfed and formula-fed infants. In a study looking at key cytokines (cell messengers and regulators of inflammatory responses) and antibody-secreting cells in breastfed and formula-fed infants, Kainonen, Rautava, & Isolauri (2013) found that anti-inflammatory cytokine levels were significantly higher in breastfed infants compared to formula-fed babies. Pro-inflammatory cytokines (TNF-alpha and IL-2) were significantly higher in formula-fed infants, with elevated concentrations of these seen throughout the first year of life. TNF-alpha has the ability to disrupt mucosal barrier function. In breastfed infants, the anti-inflammatory TGF-beta2 was significantly higher, which modulates immune response and enhances mucosal barrier function by inducing IgA production. This type of immune physiology in the breastfed infants contributes to the reduced risk of atopic disease and healthy immune function.

Infants have a functionally immature and immunonaïve gut at birth. The tight junctions of the GI mucosa take many weeks to mature and close the gut to whole proteins and pathogens. Intestinal permeability decreases faster in breastfed infants than in formula-fed infants (Catassi, Bonucci, Coppa, Carlucci, & Giorgi, 1995), with human milk accelerating the maturation of the gut barrier function while formula does not (Newburg & Walker, 2007). The open junctions and immaturity of the GI tract's mucosal barrier play a role in the acquisition of NEC, diarrheal disease, and allergy. Preterm infants experience a high risk for acquiring NEC due to their lower gastric acid production, reduced ability to break down toxins, and low levels of sIgA, which increases bacterial adherence to the intestinal mucosa. Preterm infants cannot fully digest carbohydrates and proteins. Undigested casein, the protein in infant formula, can function as a chemoattractant for neutrophils, exacerbating the inflammatory response and opening the tight junctions between intestinal epithelial cells, disrupting the integrity of the epithelium barrier, and allowing the delivery of whole bacteria, endotoxin, and viruses directly into the bloodstream (Claud & Walker, 2001). Feeding preterm infants with infant formula may produce colonization of the intestine with pathogenic bacteria, resulting in an exaggerated inflammatory response. The sIgA from colostrum, transitional milk, and mature milk coats the gut, preventing attachment and invasion of pathogens by competitively binding and neutralizing bacterial antigens. This passively provides immunity during a time of reduced neonatal gut immune function. Mothers' milk sIgA is antigen specific; that is, the antibodies are targeted against pathogens in the infant's immediate surroundings. The mother synthesizes antibodies when she ingests, inhales, or otherwise comes in contact with disease-causing

microbes. When the mother is exposed to a pathogen, M cells of the Peyer's patch in her gut-associated lymphoid tissue or tracheobronchial tree mucosa acquire the pathogen, after which the M cell presents its antigen to the B cell. The B cell migrates to the mammary epithelial cell, which secretes IgA with the antibody to the particular pathogen. The IgA enters the breastmilk and is consumed by the infant. The sIgA binds the pathogen in the infant's intestine, inhibiting its ability to infect the infant. These antibodies ignore useful bacteria normally found in the gut and ward off disease without causing inflammation.

It is important to keep the mother and her newborn baby together during their hospital stay. This practice allows the mother to enrich her milk with antibodies against bacteria and viruses to which both she and her baby are exposed. Separating mother and baby interferes with this disease defense mechanism. Feeding artificial baby milk to a newborn infant removes this protection.

The prudent clinician can avoid giving a baby infant formula in the hospital or before gut closure occurs. Once dietary supplementation begins, the bacterial profile of breastfed infants resembles that of formula-fed infants; namely, bifidobacteria are no longer dominant and obligate anaerobic bacterial populations develop (Mackie, Sghir, & Gaskins, 1999). Breastmilk ingestion creates and maintains a low intestinal pH and a microflora in which bifidobacteria are predominant and Gram-negative enteric organisms are almost completely absent. Relatively small amounts of formula supplementation of breastfed infants (1 supplement per 24 hours) result in shifts from a breastfed to a formula-fed gut flora pattern (Bullen, Tearle, & Stewart, 1977). With the introduction of supplementary formula, the flora becomes almost indistinguishable from normal adult flora within 24 hours (Gerstley, Howell, & Nagel, 1932). If breastmilk were again given exclusively, it would take 2–4 weeks for the intestinal environment to return to a state favoring the Gram-positive flora (Brown & Bosworth, 1922; Gerstley et al., 1932). Interestingly, optimal microflora in the infant might have long-term benefits if the flora of the adult is determined by events occurring in the critical period of gut colonization (Edwards & Parrett, 2002).

Other events and exposures that occur during the critical window of immune system development may combine to increase the risk and incidence of allergic disease later in life, such as cesarean delivery, prolonged labor, and infant multivitamin supplementation (Milner & Gergen, 2005). A higher incidence of atopy and allergic rhinitis was observed in adults who had received vitamin D supplementation during their first year of life (Hypponen et al., 2004). These data provide additional support for the importance of exclusive breastfeeding (Host & Halken, 2005) during the first half year of life and the avoidance of adding solid foods, infant formula, additives, supplements, or beverages to an infant's diet before maturation of the gut.

There is a strong relationship between allergic diseases and genetic and environmental factors. Differences in immune function are evident at birth, leading to the concept that prenatal factors such as maternal microbial exposure, diet, and pollutants such as cigarette smoke can modify early immune gene expression through heritable changes in genetic makeup. Certain maternal exposures may disrupt normal gene activation or silencing patterns required for normal newborn immune responses (Prescott & Nowak-Wegrzyn, 2011). The first month of life is a period of rapid maturation of the neonatal immune system and offers a window of opportunity for interventions aimed at prevention of allergy. Innate immune responses are markedly different between neonates who are exclusively breastfed during the first month of life and those who are not. Breastmilk-mediated modulation of the developing innate immune system programs for protection from subsequent disease, asthma, and atopy (Belderbos et al., 2012).

It is thought that initial sensitization to food allergens in the exclusively breastfed infant may occur from external sources such as a single feeding of infant formula. In susceptible families, breastfed infants can be sensitized to cow's milk protein by the giving of "just one bottle" (inadvertent supplementation, unnecessary supplementation, or planned supplementation) in the newborn nursery during the first 3 days of life (Cantani & Micera, 2005; Host, 1991; Host, Husby, & Osterballe, 1988). As early as 1935, Ratner recommended that isolated exposure to cow's milk be avoided in infants fed breastmilk (Ratner, 1935). Small doses of allergens can serve to sensitize an infant to subsequent challenges compared with large doses, which induce tolerance. Infants' risk of developing atopic disease has been calculated as 37% if one parent has atopic disease and as 62–85% if both parents are affected, dependent on whether the parents have similar or dissimilar clinical disease. Those infants showing elevated levels of IgE in cord blood irrespective of family history are also considered to be at high risk (Chandra, 2000). The incidence of cow's milk protein allergy is lower in exclusively breastfed infants compared with formula-fed or mixed-fed infants, with about 0.5% of exclusively breastfed infants showing reproducible clinical reactions to cow's milk protein (Vandenplas et al., 2007). In breastfed infants at risk, exclusive breastfeeding for at least 4 months or breastfeeding with hypoallergenic formulas if medically needed to supplement breastfeeding decreases the risk of atopic dermatitis (Greer, Sicherer, & Burks, 2008).

If atopic disease associated with cow's milk allergy occurs, partially hydrolyzed formula is not recommended because it contains potentially allergenic cow's milk peptides. Different hydrolysates have differing effects on atopic disease. Extensively hydrolyzed casein-based formula may be more advantageous if needed as a supplement for infants at risk for allergy development (von Berg et al., 2003). Miniello et al. (2008) recommend that if supplemental formula feeding is needed, infants from atopic families should be supplemented with a hydrolyzed infant formula for the first 6 months of life. High-risk infants without a history of eczema in a primary relative may receive a protective effect from less expensive partially hydrolyzed formula. Those infants who have first-degree relatives with eczema should receive an extensively hydrolyzed formula. Soy formula is not recommended for the prevention of atopy in infants at high risk of developing allergy (Osborn & Sinn, 2006). Rozenfeld, Docena, Añón, and Fossati (2002) demonstrated that a monoclonal antibody specific to casein (a bovine milk protein) displayed affinity to a component of glycinin, an ingredient in soy-based formulas.

Cross-sensitization between protein sources is well established. Among infants with cow's milk protein allergy, 13–20% have allergies to beef (Martelli, De Chiara, Corvo, Restani, & Fiocchi, 2002). Solid foods should not be introduced until 6 months of age; the introduction of dairy products should be delayed until 1 year of age; and the mother should consider eliminating peanuts, tree nuts, cow's milk, eggs, and fish from her diet (American Academy of Pediatrics [AAP], Committee on Nutrition, 2000; Zeiger, 1999). A 7-day washout of milk proteins is required when instituting a restricted diet, delaying the expected clinical response by the infant (Brill, 2008). A maternal elimination diet may also need to include the elimination of beef and may need to be continued for at least 2 weeks, and up to 4 weeks in cases of atopic dermatitis or allergic colitis. If symptoms improve or disappear during the elimination diet, one food per week can be reintroduced to the mother's diet. If symptoms do not reappear upon reintroduction of a particular food, the mother should begin consuming it again. If symptoms reappear, it should continue to be eliminated from her diet during the course of breastfeeding.

In susceptible families, early exposure to cow's milk proteins or the absence of breastfeeding can increase the risk of the infant or child developing insulin-dependent diabetes mellitus (type 1, or IDDM) (Karjalainen et al., 1992; Mayer et al., 1988) and type 2 diabetes mellitus (Young et al., 2002). Type 1 diabetes is one of the most common chronic diseases in childhood. It results from the autoimmune destruction of the insulin-producing beta cells in the pancreas following a variable subclinical length of time where autoantibodies against the beta cells antigens are present. Breastfeeding for 12 months or longer predicts a lower risk of type 1 diabetes as well as a lower risk of progression from islet autoimmunity to type 1 diabetes in susceptible infants (Lund-Blix et al., 2015).

The human insulin content in breastmilk is significantly higher than the content of bovine insulin in cow's milk. Insulin content in infant formulas is extremely low to absent. Insulin supports gut maturation. In animal models, oral administration of human insulin stimulates the intestinal immune system, thereby generating active cellular mechanisms that suppress the development of autoimmune diabetes. The lack of human insulin in infant formulas may break the tolerance to insulin and lead to the development of type 1 diabetes (Vaarala, Paronen, Otokoski, & Akerblom, 1998). The avoidance of cow's milk protein during the first several months of life may reduce the later development of IDDM or delay its onset in susceptible individuals (AAP, Work Group on Cow's Milk Protein and Diabetes Mellitus, 1994). Infants who are exclusively breastfed for at least 4 months have a lower risk of seroconversion leading to beta-cell autoimmunity. Short-term breastfeeding (less than 2–3 months) and the early introduction of cow's milk-based infant formula may predispose young children who are genetically susceptible to type 1 diabetes to progressive signs of beta-cell autoimmunity (Kimpimaki et al., 2001). Holmberg and coworkers (2007) concluded that positivity for beta-cell autoantibodies in children from the general population was associated with a short duration of both total and exclusive breastfeeding as well as an early introduction of formula. Sensitization and development of immune memory to cow's milk protein is the initial step in the etiology of IDDM (Kostraba et al., 1993). Sensitization can occur with very early exposure to cow's milk before gut cellular tight junction closure takes place. It can also occur with exposure to cow's milk during an infection-caused GI alteration when the mucosal barrier becomes compromised, allowing antigens to cross and initiate immune reactions. Sensitization can take place if the presence of cow's milk protein in the gut damages the mucosal barrier, inflames the gut, or destroys binding components of cellular junctions or if another early insult with cow's milk protein leads to sensitization (Savilahti, Tuomilehto, Saukkonen, Virtala, & Akerblom, 1993). Of further importance is the fact that exposure to infant cereal during the first 3 months of life in genetically predisposed infants significantly increases the risk of developing diabetes (Norris et al., 2003; Ziegler, Schmid, Huber, Hummel, & Bonifacio, 2003).

The IgG immune complexes found in breastmilk function as potent inducers of tolerance to airborne aerosolized antigens to which the mother has been sensitized, providing antigen-specific protection from asthma in the infant (Mosconi et al., 2010). Silvers and colleagues (2012) analyzed 1,105 infants over 6 years focusing on breastfeeding, wheezing, and asthma. Each month of exclusive breastfeeding was associated with significant reductions in current asthma from 2 to 6 years, as was any amount of breastfeeding. The protective effect of breastfeeding against asthma was of even more importance in atopic children, in whom exclusive breastfeeding for 3 or more months reduced asthma at ages 4, 5, and 6 years by 62%, 55%, and 59%, respectively. Dogaru and colleagues (2012) found that breastfeeding had a positive

Table 1-1 Correlation of Number of Feedings in First 24 Hours and Bilirubin Levels

Number of Feedings	Bilirubin Levels at 6 Days of Age
4 times in first 24 h	26% with elevated bilirubin levels on day 6 (12–14 mg/dL)
7–8 times in first 24 h	12% with elevated bilirubin levels on day 6
> 9 times in first 24 h	None with elevated bilirubin levels on day 6

Data from Yamauchi, Y., & Yamanouchi, H. (1990). Breastfeeding frequency during the first 24 hours after birth in fullterm neonates. *Pediatrics*, 86, 171–175.

effect on lung function in school-aged children. There was no detrimental effect of breastfeeding on children whose mothers had asthma. In fact, children of asthmatic mothers had better lung function if they had been breastfed, with a dose–response relationship with the duration of breastfeeding. Breastfeeding may have a direct positive effect on lung growth.

Avoid giving the infant extra formula, water, or sugar water in an attempt to influence bilirubin levels. In the hospital it is important to ensure 8–12 feedings each 24 hours. Bilirubin levels correlate inversely with the number of feedings during the first 24 hours (**Table 1-1**) (Yamauchi & Yamanouchi, 1990). Bilirubin levels also correlate inversely with the number of feedings over the first 3 days of life (DeCarvalho, Klaus, & Merkatz, 1982), as in the following examples:

- If the average number of feedings per day is 6, day 3 bilirubin levels would be at 11 mg/dL.
- If the average number of feedings per day is 6.8, day 3 bilirubin levels would be at 9.3 $\pm$ 3.5 mg/dL.
- If the average number of feedings per day is 10.1, day 3 bilirubin levels would be at 6.5 $\pm$ 4.0 mg/dL.
- If the average number of feedings per day is 11, day 3 bilirubin levels would be at 5 mg/dL.

Further, bilirubin levels correlate inversely with the amount of water or glucose water given to breastfed newborns (Nicoll, Ginsburg, & Tripp, 1982). The more water or sugar water given to breastfed infants, the higher the bilirubin levels on day 3. Bilirubin functions as an antioxidant to protect cell membranes. Breastfed infants have higher levels of bilirubin than formula-fed infants because they are supposed to. Artificially lowering normally elevated bilirubin levels when feeding babies infant formula has not been shown to be beneficial.

NUTRITIONAL COMPONENTS

Water

Water makes up the majority (87.5%) of human milk. All other components are dissolved, dispersed, or suspended in water. An infant receiving adequate amounts of breastmilk will automatically consume his or her entire water requirement. Even in hot arid or humid climates, human milk provides 100% of water needs (Ashraf, Jalil, Aperia, & Lindblad, 1993).

Clinical Implications

Because human milk with its low solute load provides all the water an infant needs, breastfed infants do not require additional water. Consuming more water than needed can suppress the infant's appetite (especially if the water contains dextrose) and reduce the number of calories the infant receives, placing

him or her at risk for hyperbilirubinemia and early weight loss. Sterile water has no calories; 5% dextrose water has 5 calories per ounce, whereas colostrum has 18 calories per ounce. An infant receiving an ounce of sugar water in place of an ounce of colostrum will experience a two-thirds deficit in calories.

Large amounts of low-solute water given to an infant over a short period of time can contribute to oral water intoxication, swelling of the brain, and seizures (Keating, Shears, & Dodge, 1991). Infants under one month of age have a lower glomerular filtration rate and cannot excrete a water load rapidly, making them more susceptible to oral water intoxication when given large water supplements. Oral water intoxication is more commonly seen in formula-fed infants whose caregivers use water bottles to extend the time between feedings or dilute formula supplies to make them last longer. This condition, however, can also occur in breastfed infants. A combination of factors can place the infant at risk for water intoxication, such as administration of large amounts of hypotonic intravenous (IV) solutions to laboring mothers (Tarnow-Mordi, Shaw, Liu, Gardner, & Flynn, 1981), addition of oxytocin by IV (Singhi, Chookang, Hall, & Kalghangi, 1985), and a large oral intake of fluid during labor (Johansson, Lindow, Kapadia, & Norman, 2002). A fluid shift to the fetus plus the birth-related surge in circulating vasopressin (the antidiuretic hormone) in the infant (Leung et al., 1980) can contribute to a water-sparing reaction or water retention in the infant. Excessive water in the infant can artificially inflate the birth weight, causing undue concern about large weight losses as the infant experiences diuresis or eliminates this excess water.

The mistaken belief that breastfed infants need supplemental water to prevent dehydration, hyperbilirubinemia, hypoglycemia, and weight loss disrupts breastfeeding, and the water is often offered simply for convenience (Williams, 2006). Glover and Sandilands (1990) reported that infants who received glucose water supplementation in the hospital lost more weight and stayed in hospital longer than infants who did not receive supplementation. Ruth-Sanchez and Greene (1997) described a 3-day-old breastfed infant who was given 675 mL (22.8 oz) of dextrose water by nurses and the mother in the 24 hours before NICU admission for resulting seizure activity. Infants who are breastfeeding adequately should not be offered additional water no matter what the climate (Almroth & Bidinger, 1990; Cohen, Brown, Rivera, & Dewey, 2000; Sachdev, Krishna, Puri, Satyanarayana, & Kumar, 1991).

Despite data discouraging the practice, giving water or sugar water to breastfed infants persists. In 2007, to characterize maternity practices related to breastfeeding, the CDC (2007) conducted the first national Maternity Practices in Infant Nutrition and Care (mPINC) survey. Survey responses were received from 2,687 hospitals and birthing facilities. When asked whether healthy, full-term, breastfed infants who receive supplements are given glucose water or water, 30% of facilities reported giving feedings of glucose water and 15% reported giving water, practices that are not supportive of breastfeeding. This practice is still prevalent in hospitals, with results from the 2009 mPINC survey showing that 25% of hospitals persist in engaging in the non-evidence-based practice of giving sterile water or glucose water to newborn breastfed infants. The 2013 mPINC survey, however, showed that this practice is now decreasing: An average of 12% of the surveyed hospitals reported giving healthy breastfed infants water or glucose water. Nevertheless, this rate was much higher—more than 20%—in the West North Central and East South Central regions of the United States (Centers for Disease Control and Prevention, 2014b). Data from the Infant Feeding Practices Study II (a survey of mother-reported infant feeding patterns) revealed that 13% of infants received sugar water while in the hospital and 10% of the infants

were receiving plain water at the age of 1 month (Grummer-Strawn, Scanlon, & Fein, 2008), even when findings show that infants who do not consume solid foods have no need of solute-free water (Scariati, Grummer-Strawn, & Fein, 1997).

Mothers with low confidence levels with regard to their ability to breastfeed are vulnerable to the plethora of advice offered to them, even if the advice is misguided or incorrect, such as suggestions to offer supplementary or complementary water feedings (Blyth et al., 2002). If they perceive this advice as an indication of insufficient milk, they are significantly more likely to wean. Early water supplementation is associated with the increased likelihood that water-supplemented infants will receive infant formula during the first month of life. Mixed feedings often herald early weaning from the breast and reduce the disease-protective abilities found with exclusive breastfeeding. Giugliani, Santo, de Oliveira, and Aerts (2008) found that infants who received water or herbal teas in the first 7 days of life were more likely to have infant formula introduced during the first month. Wojcicki and colleagues (2011) reported similar outcomes. Infants who received water or teas in the first 7 days of life were 3 times more likely than other infants to receive non-breastmilk fluids by 4 weeks of age.

Mulder and Gardner (2015) proposed a newborn hydration model for understanding newborn hydration immediately following birth (**Figure 1-2**). It has been common practice to supplement a breastfed infant when newborn weight loss reaches more than 7% of birth weight because that has been the threshold used as an indicator of a water deficit or dehydration. Using only this indicator during the first 24–60 hours following birth does not take into account the normal process of newborn diuresis, especially if the mother has received large amounts of IV fluids during labor. During pregnancy, the mother may retain as much as 6 to 8 liters of water, causing the fetus to be "over-hydrated" because the fetus remains in fluid and electrolyte balance with the mother. Following birth, the infant's high levels of arginine vasopressin (which increases water retention) abruptly decrease, heralding the start of physiological diuresis. This point may be clinically apparent in infants with more than a 7% weight loss when they demonstrate significantly more voids than newborns with smaller losses. Researchers have shown that infants with higher weight losses produced more voids during the early hours and days following birth (Chantry, Nommsen-Rivers, Peerson, Cohen, & Dewey, 2011; Mulder, Johnson, & Baker, 2010). Newborns with

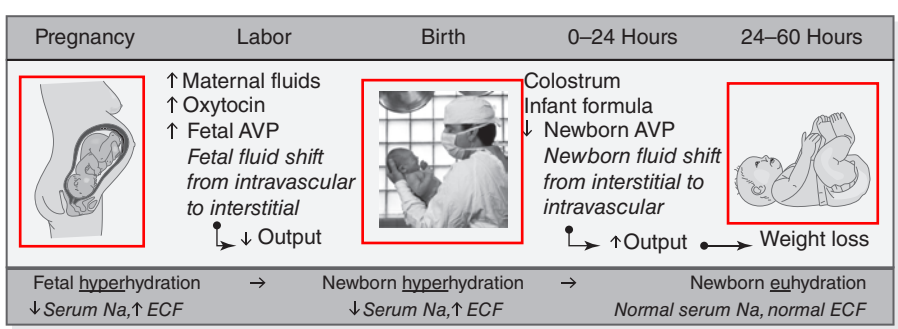

Figure 1-2 Healthy newborn hydration model.

Modified from Mulder, P. J. & Gardner, S. E. (2015). The healthy newborn hydration model: A new model for understanding newborn hydration immediately after birth. *Biological Research for Nursing, 17,* 94–99. First published on April 15, 2014 doi:10.1177/1099800414529362

exposure to high maternal fluid intake during labor may need to lose more than 7% of their birth weight to achieve normal body water content. In contrast, infants with low fluid reserves at birth may encounter under-hydration (even with less than a 7% weight loss), suggesting a better indicator than just percentage of weight loss is needed to determine newborn hydration status.

Mulder and Gardner (2015) suggest using serum sodium measurement along with daily weight loss as a more accurate means of determining hydration status and avoiding the use of supplements when they are not necessary. Serum sodium would be measured in cord blood to establish the baseline hydration status; it would be measured again at 24 hours following birth, with a serum sodium sample being obtained from the heel stick for the metabolic screen. Weight loss patterns should show a greater weight loss in the first 24 hours after birth, followed by a continued weight loss in the second 24 hours, with the lowest point being reached at 3 days. After this time the infant should be gaining weight due to the occurrence of lactogenesis II.

Flaherman and colleagues (2015) analyzed hourly weight-loss patterns of 108,907 healthy, term, exclusively breastfed newborns from 6 to 72 hours of age for vaginally delivered infants and from 6 to 96 hours for those born via cesarean section. Almost 5% of vaginally delivered newborns and almost 10% of those delivered by cesarean section had lost more than 10% of their birth weight by 48 hours after birth. The researchers used their data to create a weight-loss nomogram to help inform clinical care; it is available at http://www.newbornweight.org. However, they did not take the influence of maternal IV fluids during labor into account. Diuresis of large amounts of fluid or large meconium stooling can also result in large weight losses. Other parameters must be considered as well, such as amount of colostrum transferred and number and weight of voids and stools, before a supplementation intervention is undertaken.

Healthcare providers must clearly understand the unwanted outcomes of excessive water supplementation and adequately convey these concerns to parents. An approach to help eliminate water supplementation is as follows:

- Teach and assess proper positioning, latch, and milk transfer.
- Document swallowing and ensure that the mother knows when her baby is swallowing milk.
- Avoid using sterile water or dextrose water. If a breastfed infant requires supplementation, use expressed colostrum/milk.
- Educate the parents and extended family regarding the hazard of giving young breastfed babies bottles of water or other fluids, even in hot weather.
- Avoid placing water bottles in the infant's bassinet in the hospital.
- Remind mothers that babies also nurse at the breast for thirst, frequently coming off the breast after only a few minutes of nursing.
- If a baby is latched but not swallowing adequately, have the mother use alternate massage (massage and compress the breast during pauses between sucking bursts) to sustain sucking and swallowing.
- Maternal consumption of water in excess of thirst does not increase milk production and can cause the mother to produce less milk (Dusdieker, Booth, Stumbo, & Eichenberger, 1985).

Lipids

Milk lipids (among other components) have generated intense interest from numerous studies showing that formula-fed infants and children demonstrate less advanced cognitive development and poorer psychomotor development compared with breastfed children (**Box 1-1**). Guxens and colleagues (2011)

found that duration of exclusive breastfeeding was associated with an increase of 0.37 points on mental development scores per month of exclusive breastfeeding. Higher levels of docosahexaenoic acid (DHA) and n-3 polyunsaturated fatty acids and higher ratios between n-3/n-6 polyunsaturated fatty acids in colostrum and breastmilk were associated with higher infant mental scores. Infants whose mothers had high levels of total n-3 polyunsaturated fatty acids such as DHA and a longer duration of breastfeeding had significantly higher mental scores than infants whose mothers had lower amounts of these fatty acids and shorter durations of breastfeeding. Each month of breastfeeding has been associated with an increase of 0.16 IQ points (Kanazawa, 2015). Gustafsson, Duchen, Birberg, and Karlsson (2004) found that colostrum levels of long-chain polyunsaturated fatty acids (LCPUFAs) were significantly associated with cognitive development at 6.5 years. A number of components found in human milk that are absent from unsupplemented formulas are thought to contribute to cognitive deficits seen in non-breastfed infants, including particular LCPUFAs.

Box 1-1 Artificially Fed Infants Demonstrate Different Neurodevelopment and Cognitive Outcomes

- A different brain composition than breastfed infants (Uauy, 1990)
- Reduced concentrations of brain sialic acid, leading to potential deficits in neurodevelopment and cognition (Wang, McVeagh, Petocz, & Brand-Miller, 2003)
- Poorer neurobehavioral organization at 1 week of age (Hart, Boylan, Carroll, Musick, & Lampe, 2003)
- Less mature brain development within the first 2 months of life (Herba et al., 2012)
- Lower neurodevelopmental response at 4 months of age (Agostoni, Trojan, Bellu, Riva, & Giovannini, 1995)
- Lower cognitive development observed from 6 months through 16 years (Anderson, Johnstone, & Remley, 1999)
- Lower mental development and psychomotor development scores at 12 months (Agostoni, Marangoni, Giovannini, Galli, & Riva, 2001)
- Lower probability of scoring in the upper quartile for the Mental Development Index and psychomotor Index on the Bayley Scales of Infant Development (Andres et al., 2012)
- Less mature nervous systems at 1 year of age and attainment of near-adult values of central and peripheral conduction later than breastfed infants (Khedr, Farghaly, El-Din Amry, & Osman, 2004)
- Lower mental development scores at 18 months (Florey, Leech, & Blackhall, 1995)
- Poorer developmental outcome of very-low-birth-weight infants at 18 months of age (Vohr et al., 2006)
- Lower mental development scores at 2 years of age (Morrow-Tlucak, Haude, & Ernhart, 1988)
- Lower cognitive development at 3 years of age (Bauer, Ewald, Hoffman, & Dubanoski, 1991; Johnson & Swank, 1996)
- Lower cognitive function of very-low-birth-weight infants at 5 years of age (Tanaka, Kon, Ohkawa, Yoshikawa, & Shimizu, 2009)
- Less likely to have achieved a good level of overall educational achievement at age 5 years (Heikkila, Kelly, Renfrew, Sacker, & Quigley, 2014)

- Lower IQ scores over the entire preschool period of time (Jedrychowski et al., 2012)
- Lower IQ scores at 6.5 years (Kramer et al., 2008)
- Lower IQ scores at 7 years (Lucas, Morley, Cole, Lister, & Leeson-Payne, 1992)
- Twice the rate of minor neurological dysfunction at 9 years (Lanting, Fidler, Huisman, Touwen, & Boersma, 1994)
- Significantly lower test scores in reading and mathematics in 9-year-old children (McCrory & Layte, 2011)
- Lower IQ scores at 11–16 years (Greene, Lucas, Livingstone, Harland, & Baker, 1995)
- Lower IQ scores and lower attainment in school at 18 years (Horwood & Fergusson, 1998)
- Lower IQ scores, educational attainment, and income at age 30 years (Victora et al., 2015)
- Lower cognitive development when born small for gestational age (Rao, Hediger, Levine, Naficy, & Vik, 2002; Slykerman et al., 2005)
- Increased risk for specific language impairment (Tomblin, Smith, & Zhang, 1997)
- Half the DHA as in the brain of a breastfed infant (Cunnane, Francescutti, Brenna, & Crawford, 2000)
- Significantly lower DHA in the gray and white matter of the cerebellum (coordinates movement and balance) (Jamieson et al., 1999)
- Slower brainstem maturation in preterm infants (Amin, Merle, Orlando, Dalzell, & Guillet, 2000)
- Increased abnormalities in neurobehaviors of preterm infants (Brown, Doyle, Bear, & Inder, 2006)
- Poorer stereoacuity at 3.5 years and at 6 years of age (Singhal et al., 2007; Williams, Birch, Emmett, Northstone, & Avon Longitudinal Study of Pregnancy and Childhood Study Team, 2001)
- Suboptimal quality of general movements that correlate with poorer neurobehavioral condition of children at school age (Bouwstra et al., 2003)
- Increased gross motor delay (Sacker, Quigley, & Kelly, 2006)
- Poorer speech processing with the potential for less optimal linguistic and cognitive development (Ferguson & Molfese, 2007)
- Lower IQ scores in young adults (Mortensen, Michaelsen, Sanders, & Reinisch, 2002)
- Lower general intelligence and cortical thickness in adolescents who had been formula fed (Kafouri et al., 2012)
- Lower child development test scores; subsequent schooling and other experiences during adolescence did not eliminate the breastfeeding gap that appeared in very early childhood (Huang, Peters, Vaughn, & Witko, 2014)

Many classes of lipids and thousands of subclasses exist. The fat content of human milk varies widely, ranging from 3.5% to 4.5% (2–9 g of lipids per 100 mL). It is influenced by a number of factors (**Table 1-2**).

Lipids provide a well-tolerated energy source, contributing approximately 50% of the calories in milk. They provide essential fatty acids, lipid-soluble vitamins, and cholesterol. The milk fat is formed from circulating lipids that are derived from the maternal diet and from maternal body stores. Maternal body fat stores with a relatively slow turnover contribute greatly to the formation of human milk lipids. Short-term variations in dietary fat composition and consumption are somewhat buffered metabolically by maternal fat stores, resulting in a fairly constant LCPUFA content in the milk (Koletzko et al., 2001).

Table 1-2 Factors Influencing Human Milk Fat Content and Composition

Factor	Influence
During a feeding	Rises over the course of a feeding. This was further explained when the fat content of the milk was measured before and after every feed for 24 hours. Rather than fat content being related to the presence of foremilk or hindmilk, the fat content was related to the degree of fullness of the breast. As the breast is progressively drained, the fat content in the milk increases (Daly, Di Rosso, Owens, & Hartmann, 1993).
Volume	Lower milk fat content with higher volumes of milk.
Number of days postpartum	Phospholipid and cholesterol levels are highest in early lactation.
Diurnal rhythm	Varies.
Length of gestation	LCPUFA secretion increases with shortening length of gestation.
Parity	Endogenous fatty acid synthesis decreases with increased parity.
Maternal diet	Can change the LCPUFA profile as well as medium-chain fatty acids (increases with a low-fat diet).
Length of time between feeds	The shorter the interval, the higher the fat concentration.
Maternal energy status	A high weight gain in pregnancy is associated with increased milk fat.
Maternal age	Fat content in colostrum is higher in mothers older than 35 years of age (Lubetzky, Sever, Mimouni, & Mandel, 2015).
Method of milk expression	Manually expressed milk has a higher fat content than milk expressed by an electric pump during the first 72 hours postpartum (Mangel et al., 2015).
Smoking	Active maternal smoking decreases the fat content. Passive smoking (second-hand smoke) exposure reduces milk–lipid profiles (Baheiraei et al., 2014).

Data from Picciano, M. F. (2001). Nutrient composition of human milk. *Pediatric Clinics of North America, 48*, 53–67.

The long-term diet of the mother thus influences milk fat composition. Milk phospholipids contribute to the lipid composition of human milk. Among the several classes of sphingo- and glycolipids are gangliosides, which contribute to the host defense by binding bacterial toxins. Triacylglycerols account for more than 98% of the lipids in milk. The composition of triacylglycerols is usually shown in terms of the kinds and amounts of fatty acids. A shorthand notation is commonly used when discussing fatty acids. The chemical formula is abbreviated by stating the number of carbons to the left of the colon and the number of double bonds to the right of the colon:

- 16:0 palmitic acid
- 18:2 linoleic acid
- 20:4 arachidonic acid (AA)
- 22:6 docosahexanoic acid (DHA)

Unlike breastmilk, unsupplemented infant formula does not contain the LCPUFAs DHA and AA. These two fatty acids are found in abundance as structural lipids in the infant's brain, retina, and central nervous system. Because the animal butterfat of cow's milk formula is replaced with plant oils, human milk and formula have quite different fatty acid profiles. Infant formulas typically contain soy oil, corn oil, sunflower oil, and tropical oils such as palm and coconut oils; these oils may be well absorbed but are not used by the brain in the same way LCPUFAs are from human milk.

Concentrations of DHA and AA in human milk are highly variable and depend on the amount of these preformed fatty acids in the mother's diet and their biosynthesis from precursors (Brenna et al., 2007). Higher DHA values have been identified in preterm mothers' milk compared with those found in term human milk, underscoring the importance of using the mother's own milk to feed her preterm infant (Bokor, Koletzko, & Decsi, 2007). Because there appears to be a higher concentration of DHA in preterm milk, preterm infant formula supplemented with DHA may not contain high enough levels compared with preterm human milk. Parity has an influence on milk lipid concentration, with milk lipid content increasing with subsequent infants at least up to the third delivery (Bachour, Yafawi, Jaber, Choueiri, & Abdel-Razzak, 2012). Genetic polymorphisms (natural variations in a gene, DNA sequence, or chromosome) influence the activity of enzymes involved in the metabolism of polyunsaturated fatty acids (PUFAs) in both the mother and the infant (Glaser, Heinrich, & Koletzko, 2010). For example, the genes FADS1 and FADS2 play an important role in determining the PUFA levels in breastmilk or in the infant's ability to convert precursor fatty acids into their long chain derivatives (Moltó-Puigmartí et al., 2010; Xie & Innis, 2008). This may partially explain the differences in PUFA levels among mothers and the amounts of PUFA ultimately available to their infants. Differences in the fatty acid composition of breastmilk have also been noted in women with eczema and/or respiratory allergy. Johansson, Wold, and Sandberg (2011) reported that lower levels of several PUFAs, including DHA, were seen in the milk of mothers with eczema and/or respiratory allergies, in spite of high amounts of maternal fish intake in the diet. This finding could be the result of dysfunction in the enzymes associated with converting shorter chain fatty acids into longer chain PUFAs. Lower levels of the n-3 fatty acids in allergic women could also be due to the body's enhanced consumption of PUFAs during allergic inflammation within the allergic process.

LCPUFAs have been added to term and preterm formulas in an attempt to provide infants with an exogenous source of these fully formed fatty acids. Infant formulas are supplemented with differing amounts of DHA and AA that are typically based on the average amount found in term human milk. The source of the DHA and AA varies. Infant formulas in the United States use DHA from fermented microalgae (*Crypthecodiunium cohnii*) and AA from soil fungus (*Mortierelle alpina*). These ingredients are new to the food chain and in animal studies showed side effects such as fat loss through stool, oily soft stools (steatorrhea) in acute toxicity tests, higher liver weights in male rats, and increased fetal and neonatal undeveloped renal papilla and dilated renal pelvises (Life Sciences Research Office Report, 1998). Little evidence exists showing that supplementing formula with LCPUFAs confers any significant long-term benefit to term or preterm infants (Life Sciences Research Office Report, 1998; Simmer, 2002a, 2002b). The results of most of the well-conducted, randomized, controlled trials have not shown beneficial effects of LCPUFA supplementation of infant formula on the physical, visual, and neurodevelopmental outcomes of term or preterm infants. Routine supplementation of infant formula with LCPUFA to improve the physical, neurodevelopmental, or visual outcomes of term or preterm infants cannot be recommended based on the current evidence (Simmer, Patole, & Rao, 2008; Simmer, Schulzke, & Patole, 2008).

Because of concerns regarding the safety and effectiveness of the DHA/AA additive, the U.S. Food and Drug Administration (FDA) and Health Canada commissioned the Institute of Medicine to evaluate the process used to determine the safety of new ingredients added to infant formulas. The Institute of Medicine's subsequent report noted a number of shortcomings, including the absence of a structured approach to monitoring side effects after the new formula had been introduced to the market (see the additional reading list at the end of this chapter for information on the full report).

The bioactive fatty acids DHA and AA, when consumed in human milk, are part of a complex matrix of other fatty acids. Important physiological considerations related to this matrix are not accounted for by the simple addition of nonhuman LCPUFAs to infant formula. Many concerns have been raised about these additives (Heird, 1999):

- Supplementation with highly unsaturated oils increases the susceptibility of membranes to oxidant damage and disrupts the antioxidant system. Damage from oxygen radicals can provoke diseases thought to be related to oxidant damage, such as NEC, bronchopulmonary dysplasia, and retrolental fibroplasia (Song, Fujimoto, & Miyazawa, 2000; Song & Miyazawa, 2001). LCPUFA administration has effects on retinol and alpha-tocopherol metabolism (Decsi & Koletzko, 1995).
- Oxidation of PUFAs is enhanced by storage time, temperature, and light exposure. End products of PUFA oxidation occur in large amounts in infant formula, including malondialdehyde (MDA), 4-hydroxyhexanal (4-HHE) specific to the oxidation of DHA, and 4-hydroxynonenal (4-HNE) specific to the oxidation of AA (Genot & Michalski, 2010). Even a few minutes after powdered formula is prepared, substantial amounts of MDA, 4-HHE, and 4-HNE are present, especially in formula enriched with added PUFAs. Human milk is remarkably stable against oxidation. Even though 7 times the amount of vitamin E is found in breastmilk as an antioxidant, infant formula shows extremely high levels of these oxidative end products, especially after open storage (Michalski, Calzada, Makino, Michaud, & Guichardant, 2008). In animal studies, these oxidative end products have been associated with accumulation in the liver and the development of chronic intestinal disorders or cancers. Both 4-HHE and 4-HNE are capable of altering insulin signaling. The question remains as to whether chronic exposure to 4-HHE and 4-HNE from oxidized lipids in infant formula might cause deleterious effects on infant metabolism (Michalski, 2013). Higher levels of PUFAs in muscle cell membranes have been related to increased insulin sensitivity (Pan, Hylbert, & Storlien, 1994).
- There is a possible effect on gene transcription (Clarke & Jump, 1996).
- High-fat supplementation of formula and commercial jarred baby foods has raised the concern that these additives may contribute to the obesity epidemic (Massiera et al., 2003).
- Increasing DHA fortification of commercial baby food adds to the concerns about excessive intake and/or imbalanced ratios of n-6 and n-3 fatty acids.
- Imbalanced ratios of fatty acids can result in altered growth patterns (Carlson, Cooke, Werkman, & Tolley, 1992; Carlson, Werkman, Peeples, Cooke, & Tolley, 1993).
- Many studies have insufficient sample sizes to determine any functional benefit or safety profile; comparison of research results is confounded by the use of different sources of DHA and AA,

different amounts and ratios of these fatty acids, different compositions of the base formulas, and different lengths of time the study formulas were consumed (Koo, 2003).

- Most studies compare supplemented versus unsupplemented formulas to each other but lack a control group of exclusively breastfed infants; many have high attrition rates.
- The accuracy and reliability of the tests used to determine visual and cognitive effects of LCPUFAs during the first 2 years are controversial.
- Enrollment criteria for most studies typically excluded sick infants, twins and higher order multiples, and most infants with any type of problem. This population choice may leave doubts about the suitability of fatty acid–supplemented formula for these infants, regardless of the source of the LCPUFAs. Many of these studies did not include newborn infants or infants during the early days and weeks following birth.
- Meta-analysis of randomized trials suggests that any functional benefit in visual development or neurodevelopment from LCPUFA supplementation of infant formula is likely to be of minor clinical significance, at least for the term infant (Koo, 2003).
- Little evidence indicates that LCPUFA-containing infant formula provides clinically significant improvements in vision and intelligence in healthy term infants. The 25% higher cost can place a significant burden on a family's budget and on public nutrition programs.
- Human milk contains LCPUFAs other than DHA and AA that can be converted to DHA and AA and affects the conversion of alpha-linolenic and linoleic acid to DHA and AA. Their presence may partially explain the apparent need for greater amounts of DHA and AA in formula to achieve the same plasma lipid content of these fatty acids observed in human milk–fed infants (Clandinin et al., 1997).
- Breastmilk contains lipases that enhance fat digestion in breastfed infants; it is a complex matrix, containing numerous bioactive components, hormones, and live cells not found in infant formula. Important physiological considerations relative to the matrix are not accounted for by the simple addition of LCPUFAs to infant formula (Office of Food Additive Safety, 2001).

Clinical Implications

The provision of breastmilk during the period of brain development is important for several reasons:

- IQ studies are remarkably consistent in their demonstration of higher IQ scores that are dose dependent relative to the number of months a child has been breastfed.
- The brain composition of formula-fed infants is measurably and chemically different; namely, DHA levels remain static in formula-fed infants but rise in breastfed infants (Farquharson, Cockburn, Patrick, Jamieson, & Logan, 1992).
- Unsupplemented formula contains no DHA or AA, just their precursors, linolenic and linoleic acid. Infants must rely on an immature liver to synthesize enough of these LCPUFAs to meet the needs of the developing brain.
- Supplemented formulas have unknown side effects:
    1. Fermented microalgae and soil fungus could contain contaminants from the fermentation and oil extraction process, such as hexane residue.

2. Positional distributions of plant-based LCPUFAs on triacylglycerols are different from those of human milk triglycerides. Triacylglycerols or triglycerides are the form in which the body stores fat. They consist of a glycerol spine with three attached fatty acids. The location of the fatty acid on the glycerol spine is identified by stereospecific numbering (sn). Fatty acids are not randomly distributed among the three positions but selectively placed, with human fatty acids having preferences for binding at certain positions. Most saturated fatty acid in human milk is palmitic acid, which is about 20–25% of the total fatty acids in human milk. Palmitic acid is predominantly found in the sn-2 position (Lopez-Lopez, Lopez-Sabater, Campoy-Folgoso, Rivero-Urgell, & Castellote-Bargallo, 2002; Straarup, Lauritzen, Faerk, Høy, & Michaelsen, 2006; Valentine et al., 2010). Palmitic acid in infant formulas, where vegetable oils are the main constituents of infant formula fat, is predominantly found in the sn-1 and sn-3 positions. When palmitic acid is in the sn-2 position, it is well absorbed, but in the sn-1 or sn-3 position, it is released as free fatty acids and forms insoluble soaps that can produce harder stools and constipation in infants.
    a. Infant formula strives to match the overall fatty acid composition of human milk, but it cannot duplicate the triacylglycerol structure that alters lipid metabolism in infants not fed human milk (Nelson & Innis, 1999). Even though the formula is supplemented with DHA and AA, the shape of the molecule is different from that of the DHA and AA found in human milk.
    b. AA and DHA in human milk are present in the sn-1 or sn-2 positions but can be present in all three positions in the single-cell oils (Myher, Kuksis, Geher, Park, & Diersen-Schade, 1996). In human milk, 55% of DHA is found in the sn-2 position.
    c. Human milk triglycerides usually contain no more than one molecule of DHA or AA, whereas some single-cell triglycerides contain two or even three such molecules. Most of the LCPUFAs in formula are located in the outer positions of the triacylglycerol molecule, placing them at potential risk for slow and low absorption (Straarup et al., 2006).
    d. DHA and AA added to infant formula can act differently in the body from human DHA and AA, depending on where and in what proportion they are found on the triglyceride molecule. It is unknown how these differences in molecular shape and triglyceride positioning could affect their metabolism and functioning.

- Fungal sources of AA pose a risk of introducing mycotoxins that could act as opportunistic pathogens in an immunocompromised host.
- Fungal and microalgal oil supplements have been shown to cause a dose-dependent increase in excess gas and belching in adults (Innis & Hansen, 1996).
- The National Alliance for Breastfeeding Advocacy has received numerous complaints of infants experiencing watery explosive diarrhea, diaper rash, excessive foul-smelling gas, and abdominal cramping from ingesting infant formula with the highest levels of LCPUFA supplementation. Also reported was obesity in 6-month-old infants who initially breastfed but had DHA/AA-supplemented formula added to their diet as a supplement, followed by infant cereal at 4 months of age.
- The FDA has received approximately 100 reports from healthcare providers and parents describing adverse reactions to DHA/AA-supplemented formulas. Reports state that numerous

side effects such as vomiting and diarrhea disappeared as soon as the infant was switched to a formula without these additives (Vallaeys, 2008). Some infants may have more difficulty digesting triglycerides, with multiple DHA molecules occupying two or three places on the glycerol spine. Also, a possible contributor to variations in how infants handle these oils is the fact that they contain 40–50% DHA and AA, with the remaining components being high-oleic sunflower oil, diglycerides, and nonsaponifiable materials.

- Studies on LCPUFA-supplemented formula have shown no consistent beneficial outcomes (Follett, Ishii, & Heinig, 2003).
- Neural maturation of formula-fed preterm infants shows a deficit compared with those fed human milk.
- Delayed maturation in visual acuity can occur in both term and preterm formula-fed infants. This delay may affect other mental and physical functions linked to vision in later development (Birch et al., 1993).

The sterol content of human milk ranges from 10 to 20 mg/dL, rising over the course of the lactation, with cholesterol as the major component. Cholesterol is an essential part of all membranes and is required for normal growth and functioning. Breastfed infants' serum cholesterol levels are higher than those of formula-fed infants. This difference may have a long-term effect on the ability of the adult to metabolize cholesterol. Cholesterol is part of and necessary for the laying down of the myelin sheath that is involved in nerve conduction in the brain. Formula contains little to no cholesterol. Information for parents pertinent to the fat component of human milk includes the following:

- Inform parents of the developmental and cognitive differences between infants fed formula versus breastmilk. The average 8-point IQ score elevation in breastfed infants can be the difference between optimal and suboptimal functioning, especially in disadvantaged environments. Eight points is one-half of a standard deviation and is thought to provide a buffer to the neonatal brain from such detrimental factors as maternal smoking, maternal polychlorinated biphenol (PCB) ingestion, and lead ingestion in infancy. One IQ point has been estimated to be worth $14,500 in economic benefits from improved worker productivity (Grosse, Matte, Schwartz, & Jackson, 2002).
- The fatty acid composition of breastmilk can be altered by the maternal diet. Lowering the amounts of trans-fatty acids from hydrogenated fats and increasing the amounts of omega-3 fatty acids from eggs and fish are both beneficial.
- Teach mothers to allow the baby to finish the first side before switching to the second breast. This allows the baby to self-regulate his or her intake, receive the maximum amount of calories (from fat levels that rise at the end of a feeding), and avoid low-calorie, high-lactose feedings that result from placing time limits on the first breast "so the baby will take the second side."
- For infants experiencing slow weight gain, mothers can be guided to finish the first side by using alternate massage before offering the second side and to shorten the intervals between daytime feeds to increase the fat content of the milk.
- It was previously thought that the highest fat content in breastmilk occurred in breastmilk obtained immediately after the feeding begins. Following the initial increase in milk fat content

at the end of a feeding or pumping session, research has shown that the highest levels of both milk fat and live cells in breastmilk appear at 30 minutes after the feeding ends (Hassiotou et al., 2013). Maximum fat levels at 30 minutes post-feed were 1.5- to 8-fold higher compared to prefeeding values. These findings have implications for when milk should be expressed or feedings at breast should occur in the presence of low milk production or infant weight gain issues. It would seem prudent that, with preterm infants or any infants requiring a higher fat content milk for improved weight gain, mothers be instructed to feed again or pump at 30 minutes post feed to maximize milk fat content and improve milk production.

- When breastmilk is stored in the refrigerator or freezer, the fat rises to the top of the container. This can be skimmed off and given to a slow-gaining or preterm infant for extra calories. This milk can be quite calorie dense at 26–28 calories per ounce.
- The prevalence of smoking among pregnant women is between 15% and 20% (Mackay, Eriksen, & Shafey, 2006) and is of importance, as smoking decreases the fat content of breastmilk. Smoking has been associated with earlier weaning (Andersen et al., 1982), decreased milk production, and a lower fat content and lower long-chain fatty acid content in the first 6 months of lactation (Agostoni et al., 2003). Agostoni and colleagues (2003) also showed that smoking in early pregnancy was associated with lower milk fat content later during the first months of lactation. This delayed effect (at least 5 months) may be related to toxicants from smoking being stored in maternal fat tissues (particularly the breasts), which when mobilized during lactation find their way into the milk. Hopkinson, Schanler, Fraley, and Garza (1992) reported that milk fat content was 19% lower in mothers who smoked. Vio, Salazar, and Infante (1991) described milk volume, fat content, and infants' average weight gain over a 14-day study of maternal smokers and nonsmokers. Mean milk volume of nonsmokers was 961 $\pm$ 120 g/day, whereas the mean volume of maternal smokers was 693 $\pm$ 110 g/day. Fat concentration of nonsmokers' milk was 4.05%, whereas that of smokers was 3.25%. Infants' average weight increase over the 14-day study period was 550 $\pm$ 130 g (19.6 oz) for children of nonsmokers and 340 $\pm$ 170 g (12.1 oz) for children of smokers. Bachour and colleagues (2012) reported that smoking was associated with a 26% decrease in milk lipids, a 12% decrease in milk protein, and a slower infant growth rate. Infants whose mothers smoke need careful follow-up and frequent weight checks. These infants may need more frequent feeding.

Protein

Human milk proteins, like so many other milk components, exert multiple physiological activities, including enhancement of the immune system; defensive duties against pathogenic bacteria, viruses, and yeast; and the development of the gut. New research now shows that human milk contains at least 761 distinct proteins, including low-abundance and minor protein fractions (D'Alessandro, Scaloni, & Zolla, 2013). The protein concentration of human milk is high during the colostrum period, leveling off to about 0.8–1.0% in mature milk. It is also higher in preterm milk initially than in term milk. Infant formula can have as much as 40% more protein than human milk due to the reduction in digestibility, bioavailability, and efficiency of utilization of cow's milk proteins. Higher concentrations of serum insulin are seen in formula-fed infants, possibly due to elevated levels of insulinogenic amino acids such as valine, leucine,

and isoleucine. The long-term consequences of this early hyperinsulinemia are unknown, but the condition might increase the risk of diabetes and obesity in formula-fed infants (Lonnerdal, 2014). Mothers who smoke have been shown to have a 12% decrease in milk proteins (Bachour et al., 2012). Smoking decreases the basal prolactin levels at birth and during lactation, which might partially explain the lower protein levels in smokers' milk because prolactin induces the expression of milk protein–encoding genes. Milk proteins have classically been divided into casein and whey proteins. A third group of proteins, called mucins, surrounds the lipid globules in milk and contributes only a small percentage of the total protein content.

Caseins of human milk constitute 10–50% of the total protein, with this percentage rising over the course of the lactation. Colostrum and preterm milk either do not contain or are very low in casein. Casein gives milk its characteristic white appearance. This easily digested protein provides amino acids and aids in calcium and phosphorus absorption in the newborn. Bovine casein is less easily digested in human infants and may not exert the same effect that human milk casein does. The casein protein that predominates in cow's milk forms a tough rubbery curd in the stomach of an infant and requires a longer time to break down and digest. The whey-to-casein ratio in human milk changes over the course of lactation from 90:10 in the early milk, to 60:40 in mature milk, and to 50:50 in late lactation, and as a consequence of the continually changing ratio, there is really no fixed ratio of casein to whey in human milk (Lonnerdal, 2003). By comparison, these ratios do not change in infant formula and can, in fact, be directly opposite that of human milk.

Maternal dietary intake of protein can influence differing protein fractions in human milk (Lopez Alvarez, 2007). In a well-nourished population of mothers with widely varying differences in protein and energy intake, the nitrogen component as well as the protein fraction in their breastmilk remained unaffected (Boniglia, Carratu, Chiarotti, Giammarioli, & Manzini, 2003). However, in a malnourished sample of Colombian mothers, those who did not receive sufficient protein intake experienced a two-thirds reduction in protein content of early milk and diminished C4 complement, IgA, and IgG (Picciano, 1998). In a study that sought to improve the quality of breastmilk through protein supplementation, Chao et al. (2004) supplemented mothers with a protein-rich chicken extract from the 37th week of gestation until 3 days postpartum. Total protein in colostrum did not significantly change; however, dietary supplementation resulted in up to a 35% increase in breastmilk lactoferrin, a 62% increase in epidermal growth factor, and a 196% increase in TGF-beta 2.

Whey proteins are very diverse. Alpha-lactalbumin and lactoferrin are the chief fractions. Human alpha-lactalbumin has a high nutritional value and has been shown to have antitumor activity (Hákansson, Zhivotovsky, Orrenius, Sabharwal, & Svanborg, 1995). Alpha-lactalbumin can appear as a large complex in human milk and is modified in the infant's stomach into a molecular complex called HAMLET (human alpha-lactalbumin made lethal to tumor cells) and in this form has been shown to kill transformed cells (Gustafsson et al., 2005; Newburg, 2005) as well as 40 different carcinoma and lymphoma cell lines (Svensson, Hákansson, Mossberg, Linse, & Svanborg, 2000). HAMLET has been suggested as a possible reason why breastfeeding results in lower rates of childhood leukemia and a reduced incidence of breast cancer (Hanson, 2004). Lactoferrin is an iron-binding protein. Colostrum contains 5–7 g/L of lactoferrin, which gradually decreases over time. A 1-month-old infant consumes about 260 mg/kg/day of lactoferrin and at 4 months about 125 mg/kg/day (Butte et al., 1984). Lactoferrin promotes the growth of intestinal epithelium and has been thought to exert its bacteriocidal effect through withholding iron

from iron-requiring pathogens. This type of inhibition can be reversed by the addition of iron in excess of the binding capacity of lactoferrin. It may, however, have a more important effect, which is to alter the properties of the bacterial cell membrane, making it more vulnerable to the killing effects of lysozyme. Immunoglobulins are part of the whey protein fraction, as are enzymes.

A minor portion of the proteins in human milk reside in the lipid fraction, as an integral part of the membrane surrounding the fat globules. The milk fat globule membrane (MFGM) contains sphingomyelin, gangliosides, sialic acid, and cholesterol, all of which are involved in brain myelination and function. The MFGM is lacking in most formulas because this fraction is lost during the dairy's processing of cow's milk.

S100B protein, brain-derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF) are little known proteins with interesting effects. These factors are critical molecules that support the process of neuronal growth, development, protection, and repair, and the modulation of learning and memory. BDNF plays an important role in the development of the enteric nervous system, defense against intestinal infection, and modulation of GI motility. GDNF has been shown to support the development of human enteric nervous system and intestinal epithelial barrier integrity (Li, Xia, Zhang, & Wu, 2011).

Immunoglobulins are members of the defense agent team in breastmilk. The predominant immunoglobulin in human milk is immunoglobulin A (IgA). Concentrations of IgA are highest in colostrum and gradually decline to a plateau of about 1 mg/mL for the duration of lactation. The infant's approximate mean intake of IgA is 125 mg/kg/day at 1 month and 75 mg/kg/day by 4 months (Butte et al., 1984). Breastfeeding actively stimulates and directs the immune response of the breastfed infant. Vaccine responses to oral polio virus vaccine and parenteral tetanus and diphtheria toxoid vaccines are enhanced by breastfeeding, with formula-fed infants sometimes showing lower antibody levels to their immunizations (Hahn-Zoric et al., 1990; Pickering et al., 1998). Intestinal dysbiosis may play a role in the infant's response to oral and parenteral vaccines. High abundance of gut *Bifidobacterium*, as seen in breastfed infants, has been associated with higher responses to oral and parenteral vaccines and a larger thymus. High abundance of *Clostridia*, *Enterobacteriales*, and *Pseumonadales*, as seen in the guts of formula-fed infants, has been associated with neutrophilia (increased white cells associated with inflammation and infection) and lower vaccine responses (Huda et al., 2014).

Human milk also contains numerous enzymes. Some function in the mammary gland, some act in the infant, and some have unknown functions. Many are involved in the digestive process, whereas others function in defense against disease. For example, lysozyme is active against the human immunodeficiency virus and plays a role in the antibacterial activity of human milk, showing the most effect against Gram-positive bacteria. High concentrations are present throughout lactation, whereas concentrations are several orders of magnitude lower in bovine milk. Whereas secretory IgA and lactoferrin levels decrease after the early period of lactation, lysozyme levels remain higher during the 6-month to 2-year period of lactation than they were during the first month of breastfeeding.

Platelet-activating factor acetylhydrolase (PAF-AH) plays an important role in the prevention of NEC, an often fatal bowel disease in preterm infants. PAF is a potent ulcerative agent in the GI tract. NEC can be induced within hours after administration of PAF in experimental animals. PAF-AH hydrolyzes

PAF to produce an inactive form, thereby helping to prevent the development of NEC in infants receiving breastmilk (Furukawa, Lee, & Johnston, 1993). It is interesting to note that among the species studied, the only one devoid of milk PAF-AH was bovine milk; thus, cow's milk products cannot substitute for human milk (Park, Bulkley, & Granger, 1983).

There is also a connection between the breastmilk protein known as neuregulin-4 (NRG4) and protection against NEC. NRG4 promotes epithelial cell survival, with NRG4 receptors being present in the developing human intestine. NEC is known to be characterized by the loss of specialized intestinal cells known as Paneth cells, which help protect the gut from damage from pathological organisms and maintain healthy populations of intestinal stem cells. Such stem cells are essential to facilitate the intestine's ongoing ability to regenerate cells lost to damage and disease. Both animal studies and studies on human cell lines have shown that when fed or exposed to infant formula, Paneth cells are lost; in contrast, when fed or exposed to human milk, Paneth cells are maintained. Given that NRG4 is found only in breastmilk and not in infant formula, infants fed formula are missing out on a protective mechanism for the immature gut. Thus, when a formula-fed infant encounters a NEC trigger such as intestinal infection or injury, he or she may be at an increased risk for acquiring NEC (McElroy et al., 2014).

The nonprotein nitrogen fraction of human milk accounts for 20–25% of the total nitrogen found in human milk. It is made up of peptides, urea, uric acid, ammonia, free amino acids, creatine, creatinine, nucleic acids, nucleotides, polyamines, carnitine, choline, amino alcohols of phospholipids, amino sugars, peptide hormones, and growth factors. Nucleotides have received increased attention because some infant formulas have been supplemented with them. Human milk has a specific content of free nucleotides that differs from the mix found in cow's milk. Nucleotides are involved in the modulation of the immune system, the intestinal microenvironment, and the absorption and metabolism of nutrients. The total nucleotide content of human milk, if nucleic acid content is included, greatly exceeds the levels found in formula supplemented with nucleotides. Although nucleotide-supplemented formula is marketed as "being closer to breastmilk," it remains unproven whether this contributes to decreased morbidity, or only increased revenues.

Clinical Implications

Cow's milk serves as the base of most infant formulas. The prevalence of cow's milk allergy has been placed at between 2% and 5% of infants (Host, Jacobsen, Halken, & Holmenlund, 1995). Risk factors include a family history of atopy and early dietary exposure to cow's milk. The age at onset is directly correlated with the time of introduction of infant formula (Oski, DeAngeles, Feigin, & McMillan, 1994). Even exclusively breastfed infants can develop symptoms of cow's milk protein intolerance that may respond to elimination of the offending agent from the mother's diet.

Because of the high cross-reactivity to soy protein, the potential for soy allergy is 10–14%; thus, soy protein is not used for infants with documented cow's milk allergy. Soy formula neither reduces allergic symptoms nor delays allergies (Chandra & Hamed, 1991). Soy formula has no advantage as a supplement to breastmilk, has no proven value in the prevention or management of infant colic or fussiness, and is indicated only for infants with galactosemia or hereditary lactase deficiency (extremely rare), or when the family prefers a vegetarian diet (Bhatia & Greer, 2008). Although cow's milk protein–based lactose-free

formulas are available, they have not been shown to have any clinical impact on colic, growth, or development, and they are not recommended for infants with diarrhea (Heyman, 2006). Breastfed infants should be continued on human milk during episodes of diarrhea. Human milk proteins play a critical role in directing the construction of the immune system. Infants fed soy formula have the lowest antibody titers to their vaccines, and those fed cow's milk–based formula are still below breastfed infants both in antibody titers and incidence of illness (Zoppi et al., 1983). Some soy formula–fed infants have had to be reimmunized after soy formula feeding. Compared with girls fed non–soy-based infant formula or milk (early formula), early soy-fed girls were at a 25% higher risk of earlier menarche (Adgent et al., 2012). It is recommended that the clinician take the following steps:

- Prenatally, help parents understand the importance of exclusive breastfeeding for about 6 months, especially those with a family history of allergies and diabetes.
- Avoid giving bottles of formula (cow's milk based or soy based) in the hospital. Even one bottle can sensitize a susceptible infant and provoke an allergy later when challenged again.
- Encourage frequent breastfeeding, 8–12 times every 24 hours right from the start. Colostrum contains large amounts of protein, especially the sIgA that helps protect the infant against disease. These protein levels in colostrum also serve to prevent hypoglycemia.

Carbohydrates

The principal carbohydrate in human milk is lactose (galactose + glucose), with other carbohydrates occurring in smaller amounts, such as oligosaccharides, monosaccharides, and peptide-bound and protein-bound carbohydrates. Lactose is the most abundant component of human milk (70 g/L). It is thought to have several functions:

- Lactose favors the colonization of the infant's intestine with microflora that competes with and excludes pathogens.
- Infants undergo rapid brain development during the nursing period, with the natural period of exclusive breastfeeding coinciding with the most rapid period of brain development. Myelination requires large amounts of galactosylceramide (galactocerebrosides) and other galactolipids that are major components of this growth. The infant liver may be unable to synthesize all the galactolipids needed at this time. Milk galactose is most likely present to ensure an adequate supply of galactocerebrosides for optimal brain development. Brain growth and development during this time are known to be vulnerable to many types of nutrient deficiencies. This vulnerability has important ramifications for infants on artificial diets, especially when lactose is removed from their sole source of nutrition, as with soy formula or cow's milk–based formula that has had the lactose removed.
- Lactose enhances calcium absorption. Infants fed cow's milk–based formula with the lactose removed show reduced calcium absorption (Abrams, Griffin, & Davila, 2002). Lactose levels are quite stable, showing little or no change in response to a wide variety of environmental or dietary challenges. Infants are well suited to use lactose because lactase, a brush border intestinal enzyme that digests lactose, is present by 24 weeks of fetal life. Lactase levels increase

throughout the last trimester of fetal life, reaching concentrations at term that are two to four times the levels seen at 2–11 months of age. One hypothesis suggests that a relationship exists between the relative size of the brain and the level of lactose in a species' milk. Humans, with their large brains, have very high lactose levels in their milk compared with other species.

Oligosaccharides are biologically active carbohydrates. More than 200 neutral and acidic oligosaccharides have been identified to date at levels of approximately 12–14 g/L (1.2–1.4 g/dL), which represents 1% of human milk and 10% of its caloric content. This makes these carbohydrates collectively the third largest solid component of human milk. Oligosaccharides are essentially indigestible by the infant's gut mucosa and are not utilized as a macronutrient. Instead, they are delivered to the gut intact, where they nourish the infant's gut microbiota, thereby acting as the infant's first prebiotic. An individual mother's milk can contain anywhere from a few dozen to more than a hundred different oligosaccharides. The concentration of oligosaccharides in cow's milk is about 20-fold lower than that found in human milk (Veh et al., 1981). Oligosaccharides have water-soluble cell surface analogs that can inhibit enteropathogen binding to host cell receptors. Oligosaccharides can inhibit the binding to their intestinal cell receptors of such bacteria as *Streptococcus pneumoniae*, *Haemophilus influenzae, E. coli*, and *Campylobacter jejuni*. They essentially act as decoys through their ability to mimic intestinal cell receptors, preventing bacteria from attaching to their respective receptors in the host cells.

Milk oligosaccharides also contain human blood group antigens, with women from different blood group types exhibiting distinct patterns.

Lactating mothers differ genetically in their ability to produce protective oligosaccharides and thus may influence their breastfed infant's susceptibility to enteric disease (Morrow et al., 2004; Uauy & Araya, 2004). Newborns fed breastmilk from mothers who exhibit higher levels of certain oligosaccharides may be better protected against certain pathogens such as *E. coli* than infants whose mother's milk contains a genetically programmed lower amount of such disease fighters (Thurl et al., 2010). This distinction is thought to be related to the Lewis blood group to which a mother belongs. Four human milk groups have been identified based on the Lewis blood group system. Each of these groups produces different amounts and kinds of oligosaccharides that appear in the respective mother's breastmilk (Zivkovic, German, Lebrilla, & Mills, 2011).

Oligosaccharide composition also depends on the mother's secretor status. Mothers whose milk-making cells turn "on" the FUT2 gene produce a different set of milk sugars that are more protective than those produced in the absence of a functioning FUT2 gene. Those mothers with a functional FUT2 gene are called "secretors." In contrast, "nonsecretors" have a disabling mutation in the FUT2 (α-1,2-fucosyltransferase) gene, such that they produce sugars with different linkages in their milk. Approximately 20% of mothers are nonsecretors. Both secretors and nonsecretors produce the lactose and fucose molecules, but an important difference is how the fucose attaches to the lactose. If the fucose attaches via an α-1,2 linkage, the sugar is called 2′-FL and has a variety of protective actions; if the fucose links via an α-1,3 linkage, the sugar is called 3′-FL and loses some of its defensive ability, rendering it less protective for the infant. Researchers have shown that bifidobacteria are established earlier and more often in infants fed by secretor mothers than in infants fed by nonsecretor mothers (Lewis et al., 2015). In Lewis et al.'s (2015) study, infants fed by nonsecretor mothers had 10 times fewer bifidobacteria and

were delayed in the establishment of bifidobacteria-laden microbiota, possibly due to difficulties in the infant acquiring a species of *Bifidobacterium* that is able to consume the specific milk oligosaccharides provided by the mother.

This finding is true for the milk of preterm mothers as well. Preterm human milk tends to have a lower lactose content than term milk. High concentrations of oligosaccharides exist in the milk of certain Lewis blood group preterm mothers. The use of donor-pooled milk is of benefit rather than single donor milk because the pooled milk is likely to average out the large interindividual variations in oligosaccharide amounts and composition (Gabrielli et al., 2011).

Infant formulas have an oligosaccharide-bound sialic acid content that is 10 to 27 times lower than that of human milk because the manufacturing process for infant formulas has virtually eliminated this component from the finished product (Martin-Sosa, Martin, Garcia-Pardo, & Hueso, 2003). Formula-fed infants have a reduced intake and number of oligosaccharides than human milk–fed infants in their stool and urine, which also differ in composition from those of breastfed infants (Hanson, 2004). Many infant formulas have been supplemented with plant-based oligosaccharides in an effort to mimic the function and outcome seen in breastfed infants, such as inducing softer stools and creating gut flora patterns similar to those of breastfed infants. One potential side effect of this type of supplementation is the possibility of bacterial translocation. The term bacterial translocation is used to describe the passage of viable resident bacteria from the GI tract to normally sterile tissues such as the mesenteric lymph nodes and other internal organs. Barrat et al. (2008) reported that in a study of rats fed a formula with added oligosaccharides and inulin, an increase in bacterial translocation occurred in the immature gut. This side effect may pose a potential infectious risk and requires further study. Oligosaccharides currently added to infant formulas are structurally different from human milk oligosaccharides (HMOs) and most likely are not functionally equivalent (Jantscher-Krenn & Bode, 2012). Xia and colleagues (2012) measured the abundance of commensal and beneficial bacteria (*Bacteroides*, *Bifidobacterium*, and *Lactobacillus*) and pathogenic bacteria (*Clostridium difficile* and *E. coli*) in breastfed infants and infants fed formula supplemented with various amounts of fructo-oligosaccharides. Results showed that formula-fed infants harbored a greater abundance of *C. difficile* and *E. coli* and similar amounts of beneficial bacteria as breastfed infants. The addition of nonhuman oligosaccharides had no significant prebiotic effect with respect to increasing beneficial bacteria or decreasing pathogenic bacteria in formula-fed infants. Some commercially available prebiotics (FOS and GOS) have been shown to stimulate the growth of pathogenic microorganisms such as *Clostridia* (Bunesova et al., 2012), which raises a question about the desirability of supplementing infant formula with these substances.

HMOs are an important source of sialic acid for the infant. Sialic acid is an integral part of the plasma membranes of nerve cells concentrated in the region of nerve endings and dendrites in the brain. The brains of breastfed infants have higher amounts of sialic acid than those of formula-fed infants (Wang et al., 2003). Formula-fed infants derive less than 25% of the amount of sialic acid that is supplied to breastfed infants through mature human milk. Formula-fed infants have a lower dietary source of sialic acid and are unable to synthesize the difference (McVeagh & Miller, 1997). Soy formulas have almost undetectable amounts of sialic acid (Wang, Brand-Miller, McVeagh, & Petocz, 2001). In animal models, an exogenous source of sialic acid increased learning performance as well as the concentration of sialic acid in the frontal cortex of the brain (Wang et al., 2007). Because breastmilk is such a rich source of sialic acid, this suggests that the rapid formation of brain gangliosides during the infant's first month depends

on the steady and abundant supply of this brain growth factor. Concentrations of several of the oligosaccharides are higher in colostrum than in mature milk, suggesting that the early days of brain growth are enhanced by the presence of particular factors in breastmilk that are vital for the proper development of the rapidly growing brain (Asakuma et al., 2007). Large amounts of sialylated oligosaccharides may be one mechanism by which breastfeeding promotes higher cognitive performance in children. Infant formula does not have sufficient amounts of Neu5Ac sialic acid (the predominant sialic acid form in healthy humans). It does, however, contain Neu5Gc, which is normally absent in humans and which has often been associated with human inflammatory diseases (Wang, Hu, & Yu, 2006).

Because oligosaccharides present in human milk are able to modulate the microbiota of breastfed infants, it might be possible that HMOs could also modulate the bacterial communities in the breast itself. While milk of secretor women is rich in 2′-fucosyllactose and other α-1,2-fucosylated HMOs, nonsecretor women lack a functional FTU2 enzyme, resulting in milk that does not contain α-1,2-fucosylated HMOs. Interestingly, some strains of *Staphylococcus*—the major bacterial contributor to mastitis—bind to 2′-fucosyllactose (Lane, Mehra, Carrington, & Hickey, 2011). Thus, susceptibility to acquire mastitis might be determined not only by the bacterial composition of the milk, but also by the blood group and corresponding type of HMOs contained in each individual mother's milk (Jeurink et al., 2013). In essence, some mothers can be protected from mastitis by their own HMOs.

Clinical Implications

Human milk provides several tiers of protection from pathogens, and those tiers have the potential to work synergistically. The processing of infant formulas based on cow's milk utilizes procedures that exclude from the final product colostrum, MFGM, and fractions that contain DNA. Milk oligosaccharides from other species confer protection to the young of that species. Random additions of a few synthesized, structurally different oligosaccharides to infant formula would not be expected to generate a tier of disease protection because those oligosaccharides in breastmilk are unique to human milk and have not been replicated synthetically. Reassure parents that it is uncommon for infant fussiness to be related to lactose intolerance. Primary lactose intolerance (lactase deficiency) is extremely rare. Weaning a breastfed infant to a lactose-free formula removes all layers of disease protection and changes the nature of the nutrient supply to the brain. There is a 10-fold difference in sialic concentrations among different types of formulas, with none containing more than 25% of the brain builders found in human milk. The oligosaccharides contained in some infant formulas are synthesized by bacterial enzymes or isolated from plants and lack fucose and sialic acid. These components are essential to realize the beneficial effects of HMOs. The oligosaccharides in formula are unable to mimic the structure-specific effects of the HMOs and may leave formula-fed infants without essential brain nutrients.

Vitamins

The water-soluble vitamins in human milk are ascorbic acid (vitamin C), thiamin (vitamin B_1), riboflavin (vitamin B_2), niacin, pyridoxine (vitamin B_6), folate, pantothenate, biotin, and vitamin B_{12}. The concentration of water-soluble vitamins in human milk shows variations reflecting the stage of lactation, maternal intake, and delivery before term. The breast cannot synthesize water-soluble vitamins, so their origins lie in the maternal plasma, derived from the maternal diet. Concentrations are generally lower in the early days of breastfeeding compared with those found in mature milk. In mothers who are adequately nourished,

maternal supplementation in higher than physiological doses either has no effect or is transient. Maternal vitamin supplementation generally shows benefits only when the mother herself is malnourished.

Storage and handling of expressed human milk can alter some of the vitamin components in it. Total ascorbic acid (vitamin C) levels decreased on average by one-third after 24 hours of storage at 4°C (39.2°F), with wide variations between individual mothers (Buss, McGill, Darlow, & Winterbourn, 2001). Francis, Rogers, Brewer, Dickton, and Pardini (2008) found that various bottle systems showed measurable decreases in the mean concentration of ascorbic acid over a 20-minute sampling period (the approximate time of a feeding). Those bottles with the largest milk-to-air interface had the greatest decreases in mean concentration of ascorbic acid over time. The air moving through the milk and the formation of bubbles on the surface of the milk could be factors in the observed decreases of ascorbic acid concentration. Ascorbic acid is also degraded by exposure to light, suggesting that tinted bottles for expressing, storing, and feeding human milk might be appropriate. Those infants who are solely dependent on bottle-feeds for their total ascorbic acid intake by formula or breastmilk, especially high-risk or preterm infants, may need their ascorbic acid status evaluated. Caregivers (parents and health professionals) should avoid shaking the bottle, may wish to use bottle systems with lower milk-to-air surface interfaces, and may wish to consider using tinted bottles for expressing, storing, and feeding purposes.

Micronutrients such as vitamins C and E are essential to the health of the infant's antioxidant defense system. Mothers who were supplemented with 500 mg of vitamin C and 100 mg of vitamin E showed a significant increase in the antioxidant capacity of their breastmilk (Zarban et al., 2015), While a healthy balanced diet may outweigh single supplements, mothers with a diet deficient in vitamins C and E may benefit from supplementation to help enrich their milk with these antioxidants.

Vitamin B_{12} is needed by the infant's developing nervous system. This vitamin occurs exclusively in animal tissue, is bound to protein, and is minimal to absent in vegetable protein. A mother consuming a vegan diet, without meat or dairy products, may have milk deficient in vitamin B_{12}. Infants who present with infections, pallor, hypotonia, neurodevelopmental delays, refusal to suck, failure to thrive, hematological issues, or fatigue may benefit from a check of their vitamin B_{12} levels. Mothers should be asked if they are consuming a vegetarian diet and should also have their vitamin B_{12} status evaluated (Akcaboy et al., 2015).

The fat-soluble vitamins are A, D, E, and K. Vitamin A and its precursors, known as carotenoids (beta-carotene), occur at twice the levels in colostrum as in mature human milk. The level of vitamin A in the milk of well-nourished mothers delivering prematurely is even higher. This vitamin is important in infant growth and development. An inverse relationship exists between the risk of morbidity and mortality and vitamin A status. Even after breastfeeding is discontinued, it appears to confer a protective effect due to some of the vitamin A provided by breastmilk being stored in the child's liver.

Vitamin D comprises a group of related fat-soluble compounds with antirachitic (rickets) activity. Vitamin D is not actually a vitamin or a nutrient but a precursor of a steroid hormone formed when the skin is directly exposed to ultraviolet B radiation in sunlight. It is essential for the normal absorption of calcium from the gut. There are two forms of vitamin D, D_2 (ergocalciferol, which is synthesized by plants) and D_3 (cholecalciferol, which is synthesized by mammals). The main source of vitamin D for humans is through its synthesis in the skin when exposed to ultraviolet B radiation in the range of 290–315 nm, with less than 10% derived from dietary sources. The most common food source of vitamin D is the plant steroid ergosterol, the liver and oils of some fatty fish, and foods fortified with vitamin D such as milk, orange juice, margarine, and cereals. Although vitamin D is synthesized in the skin upon exposure to

Box 1-2 Details on Vitamin D

- Breastmilk contains an average vitamin D content of 26 IU/L (range, 5–136 IU) in a vitamin D–sufficient mother (Institute of Medicine, Food and Nutrition Board, & Standing Committee on the Evaluation of Dietary Reference Intakes, 1997).
- Infant formula contains 400 IU (10 mg) per liter (Life Sciences Research Office Report, 1998).
- If an infant consumes an average of 750 mL/day of breastmilk, exclusive breastfeeding without sun exposure would provide a range of 11–38 IU/day of vitamin D, which is below the recommended minimum intake of 400 IU/day (Wagner, Greer, American Academy of Pediatrics Section on Breastfeeding, & American Academy of Pediatrics Committee on Nutrition, 2008).
- An adequate intake level of vitamin D for infants with some sunlight exposure has not been established, but breastfed infants with limited sunlight exposure have not been shown to develop rickets.
- The cost of averting a single case of rickets by universally dosing infants with vitamin D could be between $252,614 and $958,293 per case (Vitamin D Expert Panel, & Centers for Disease Control and Prevention, 2001).
- The cost to breastfeeding initiation and duration rates has not been accounted for, nor has the concern that formula manufacturers will promote their products in such a way as to imply that breastmilk is inadequate and that their products should be used to prevent a condition caused by the use of "deficient" breastmilk (Heinig, 2003).

sunlight, many people derive much of their vitamin D from foods that are supplemented or enriched with it. Levels of this vitamin vary in breastmilk and are often reported as being inadequate (**Box 1-2**).

Human milk is not necessarily deficient in vitamin D. Approximately 20% of maternal circulating vitamin D is transferred to the infant through the mother's milk. Infants suffering from vitamin D deficiency and rickets do so from a deficit of exposure of the skin to sunlight or may be born deficient if their mother was also deficient during the pregnancy. Vitamin D content in human milk is sufficient when the mother's levels are sufficient (Henderson, 2005). Attaining sufficient and safe sunlight exposure for infants has been complicated by the recommendation from the AAP to keep infants less than 6 months of age out of direct sunlight and to use sunscreen on older infants and children (AAP & Committee on Environmental Health, 1999). Rickets and poor bone mineralization are rare in breastfed infants but do occur. Vitamin D insufficiency and actual deficiency can occur in breastfed infants who are not supplemented with extra vitamin D (Merewood et al., 2012). Incidents of vitamin D deficiency in its extreme form, rickets, are still reported. Rickets is primarily associated with dark-skinned children on vegetarian diets, dark-skinned infants exclusively breastfed beyond 3 to 6 months of age, premature infants, and infants born to mothers who are vitamin D deficient themselves (Misra, Pacaud, Petryk, Collett-Solberg, & Kappy, 2008). Greer and associates (1982) randomized infants into a group receiving a placebo and a group receiving a supplement of 10 mg of vitamin D daily. The bone-mineral content of the placebo group was significantly lower in the first few months after birth but at the end of the first year was actually higher than that in the supplemented group.

Exclusive breastfeeding results in normal infant bone-mineral content when maternal vitamin D status is adequate (Greer & Marshall, 1989), when neonatal stores are normal, and when the infant is regularly exposed to sunlight. Specker, Valanis, Hertzberg, Edwards, and Tsang (1985) reported that

30 minutes of sun exposure per week for infants wearing only a diaper and 2 hours of sun exposure per week for fully clothed infants without a hat maintained vitamin D levels of greater than 27.5 nmol/L. Merewood and colleagues (2012) showed that as little as 10 minutes outside once per week is protective against vitamin D deficiency. However, the duration of sun exposure that is necessary for differing categories of infants (e.g., dark skinned, living at differing latitudes, clothed or just diapered) to maintain vitamin D levels at greater than 50 nmol/L, the currently accepted level for vitamin D sufficiency in children, is undetermined. Darkly pigmented infants require a greater exposure to sunshine to initiate the synthesis of vitamin D in the skin (Clemens, Adams, Henderson, & Holick, 1982). It has been shown that 400 IU/day of vitamin D maintains serum concentrations at greater than 50 nmol/L in exclusively breastfed infants (Wagner, Hulsey, Fanning, Ebeling, & Hollis, 2006). Reports in the literature of confirmed rickets in breastfed infants (Kreiter et al., 2000; Shah, Salhab, Patterson, & Seikaly, 2000) have led to a recommendation by the AAP that all breastfed infants be supplemented with 400 IU of vitamin D per day beginning in the first few days of life (Wagner et al., 2008). Controversy has been generated by this recommendation and the concomitant urging by the AAP that people limit their exposure to sunlight to reduce the incidence of skin cancer. Vitamin D status in infants also depends on numerous factors:

- Is the infant being exclusively breastfed? Is he or she being given medications, other foods, or drinks that could interfere with absorption of nutrients such as calcium? Chronic calcium deficiency increases vitamin D metabolism with secondary vitamin D deficiency (Clements, Johnson, & Fraser, 1987).
- What was the vitamin D status of the mother during her pregnancy? Maternal vitamin D concentrations largely determine the vitamin D status of the fetus and newborn infant. A mother deficient in vitamin D during her pregnancy will give birth to a vitamin D–deficient infant or an infant who will reach vitamin D deficiency more quickly than an infant born to a vitamin D–sufficient mother. Was the infant preterm? Preterm infants lack the necessary time to accumulate vitamin D stores. What is the mother's current vitamin D intake and exposure to sunlight?
- Is the infant's skin deeply pigmented? Approximately 90% of all reported cases of nutritional rickets have occurred in African American children, identifying a population of infants at higher risk for rickets and for whom maternal supplementation or direct supplementation of vitamin D may be more important (Hirsch, 2007). Is the infant from a poor socioeconomic background? Is he or she malnourished or does the infant have fat malabsorption? How old is the infant? Overt rickets is more common in children older than 6 months of age (Pugliese, Blumberg, Hludzinski, & Kay, 1998; Sills, Skuza, Horlick, Schwartz, & Rapaport, 1994).

A number of environmental, genetic, hormonal, nutritional, and cultural factors interact and/or overlap, putting some susceptible children at risk for rickets (Mojab, 2002):

- Maternal deficiency (prenatal and postpartum)
- Daylight hours spent indoors
- Living conditions such as residence in high latitudes and urban areas with buildings or pollution that block sunlight
- Cultural practices such as restricting postpartum women from outdoor exposure during the first month postpartum

- Dark skin pigmentation
- Use of sunscreen
- Covering the body when outside (cold climate, fear of skin cancer, cultural dress customs)

The antirachitic activity in human milk varies by season, maternal vitamin D intake, sun exposure, and race. Various levels of maternal vitamin D supplementation have been studied in an attempt to delineate how much is necessary to increase an infant's vitamin D levels to a midrange of normal through the consumption of breastmilk. Researchers have supplemented lactating mothers with 2,000–4,000 IU of vitamin D per day for 3 months, causing a significant rise in maternal vitamin D levels as well as improving vitamin D levels in breastmilk and in the recipient infants (Basile, Taylor, Wagner, Horst, & Hollis, 2006; Hollis & Wagner, 2004). Such a dose is much higher than the current Dietary Reference Intake for lactating mothers (400 IU/day). Concern has been raised regarding the safety of this practice; however, Vieth, Chan, and MacFarlane (2001) and Heaney, Davies, Chen, Holick, and Barger-Lux (2003) show that vitamin D intakes of 10,000 IU/day (250 mg) or more are safe for periods up to 5 months. Further research is necessary to determine optimal vitamin D intakes for pregnant and lactating women from both sunlight and supplements.

Delineating high-risk groups of infants suitable for supplementation such as dark-skinned, exclusively breastfed infants who spend much time indoors has been suggested as a means of providing supplemental vitamin D appropriately, while avoiding the implication that breastmilk is deficient in this substance (Weisberg, Scanlon, Li, & Cogswell, 2004). Ponnapakkam, Bradford, and Gensure (2010) performed a prospective clinical trial comparing vitamin D supplementation of breastfeeding infants with a placebo as control in southern Louisiana. Those infants in the placebo group showed borderline deficiency at the 2- and 4-month points, but by 6 months, the 25-OHD levels were comparable with those of the treated group. The authors state that there appears to be a critical time period for developing vitamin D insufficiency in infants, which is between 2 and 4 months of age. There was no measurable consequence to this transient vitamin D insufficiency, but it may indicate a period that is more critical for infants who are at a higher risk for developing rickets. They saw no evidence of a benefit of universal vitamin D supplementation for breastfed infants to prevent rickets. High-dose vitamin D supplementation (6,400 IU daily) in nursing mothers may be a workable strategy for improving the vitamin D status of both the mothers and their exclusively breastfeeding infants (Haggerty, 2011). Welch, Bergstrom, and Tsang (2000) recommend viewing vitamin D supplementation as a mechanism to ensure an adequate substrate for a hormone whose normal production has been adversely affected by the realities of modern living conditions—not as a treatment for nutritional inadequacy of human milk. Research has shown that adequate vitamin D maternal supplementation (6400 IU per day) significantly increases the antirachitic activity in breastmilk and is as effective in increasing the infant's vitamin D levels as actually supplementing the infant with 400 IU of vitamin D daily (Hollis et al., 2015; Wagner et al., 2006). Supplementing the mother also reduces the risk of any side effects from supplementing the infant directly. Katikaneni, Ponnapakkam, Ponnapakkam, and Gensure (2009) found that supplementation of infants with standard vitamin D preparations (400 IU/day) was associated with a 76% increased risk of urinary tract infections.

Vitamin E (alpha-tocopherol) functions as an antioxidant. It protects cell membranes and is required for muscle integrity. This vitamin's concentration in colostrum is higher than in mature milk because

vitamin E levels are low in the newborn and absorption is inefficient. Human milk supplies more than adequate amounts of vitamin E to the infant.

Vitamin K is essential for the formation of several proteins required for blood clotting. It is produced by the intestinal flora but takes several days in the previously sterile neonatal gut to be effective. Vitamin K stores at birth are very low, so newborns are immediately dependent on an external source for the vitamin. A deficiency of vitamin K increases the risk of a syndrome called hemorrhagic disease of the newborn. The early-onset form occurs at 2–10 days of age in 1 of every 200 to 400 newborns who do not receive additional vitamin K. The late-onset form occurs around 1 month of age in 1 of every 1,000 to 2,000 unsupplemented newborns. The most dependable method of preventing hemorrhagic disease of the newborn is an injected or oral dose of vitamin K at birth (Kleinman, 2004). Vitamin K levels in human milk respond to maternal supplements, but this response is variable and has not been well studied. Vitamin K is localized in the milk-fat globule, with hindmilk containing twofold higher vitamin K concentrations than milk collected from a full breast pumping.

Minerals and Trace Elements

The most prevalent monovalent ions in human milk are sodium, potassium, and chloride; the most prevalent divalent ions are calcium, magnesium, citrate, phosphate, and sulfate. Numerous factors affect the levels of these minerals in human milk. During pregnancy, involution, and mastitis, the junctions between the alveolar cells remain open, allowing sodium and chloride to enter the milk space, drawing water along with them. Lactose and potassium are also thought to move from the milk space to the blood. The net result is that under these conditions, milk has greatly increased concentrations of sodium and chloride and decreased concentrations of lactose and potassium. The presence of high sodium concentrations in human milk is diagnostic of either mastitis or low milk-volume secretion. Colostrum has much higher concentrations of sodium and chloride than does mature milk because the gland is undergoing the transition between pregnancy, when the junctions are open, and full lactation, when they are closed. Preterm milk shows lower concentrations of sodium and chloride, which rise to normal levels approximately 30 days postpartum.

The concentrations in milk of the major divalent ions are species specific. The calcium level increases markedly during the first few days postpartum but then decreases gradually over the course of the lactation. Citrate and phosphate concentrations rise in parallel with the sharp increase in milk volume between 2 to 4 days postpartum. The calcium-to-phosphorus ratio is lower in cow's milk (1:4) than in human milk (2:2). Lactation also affects the mother's calcium movement. Calcium uptake in the maternal duodenum is enhanced during lactation. After weaning, women who have lactated show significantly more bone in the lumbar spine than women who have not lactated (Kalkwarf, Specker, Heubi, Vieira, & Yergey, 1996).

Microminerals

Microminerals (trace minerals or trace elements) can be classified into four categories:

1. Essential: required in the diet, such as iron, zinc, copper, manganese, molybdenum, cobalt, selenium, iodine, and fluorine.
2. Possibly essential: chromium, nickel, silicon, tin, and vanadium.
3. Toxic in excess: aluminum, arsenic, cadmium, lead, and mercury. Soy-based infant formulas, hypoallergenic formulas, and formulas for premature infants have aluminum levels far in excess

of human milk (50 ng/g). Aluminum levels in cow's milk–based formulas have been measured in ranges from 10 to 3,400 ng/g; in soy-based formula, aluminum levels range from 230 to 1,100 ng/g; and in formula for preterm infants, aluminum ranges from 365 to 909 ng/g (Dabeka, Fouquet, Belisle, & Turcotte, 2011). The high aluminum content seen in infant formulas originates from both the myriad ingredients used to produce the product and aluminum from the packaging (Chuchu, Patel, Sebastian, & Exley, 2013). The immature physiologies of infants' GI tract, kidneys, and blood–brain barrier may predispose them to aluminum toxicity.

4. All other elements.

Because infants typically receive their entire nutrition from a single type of food, it is important that the proper trace elements are present and occur in the appropriate concentrations.

The iron concentration in human milk is highest during the first few days after birth and diminishes with the progression of lactation. Compared with the calculated requirements for the growing infant (8–10 mg/day), human milk appears to be relatively low in iron, at a concentration of 0.2–0.8 mg/L. In reality, the full-term infant is born with large physiological stores in the liver and hemoglobin, which, along with the iron in breastmilk, are sufficient to meet requirements for about 6 months if infants are exclusively breastfed. Approximately 50% of the iron from human milk is absorbed by the infant, compared with 2–19% from iron-fortified formula and 4% from fortified infant cereals. Iron concentrations increase during the weaning period and when women produce less than 300 mL/day after 7 months (Dewey, Finley, & Lonnerdal, 1984). The iron concentration in milk is not influenced by the maternal iron status. The infant who is exclusively breastfed for the first 6 months of life is not at risk for iron-deficiency anemia (Duncan, Schifman, Corrigan, & Schaefer, 1985). Caution has been advised with supraphysiological iron supplementation, however, because it can cause as much as a 40-fold increase in iron retention (Schulz-Lell, Buss, Oldigs, Dorner, & Schaub, 1987). Lactose, which promotes iron absorption, is present in higher concentrations in breastmilk, especially compared with commercial formulas, some of which contain no lactose at all. Breastfed infants do not suffer microhemorrhages of the bowel as some formula-fed infants do, so they will not have iron depletion through blood loss. Pisacane and coworkers (1995) studied the iron status of infants breastfed for 1 year who were never given cow's milk, supplemental iron, or iron-enriched formula. None who were exclusively breastfed for 7 months were anemic. Those breastfed exclusively for 6.5 months versus 5.5 months were less likely to be anemic. Iron supplementation of normal, healthy, full-term infants in the first 6 months therefore appears unnecessary and, in fact, increases the risk of disease by saturating lactoferrin. Supplementary foods reduce the intake of human milk and may impair iron absorption. Early introduction of highly bioavailable iron supplements, before 6 months of age, may be beneficial in a high-risk population, such as preterm infants who lack their full complement of iron stores. However, some studies have reported detrimental effects such as decreased growth and increased morbidity (Dewey et al., 2002), decreased zinc absorption (Lind et al., 2004), and altered vitamin A metabolism (Wieringa et al., 2003) when iron supplements are provided to infants who do not need them. It is possible that young infants may lack the capacity to downregulate iron absorption, either due to immaturity or to other micronutrient deficiencies, and that iron given to such infants may cause adverse effects (Hicks, Zavaleta, Chen, Abrams, & Lonnerdal, 2006). Deleterious effects of iron supplementation appear to affect infants who have adequate iron stores to begin with. Ziegler, Nelson, and Jeter (2009) conducted a study to assess the effect of universal early iron supplementation (all breastfed

infants) versus selective supplementation (only at-risk infants) from ages 1 month to 5.5 months of age. While 7 mg/day of iron caused some preservation of the infants' iron stores, the effect was modest and did not extend beyond the period of supplementation. Iron supplementation in this study significantly decreased the weight gain (but not length) of female infants. The authors concluded that iron supplementation of breastfed infants from an early age was feasible but only temporarily affected the iron status of the infants. Because of the low prevalence of iron deficiency, selective treatment of only those infants at risk for iron deficiency would be a more suitable approach to prevention of iron deficiency than universal supplementation of all breastfed infants.

Zinc is an essential component of more than 200 enzymes that have both catalytic and structural roles. This nutrient appears to play a critical role in gene expression. Many DNA-binding proteins are zinc complexes. Zinc concentration in colostrum ranges from 8 to 12 mg/mL and in mature milk from 1 to 3 mg/mL. The zinc in human milk is more efficiently utilized by infants than the zinc in cow's milk or formulas. Zinc bioavailability from soy formulas is considerably lower than that from milk-based formulas due to the phytate content of soy protein isolates. The full-term breastfed infant is at little risk for zinc deficiency. Only in rare cases have breastfed infants experienced such a deficiency, usually because of defective zinc uptake by the mammary gland. Transient zinc deficiency due to increased zinc requirements in breastfed mainly preterm infants is a condition similar to acrodermatitis enteropathica, an autosomal recessive disorder of enteric zinc absorption affecting almost exclusively nonbreastfed infants. Early recognition of the disorder and introduction of zinc supplementation rapidly reverses transient zinc deficiency. The term acrodermatitis enteropathica (AE) is used for all patients with acral dermatitis related to zinc deficiency, although it should be strictly confined to hereditary forms. Hypozincemia in infancy is divided into three types. Type I is characterized by an inherent defect in the absorption of zinc from the gut. Type II occurs because of defective secretion of zinc in mother's milk. Type III develops in preterm infants who are put on prolonged parenteral alimentation deficient in zinc.

Copper, selenium, chromium, iodide, manganese, nickel, fluorine, molybdenum, and cobalt all appear in adequate amounts in human milk. Healthy, full-term, breastfed infants require no supplementation of any of these minerals, including fluoride. However, the iodine content of the breastmilk of mothers who smoke cigarettes is lower than that of nonsmokers. Mothers who smoke have been reported to have higher levels of thiocyanate, which may reduce iodide transport into breastmilk. Infants of mothers who smoke may need to have their iodine levels monitored and to be given iodine supplements if appropriate (Laurberg, Nohr, Pedersen, & Fuglsang, 2004).

Clinical Implications

Continued research has revealed the highly complex nature of human milk. Many of the ingredients in breastmilk participate in multiple functions (**Table 1-3**). The interrelationships among the various components may be more significant than the amounts present or their levels of uptake. The ability of human milk and the act of breastfeeding to promote affiliative behavior, protect infant health, and support normal growth and development is unmatched by any other feeding system. Normal, healthy, full-term infants who are exclusively breastfed typically do not need vitamin or mineral supplements, with the exception of some high-risk infants who may need additional vitamin D or iron.

Table 1-3 Multiple Functions of the Major Nutrients of Human Milk in the Infant

Nutrients	Amount	Function
Protein		
sIgA	50–100 mg/dL	Immune protection
IgM	2 mg/dL	Immune protection
IgG	1 mg/dL	Immune protection
Lactoferrin	100–300 mg/dL	Anti-infective, iron carrier
Lysozyme	5–25 mg/dL	Anti-infective
Alpha-lactalbumin	200–300 mg/dL	Ion carrier (Ca^{2+}), part of lactose synthase
Casein	200–300 mg/dL	Ion carrier, inhibits microbial adhesion to mucosal membranes
Carbohydrate		
Lactose	6.5–7.3 g/L	Energy source
Oligosaccharides	1.0–1.5 g/L	Microbial ligands
Glycoconjugates	—	Microbial and viral ligands
Fat		
Triglyceride	3.0–4.5 g/L	Energy source
LCPUFA	—	Essential for brain and retinal development and for infant growth
FFA	—	Anti-infective

FFA, free fatty acids, produced from triglycerides during fat digestion in the stomach and intestine.
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Lactating mothers rarely need supplemental vitamins and minerals because most supplements do not appreciably affect milk nutrient concentrations. However, vegetarian mothers may need either a vitamin B_{12} supplement or consultation regarding acceptable food sources of this vitamin in their diet. Mothers who have undergone gastric bypass surgery will also need a source for vitamin B_{12} supplementation. Celiker and Chawla (2009) reported on the infant of a mother who had undergone gastric bypass surgery 6 years previous to the birth. The infant was born with congenital B_{12} deficiency. It is important that clinicians are aware that B_{12} deficiency may be congenital as well as occur as a result of being fed breastmilk deficient in vitamin B_{12}.

Although diet can affect the composition of fatty acids in the mother's milk, no definitive data support the practice of supplementing the mother with additional DHA to raise DHA levels in her milk or indicate that doing so results in any long-term benefits to her infant (Follett et al., 2003). The association between maternal supplementation with DHA and infant status is a saturable curve (Gibson, Neumann, & Makrides, 1997). As Follett and colleagues (2003) state, "Increasing supplementation of mothers is not associated with increased infant erythrocyte DHA if a level of 0.8% has been reached. Therefore, higher levels of DHA do not result in higher stores or improved function in the infant." In a review of 8 radomized controlled trials that included 1,567 women, LCPUFA supplementation of breastfeeding mothers

did not appear to improve children's neurodevelopment, visual acuity, or growth (Delgado-Noguera, Calvache, Bonfill Cosp, Kotanidou, & Galli-Tsinopoulou, 2015). Mitoulas (2000) advises that maternal supplementation of particular medium- or long-chain fatty acids may adversely affect other fatty acids such that the proportions of various fatty acids may become unbalanced, decreasing the proportion of some when others are increased. It is unknown what effect altered fatty acid profiles have on other human milk components or the recipient infant. Cheatham, Nerhammer, Asserhøj, Michaelsen, and Lauritzen (2011) examined whether fish oil supplementation during lactation affects processing speed, working memory, inhibitory control, and socioemotional development of children at 7 years of age. Early fish oil supplementation of breastfeeding mothers may actually have a negative effect on later infant cognitive abilities. The speed of cognitive processing scores were predicted by maternal n-3 LCPUFA intake during the study intervention period, which showed a negative relation (lower scores in the supplemented group). Stroop scores indicative of working memory and inhibitory control were predicted by infant erythrocyte DHA status at 4 months of age, again with a negative relation.

Fluoride supplementation is no longer recommended for infants younger than 6 months of age and only thereafter for infants living in communities with suboptimally fluoridated water supplies. Adding solid foods or infant formula before about 6 months of age may interfere with iron uptake in the breastfed infant and saturate the iron-binding capacity of lactoferrin, increasing the infant's risk of GI disease. Some commercially available bottled baby water contains added fluoride. Parents should be advised that breastfed infants do not require additional water or fluoride and should be told to check with their primary healthcare provider about the safety of such products advertised for young infants.

DEFENSE AGENTS

The immune system of human milk is a complex interplay between milk factors, the matrix of human milk, synergistic activities of defense components, differences in resident gut microflora of the infant, and individual differences in mothers and infants. Defensive characteristics of human milk are potent inhibitors of numerous diseases. As little as 2 weeks of exclusive breastfeeding reduces enterovirus infections in infants for up to 1 year (Sadeharju et al., 2007). Although breastmilk is often referred to as the infant's first immunization, it has been shown that breastfed infants also show a better developed response to a number of vaccines such as *H. influenzae* and pneumococcal (Silfverdal, Ekholm, & Bodin, 2007). Human milk provides the recipient infant with several tiers of protection against pathogens, resulting in the reduced incidence of a number of acute and chronic diseases and conditions long after breastfeeding has ceased (Hanson et al., 2002). These include the following:

- Nutrients that facilitate optimal development of the infant, including the immune system and intestinal mucosa (**Table 1-4**)
- Antibodies in the milk to specific environmental pathogens
- Broad-spectrum protective agents such as lactoferrin and fatty acids that provide a third layer of defense (see **Table 1-3**)
- Glycoconjugates and oligosaccharides
- Live cells
- Anti-inflammatory agents (**Table 1-5**)
- Immunostimulating agents

Table 1-4 Protective Components in Human Milk

Immune Protection	Function
sIgA, G, M, D, E	Specific antigen-targeted anti-infective activity
Nonspecific protection	Antibacterial, antiviral, and antimicrobial-toxin, enhancing newborn's immune system maturation
Major and minor nutrients	See Table 1-3
Nucleotides	Enhance T-cell maturation, natural killer cell activity, antibody response to certain vaccines, intestinal maturation, and repair after diarrhea
Vitamins	
A (beta-carotene)	Anti-inflammatory (scavenging of oxygen radicals)
C (ascorbic acid)	Anti-inflammatory (scavenging of oxygen radicals)
E (alpha-tocopherol)	Anti-inflammatory (scavenging of oxygen radicals)
Enzymes	
Bile salt–dependent lipase	Production of FFA with antiprotozoan and antibacterial activity
Catalase	Anti-inflammatory (degrades H_2O_2)
Glutathione peroxidase	Anti-inflammatory (prevents lipid peroxidation)
PAF acetylhydrolase	Protects against NEC (hydrolysis of PAF)
Hormones	
Prolactin	Enhances the development of B and T lymphocytes, affects differentiation of intestinal lymphoid tissue
Cortisol, thyroxine, insulin, and growth factors	Promote maturation of the newborn's intestine and development of intestinal host-defense mechanism
Cells	
Macrophages, PMNs, and cytokines	Microbial phagocytosis, production of lymphokines and lymphocytes, interaction with and enhancement of other protective agents
Cytokines	Modulate functions and maturation of the immune system

PAF, platelet-activating factor; PMN, polymorphonuclear.
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Jensen (1995) described common features of the biochemically diverse defense agents in human milk:

- An inverse relationship often exists between the production of these factors in the breast and their production by the infant over time.
- As lactation progresses, the concentrations of many of these factors in human milk decline. At the same time, the production at the mucosal sites of those very factors increases in the developing infant.
- Most components of the immunological system in human milk are produced throughout lactation and during gradual weaning.
- The factors are usually common to other mucosal sites.

Table 1-5 Anti-inflammatory Components of Human Milk

Component	Function
Vitamins	
A	Scavenges oxygen radicals
C	Scavenges oxygen radicals
E	Scavenges oxygen radicals
Enzymes	
Catalase	Degrades H_2O_2
Glutathione peroxidase	Prevents lipid peroxidation
PAF-acetylhydrolase	Degrades PAF, a potent ulcerogen
Antienzymes	
Alpha$_1$-antitrypsin	Inhibits inflammatory proteases
Alpha$_1$-antichymotrypsin	Inhibits inflammatory proteases
Prostaglandins	
PGE_1	Cytoprotective
PGE_2	Cytoprotective
Growth Factors	
EGF	Promotes gut growth and functional maturation
TGF-alpha	Promotes epithelial cell growth
TGF-beta	Suppresses lymphocyte function
Cytokines	
IL-10	Suppresses function of macrophages and natural killer and T cells
Cytokine Receptors	
TGF-alpha; RI, RII	Bind to and inhibit TGF-alpha

EGF, epidermal growth factor; IL, interleukin; PAF, platelet-activating factor; PGE, prostaglandin E; TGF, transforming growth factor.
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- They are adapted to resist digestion in the GI tract of the recipient infant.
- They offer protection via noninflammatory mechanisms.
- The agents act synergistically with one another or with defense agents produced by the body.

Types of Defense Agents

Human milk contains a potent mixture of agents that work synergistically to form an innate immune system that allows the nursing mother to protect her infant from a host of diseases.

Direct-Acting Antimicrobial Agents

- Oligosaccharides and glycoconjugates: These agents inhibit toxin binding from *Vibrio cholerae* and *E. coli* and interfere with the attachment of *H. influenzae*, *S. pneumoniae*, and *C. jejuni*.

- Proteins: Many of the whey proteins have direct antimicrobial actions. Lactoferrin competes with bacteria for ferric iron and disrupts their proliferation. A concentration of approximately 5–6 mg/mL is found in colostrum, with the concentration decreasing to 2 mg/mL at 4 weeks and to 1 mg/mL in milk thereafter. Lysozyme lyses susceptible bacteria. The approximate mean intake of milk lysozyme per day in healthy full-term infants is about 3–4 mg/kg/day at 1 month and 6 mg/kg/day at 4 months. Fibronectin facilitates the actions of mononuclear phagocytic cells. Complement components are present as well. Immunoglobulins represent important defensive agents. The predominant immunoglobulin in human milk is sIgA. IgE, the principal type of antibody responsible for immediate hypersensitivity reactions, is absent from human milk. Mucins defend against *E. coli* and rotavirus. Nucleotides are thought to enhance the growth of beneficial bacteria in the gut.
- Bifidus growth promoter.
- Defense agents: These are created from partially digested substrates from human milk. Fatty acids and monoglycerides are able to disrupt enveloped viruses (Isaacs, 2005), with lipid-induced active antiviral activity apparent in the infant's stomach within 1 hour of feeding (Isaacs, Kashyap, Heird, & Thormar, 1990).
- Leukocytes: These are living white cells present in human milk in highest concentrations during the first 2–4 days of lactation. The leukocyte component of human milk is made up of lymphocytes, macrophages, and polymorphonuclear leukocytes (neutrophils and eosinophils). Neutrophils and macrophages are the most abundant in human milk. Lymphocytes are found in human milk, with 80% of them appearing as T cells. They synthesize IgA antibody. Milk lymphocytes manufacture several chemicals, including gamma-interferon, migration inhibition factor, and monocyte chemotactic factor, all of which augment the body's own immune response.
- Anti-inflammatory agents:
  1. Factors that promote the growth of epithelium
     a. Cortisol
     b. Epithelial growth factor
     c. Polyamines
     d. Lactoferrin
  2. Antioxidants
     a. Ascorbate-like compound.
     b. Uric acid.
     c. Beta-carotene.
     d. Carotenoids such as lutein can also act as antioxidants. In the eye, certain other carotenoids (lutein and zeaxanthin) apparently act directly to absorb damaging blue and near-ultraviolet light, in order to protect the macula lutea. Because humans cannot synthesize lutein, this carotenoid must be supplied by dietary sources. Exclusively breastfed infants have six times the mean serum lutein concentration compared with infants consuming formula that is not supplemented with lutein, with four times more lutein needed in formula to achieve similar concentrations seen in breastfed neonates (Bettler, Zimmer, Neuringer, & DeRusso, 2010). With some formulas being supplemented with

these high levels of lutein, it remains unknown what side effects, if any, this might have on recipient infants.

3. Prostaglandins
4. PAF-AH
5. Immunomodulators
   a. Alpha-tocopherol.
   b. Cytokines regulate many epithelial cell functions and are at their highest in human milk when they are at their lowest in the recipient infant. Most of the cytokines that are known to be deficient in the neonate are found in significant amounts in breastmilk. Cytokines have been shown to be higher in milk samples from Asian mothers compared with African mothers and may vary according to race (Chirico, Marzollo, Cortinovis, Fonte, & Gasparoni, 2008). Some of these cytokines are IL-1B, IL-6, IL-8, and IL-10.
   c. Granulocyte-colony stimulating factor.
   d. Macrophage-colony stimulating factor.
   e. TNF-alpha, interferon, epithelial growth factor, TGF-alpha, and TGF-beta2.

CAN BREASTMILK TELL TIME?

The pineal hormone melatonin has been shown to exhibit a circadian rhythm when measured in body fluids. It has a hypnotic effect as well as a relaxing effect on the smooth muscle of the GI tract. Illnerova, Buresova, and Presl (1993) measured melatonin in human milk samples and found that it displayed a circadian rhythm. Melatonin in breastmilk that was expressed during the day was beyond the limits of detection, but breastmilk melatonin during the night was measured at 99 $\pm$ 26 pmol/L. Does breastmilk communicate time of day information to breastfed infants? Cohen Engler, Hadash, Shehadeh, and Pillar (2011) assessed the differences in the prevalence and severity of colic and nocturnal sleep between breastfed and formula-fed 2- to 4-month-old infants. They also characterized the melatonin secretion profile in human milk and formula. Their results showed that breastfed infants had a significantly lower incidence of colic attacks, lower severity of irritability attacks, and a trend for longer nocturnal sleep duration. Melatonin in human milk showed a clear circadian rhythm (higher at night) and was not measurable in infant formula.

The circadian rhythm of melatonin in breastmilk could contribute to the consolidation of infants' sleep–wake cycle until maturation of their own circadian system occurs. It might be useful to breastfeed in the dark for nighttime feedings, as exposure to light causes melatonin suppression in breastmilk. Milk banks might consider having donor mothers label day- and nighttime-expressed milk so that recipient infants can be fed with the milk that corresponds to day or night feeds (Sanchez-Barcelo, Mediavilla, & Reiter, 2011).

People with atopic eczema frequently complain of sleep disturbance, and their levels of blood melatonin are decreased in comparison to healthy subjects. Melatonin levels in breastmilk have only been reported in healthy mothers. Knowing that laughter increases natural killer cell activity in blood and free radical-scavenging capacity in saliva in healthy subjects, Kimata (2007) studied the effects of laughter on the levels of melatonin in the breastmilk of mothers with atopic eczema. Also studied was the effect

of feeding with breastmilk after laughter on allergic responses in the infants of the allergic mothers. All infants had allergic eczema and were allergic to latex and house dust mites. Mothers viewed a humorous 87-minute DVD or an 87-minute DVD on the weather. Laughter increased the levels of breastmilk melatonin in both allergic and healthy mothers. Feeding infants with milk that contained increased levels of melatonin reduced allergic responses in the infants. While laughter is often called the best medicine, it seems to have a clear place in influencing melatonin levels in breastmilk and positively affecting allergic, irritable, and colicky infants.

HUMAN MILK FORTIFICATION

Whereas the immunological factors in breastmilk are important for all infants, they are essential to preterm or ill infants whose immune systems may be immature or challenged by other health conditions. Nutrient fortification of preterm mothers' milk is seen in NICUs when an infant's needs exceed the capacity of breastmilk to provide selected nutrients in amounts that support a particular growth velocity.

Most NICUs do not use single-nutrient fortification but rely on multinutrient commercial fortifiers of differing composition. Preterm mothers' milk inhibits the growth of many bacteria, including *E. coli*, *Staphylococcus*, *Enterobacter sakazakii*, and group B *Streptococcus*; however, the addition of a cow's milk protein–based powdered human milk fortifier high in iron can neutralize the ability of human milk to kill these bacterial species (Chan, 2003). The addition to human milk of an older formulation of the same product decreased the IgA levels to *E. coli* and resulted in a 19% decrease in lysozymal activity in human milk, a measure of bacterial lysis (Jocson, Mason, & Schanler, 1997). The addition of a relatively large amount of iron directly into human milk may interfere with the ability of lactoferrin to bind iron and lyse bacterial cell walls. Quan et al. (1994) reported significant decreases in lysozyme content and IgA specific for *E. coli* when commercial fortifiers were added to fresh frozen milk. Chan, Lee, and Rechtman (2007) compared the antibacterial activity of preterm milk that had a commercial cow's milk–based high iron fortifier added to it or a human milk–based fortifier that was lower in iron. The commercial fortifier sample was shown to almost completely eliminate the bacterial inhibitory actions of the milk compared with the human milk fortifier sample, which retained its bacteriocidal activity. Fortifiers can be added to expressed hindmilk when the rate of weight gain is low. Some fortifiers can increase the osmolality of the milk, increasing the risk for GI irritation and feeding intolerance (Fenton & Belik, 2002; Rochow, Landau-Crangle, & Fusch, 2015; Srinivasan, Bokiniec, King, Weaver, & Edwards, 2004). They may also contain cow's milk protein and soy, which presents allergy as a potential side effect. The addition of human milk fortifier may temporarily delay gastric emptying and cause a short-term increase in gastric residuals and emesis.

Commercially available human milk fortifiers may add needed nutrients to mothers' expressed breastmilk but contain casein and whey proteins derived from bovine milk, lipids from both plants and microbial sources, and carbohydrates derived from plants. Although the addition of non–human milk-derived components to human milk is beneficial for promoting the growth of the preterm infant, bovine milk proteins have a different amino acid composition and are not as efficiently digested as human milk proteins. In addition, infants fed fortified human milk may exhibit higher rates of feeding intolerance and NEC. Sullivan and colleagues (2010) showed that NEC was reduced by 76% in preterm infants

whose mother's milk was fortified with a human milk-based fortifier compared with a bovine milk-based fortifier. The addition of a bovine fortifier to breastmilk is associated with an acute increase in GI tract inflammation (Panczuk et al., 2016). An alternative to fortifying human milk with commercially available human milk fortifiers is to use human milk products that are derived from donated human milk (Czank, Simmer, & Hartmann, 2010). Ganapathy, Hay, and Kim (2012) conducted a cost-effectiveness analysis of using a human milk–based fortifier compared with a bovine milk–based fortifier on the cost of NEC. They showed that an exclusively human milk–based feeding strategy saved $8,167 per infant who received a diet of exclusive human milk (expressed breastmilk plus a human milk–based fortifier). One study was done to determine the in vitro effect(s) of a bovine-based human breastmilk fortifier (HMF) on human intestinal cells. HMF increased the expression of BCL2/adenovirus E1B 19 kDa protein-interacting protein (Bnip3) and cell death. The outcome supported the hypothesis that HMF increases intestinal Bnip3 in vitro, and that the gene product triggers intestinal cell death (Diehl-Jones et al., 2015). This would raise the prospect of using caution when fortifying human milk with a nonhuman milk fortifier.

Lessaris, Forsythe, and Wagner (2000) showed that human milk fortifier differentially altered the biochemical profile of human milk with regard to TGF-alpha (a gut peptide that exerts a maturational effect on the neonatal gut) concentration and molecular mass profile. What effect this alteration in human milk biochemistry has on neonatal gut function remains unknown. The addition of iron and vitamin C to preterm human milk was shown to increase oxidative stress and reduce the content of mono- and polyunsaturated fatty acids (Friel, Diehl-Jones, Suh, Tsopmo, & Shirwadkar, 2007).

Caution should be exercised when adding a preparation to human milk, such as a fortifier, that neutralizes the milk's ability to destroy harmful bacteria, especially if the potential for bacterial contamination is contained in the fortifier itself. Fortified human milk can be stored in a refrigerator at 2–4°C (35–40°F) for no longer than 24 hours. Once fortified human milk is prepared, it can remain at room temperature for 4 hours (Telang et al., 2005). The American Dietetic Association recommends a hang time for fortified breastmilk of no longer than 4 hours at room temperature (25°C/77°F) (Robbins & Meyers, 2011). Fortified human milk should not be used if it is unrefrigerated for more than a total of 2 hours. After a bottle-feeding begins, it must be used within 1 hour or discarded.

Powdered infant formula is not sterile, including powdered human milk fortifiers. Some samples of powdered infant formula have been found to harbor *E. sakazakii* (*Chronobacter sakazaki*) (Baker, 2002). The FDA discourages the use of powdered forms in the NICU secondary to contamination risk (Himelright et al., 2002). The FDA also advises that "alternatives to powdered forms should be chosen when possible." However, in a study on the use of a new acidified liquid human milk fortifier, a number of poor infant outcomes were seen, such as poor growth, increased acidosis, increased incidence of NEC, metabolic acidosis, feeding intolerance, and diaper dermatitis (Thoene et al., 2014).

One way to avoid the unwanted side effects from use of a cow's milk–based liquid or powered fortifier is to use a donor human milk–derived fortifier, which would result in an exclusively human milk–based diet. In one study, a feeding protocol with early and rapid advancement using a human milk–based fortifier resulted in adequate growth and a low rate of extrauterine growth restriction (Hair, Hawthorne, Chetta, & Abrams, 2013).

Protein is often the limiting factor in breastmilk-fed to very preterm infants. While protein is higher during the early days of lactation, protein concentrations in mother's milk decreases quickly to the point

that it may not be sufficient for the needs of a very premature infant. Protein levels vary in maternal and banked donor milk, presenting the problem of how much protein is actually provided to an individual infant. Studies on increasing the amount of protein in fortifiers have shown better weight gain and fewer infants who remain in less than the 10th percentile for length (Miller et al., 2012). Rather than using a shotgun approach to fortification as in standard commercial fortifiers, there are two individualized methods of fortification that may provide a better outcome—adjustable fortification and targeted fortification (Arslanoglu, Moro, Ziegler, & The WAPM Working Group on Nutrition, 2010). Targeted fortification is a method that analyzes the expressed milk for the amounts of particular nutrients—in this case, protein. The amount of fortifier added to the milk is targeted such that the particular nutrient in question reaches a certain level in the milk. Adjustable fortification looks at the individual infant's metabolic response to the contents of the milk. There is no assumption of the infant's protein needs, as periodic sampling of the blood urea nitrogen levels determines adjustments needed in the level of protein intake. Adjustment in protein levels is based on the metabolic response of the infant to avoid under- or overfortification of protein (Arslanoglu, Moro, & Ziegler, 2006).

A best practice concept for the provision of human milk in the NICU is use of a centralized facility or a human milk management center that allows staff to analyze human milk, perform creamatocrits, conduct nutrient analysis, fortify milk under aseptic conditions, make skim milk, and tailor the milk to meet each infant's needs (Spatz, Schmidt, & Kinzler, 2014). One study reported use of a mobile milk cart for the preparation and fortification of breastmilk in a hospital that lacked a central space for the management of human milk nutrition for its preterm patients (Barbas, 2013). Technology for human milk analysis exists in the form of several human milk analyzers that a number of NICUs already utilize to augment their overall nutritional support plans. Given the large variability between inter-woman and intra-woman milk samples, the expressed milk of many mothers of preterm infants as well as pasteurized banked donor human milk may not contain the average 20 kcal/oz and 1.5 g/dL of protein (Adamkin & Radmacher, 2014). Precise analysis and individualized fortification give preterm infants an enhanced ability to have their nutritional needs adequately met.

MILK TREATMENT AND STORAGE

The antioxidant activity of human milk is diminished by both refrigeration and freezing, and over the course of time; however, it remains significantly higher than infant formula in antioxidant capacity despite how it is stored or when it is collected or ingested by the infant (Ezaki, Ito, Suzuki, & Tamura, 2008; Hanna et al., 2004). This is extremely important to preterm infants who are born before their antioxidant defense system is fully developed and functional (Baydas et al., 2002; Georgeson et al., 2002).

Preterm infants can experience oxidative stress from conditions such as infection and chronic lung disease and from interventions such as mechanical ventilation, oxygen therapy, IV nutrition, and blood transfusions. Some of the conditions and diseases common to preterm infants, such as NEC and retinopathy of prematurity, are often attributed to a profusion of oxidative stress and a deficiency in the oxidative defense system. Ingesting human milk rapidly increases antioxidant concentrations (Ostrea, Balun, Winkler, & Porter, 1986; Sommerburg, Meissner, Nelle, Lenhartz, & Leichsenring, 2000; Zoeren-Grobben, Moison, Ester, & Berger, 1993), partially explaining the reduced incidence of NEC and retinopathy of prematurity in infants protected by the consumption of human milk (Hylander, Strobino,

Pezzullo, & Dhanireddy, 2001). Preterm infants with a birth weight below 1,000 grams and a gestational age below 30 weeks may be at high risk of acquiring a symptomatic cytomegalovirus (CMV) infection through their mother's milk if she is CMV positive. Refrigeration and freezing of milk from preterm mothers may reduce the risk of transferring CMV to the infant but does not eliminate it (Hamprecht, Maschmann, Jahn, Poets, & Goelz, 2008). Most preterm infants remain asymptomatic when infected with CMV through breastmilk (Omarsdottir et al., 2015). One study showed that transmission of CMV from seropositive mothers via breastmilk to preterm infants did not appear to have major adverse effects on clinical outcomes, growth, neurodevelopmental status, or hearing function at 12 and 24 months corrected age (Jim et al., 2015). Inactivation of CMV can be accomplished by Holder pasteurization (heating to 62.5°C/144.5°F for 30 minutes) but can decrease the immunological components in breastmilk. Rapid high-temperature treatment of human milk (72°C/161.6°F for 5 or 15 seconds) has been shown to eliminate CMV infectivity without destroying many of the anti-infective capabilities of the milk (Lawrence, 2006). Ehlinger and colleagues (2011) studied methods to reduce CMV virus shedding into mother's milk as a method to lower the potential of postnatal CMV transmission to preterm infants. Rather than treat the milk, antibody-based maternal vaccines might prove more useful for protection against symptomatic postnatal CMV.

The goal of milk treatment and storage is to preserve the nutrient and protective properties of the milk. Heat treatment includes the following processes:

- Microwaving. Refrigerated or frozen breastmilk is sometimes microwaved by parents to quickly thaw or heat it, but microwaving for 50 seconds destroys 30.5% of the milk's IgA. Quan and coworkers (1992) found that microwaving breastmilk at 72–98°C (162–208°F) decreases the activity of lysozyme by 96% and that of total IgA by 98%. Treatment at low temperatures, 20–53°C (68–127°F), did not affect total IgA but decreased lysozyme by 19%. Subsequent *E. coli* growth 3.5 hours after treatment was 5.2 times greater than in the control at low microwave temperatures and 18 times greater at the high temperatures, showing dramatic loss of anti-infective factors. Microwaving bottles of expressed breastmilk also poses a risk of injury to the infant from hot spots in the milk, which could burn the tongue, mouth, and throat as well as cause scalding and full-thickness burn injuries to the body from exploding bottles and nipples. In spite of these microwaving hazards, one study showed that 10% of mothers heated their expressed breastmilk in a microwave (Labiner-Wolfe & Fein, 2013). Advise parents to place the bottle of expressed breastmilk under warm running water or in a bowl of warm water. Human milk is delivered to the baby at body temperature, leaving little reason to heat milk beyond 36.9°C (98.4°F).
- Pasteurization.
  - Human milk is most often pasteurized by milk banks for use as donor milk for preterm or ill infants, in special situations where the unique defense properties in human milk would be therapeutic, when infants cannot tolerate artificial baby milks, and so forth. Banked donor human milk can be obtained by prescription from any of the human milk banks listed in Appendix 1-2. Heat treatment can reduce the effectiveness of some of the defense factors in milk such as the B- and T-cell components of milk, lactoferrin, and IgA. The Human

Milk Banking Association of North America (2008) requires the use of Holder pasteurization (62.5°C/144.5°F for 30 minutes) to eliminate viral contaminants such as human immunodeficiency virus, human T-cell lymphotropic virus-1, and CMV as well as common bacterial contaminants. High-temperature, short-time processing can also be done with human milk at 70°C (158°F) or 75°C (167°F) for 15 seconds. Silvestre, Ruiz, Martinez-Costa, Plaza, and Lopez (2008) noted that the temperature applied is more important than the duration of application for preserving the bactericidal capacity of pasteurized human milk. They found that when comparing untreated milk, milk pasteurized at 63°C (145.4°F) for 30 minutes, and milk pasteurized at 75°C (167°F) for 15 seconds, growth of *E. coli* was reduced by 70.10%, 52.27%, and 36.9%, respectively. The lower milk processing temperature was preferable for preserving more bactericidal capacity. Refrigeration of this pasteurized milk did not further reduce its antibacterial properties.

- Baro and colleagues (2011) compared Holder pasteurization with high-temperature, short-time pasteurization regarding the effects on bile salt–stimulated lipase, lactoferrin, and components of the immune system. Holder pasteurization decreased the amount of bile salt–stimulated lipase and lactoferrin while the high-temperature, short-time method did not alter the activity of bile salt–stimulated lipase, lactoferrin, and IgA. Ley, Hanley, Stone, and O'Connor (2011) reported a reduction in adiponectin (32.8%) and insulin (46.1%) after Holder pasteurization of 17 different batches of donor human milk. Holder pasteurization also preserves the effects of HMOs (Bertino et al., 2008). Pasteurization can reduce the antioxidant capacity of expressed breastmilk (Silvestre, Miranda, et al., 2008). Vieira, Soares, Pimenta, Abranches, & Moreira (2011) reported a 5.5% reduction in fat and a 3.9% reduction in protein after milk was pasteurized. Valentine and colleagues (2010) showed that there was a reduction in DHA levels in pasteurized milk as well as lower concentrations of a number of amino acids. A newer high-temperature, short-time form of pasteurization has been developed that better maintains the immunological quality of the milk (Chen & Allen, 2001). The high-temperature, short-time method of milk pasteurization may hold more promise for reducing alterations of many human milk components.
- Pasteurized donor human milk is a precious resource whose supply is limited. The current standard protocol is that once pasteurized donor human milk is thawed, any remaining milk should be discarded after 24 hours to avoid microbial contamination (Jones, 2011). However, it has been shown that there is no evidence of microbial growth in pasteurized donor human milk when defrosted and stored at 4°C (39.2°F) for up to 9 days (Vickers, Starks-Solis, Hill, & Newburg, 2015). Longer storage times of defrosted donor milk could reduce waste of this valuable resource and increase the availability of human milk for vulnerable infants.
- Boiling human milk is probably the most damaging to its components (Ballard & Morrow, 2013).

- Evaporation. In an effort to engineer human milk as an appropriate fortifier for preterm milk, Braga and Palhares (2007) evaporated pasteurized human milk samples by removing 30% of the water. The pasteurized evaporated human milk met the recommended requirements of sodium,

potassium, magnesium, protein, fat, and lactose but not calcium and phosphorus requirements. Further studies are needed to see if this type of human milk manipulation is appropriate for preterm infants, especially if calcium and phosphorus were added.

- Refrigeration and freezing. Human milk can be safely stored under appropriate conditions to ensure infants receive their mother's or banked milk under a variety of conditions (Lawrence, 1999; Ogundele, 2000). Recommendations for storage times and temperatures vary among sources. The Academy of Breastfeeding Medicine recommends storage at room temperature, 16–29°C (60–85°F), for 3–4 hours as optimal; storage for 6–8 hours is acceptable under very clean conditions. Storage in the refrigerator at $<$ 4°C (39°F) for 72 hours is recommended as optimal, with a longer period of 5–8 days being acceptable under very clean conditions. In the freezer <-17°C (0°F) for 6 months is recommended as optimal, with 12 months being acceptable (Academy of Breastfeeding Medicine Protocol Committee, & Eglash, 2010). According to the Human Milk Banking Association of North America (2011), freshly expressed milk can be stored at room temperature for $<$ 6 hours, in the refrigerator for $<$ 5 days for a term infant and for $<$ 8 days for an older child, and in the freezer ideally for 3 months, optimally for $<$ 6 months, although 12 months is acceptable if in a deep freezer (−20°C/−4°F). Previously frozen milk that has been thawed in the refrigerator but not warmed can be stored at room temperature for $<$ 4 hours and in the refrigerator for $<$ 24 hours, but should not be refrozen.

  With previously frozen milk that has been brought to room temperature, the feeding should be completed within 1 hour at room temperature, then any remaining milk should be discarded. It can stay up to 4 hours in the refrigerator but should not be refrozen. If the infant has started feeding, the feeding should be completed and the milk can be placed in the refrigerator for up to 4 hours but should not be refrozen.

  Storage of milk overnight results in formation of a cream layer on top containing about 20% fat and a skim layer below it containing about 1% fat. These layers are generally mixed before being fed to the infant. However, in special situations, such as a slow-gaining or preterm infant, the top high-fat layer can be skimmed off and given to infants as physiological high-calorie supplements.

  Few data are available on storage times and conditions for fortified preterm human milk. Refrigerated fortified human milk should generally be used within 24 hours of when it was prepared (Jocson et al., 1997). Martínez-Costa et al. (2007) showed that refrigeration for 48 hours did not cause significant modifications in antibacterial properties of human milk, but storage beyond 72 hours significantly lowered the degree of bacteriolysis versus fresh milk.

  Ogundele (2002) reported that although the bactericidal activities of refrigerated samples diminished rapidly, up to two-thirds of the original activity level was maintained by freezing for up to 3 months. The ability of MFGM to adhere to suspended bacteria was gradually lost in frozen milk samples, whereas it was greatly enhanced during the first few days in refrigerated samples, before declining sharply. This study shows that loss of bactericidal activity in refrigerated milk is well compensated for by enhanced bacteria sequestration activity.

  Takci and colleagues (2012) reported that freezing at −20°C (−4°F) for 1 month did not cause statistically significant alteration in the bactericidal activity of expressed milk, but

storage for 2 months at this temperature significantly lowered the degree of bactericidal activity against *E. coli*. Bactericidal activity was protected when the milk samples were stored at −80°C (−112°F). There was no statistically significant difference in bactericidal activity of human milk against *E. coli* between freezing at −20°C and −80°C for 1 month; however, when milk was stored for 3 months, storage at −80°C was significantly more protective. Freezing at −20°C and −80°C for 1 and 3 months did not cause any significant change for bactericidal activity against *Pseudomonas aeruginosa*. Tacken and colleagues (2009) found that triglyceride (fat) and carotenoid concentrations in human milk remained stable after refrigeration and freezing as well as low-temperature microwave heating, except for lutein, which decreased after refrigeration and freezing.

Xavier, Rai, and Hegde (2011) showed that when expressed transitional and mature milk were refrigerated or frozen, the total antioxidant capacity of the milk was reduced by 10–20% for 48 hours of storage and by 15–30% for 1 week of storage. This emphasizes the importance of using fresh expressed breastmilk for preterm infants or milk that has been stored only for short periods of time to ensure that a robust antioxidant system is retained in the milk, as preterm infants are highly subject to conditions related to oxidative stress. Slutzah, Codipilly, Potak, Clark, and Schanler (2010) demonstrated that fresh mother's milk may be stored at refrigerator temperature (4°C/39.2°F) for as long as 96 hours with minimal changes and with the overall integrity of the milk during refrigerator storage being preserved.

Prolonged refrigeration of human milk for 96 hours has also been shown to maintain the milk's overall lipid composition. Bile salt–dependent lipase activity, LCPUFAs, and medium-chain saturated fatty acid concentrations were unaffected during the 96 hours of refrigerator storage in one study, as was the milk's overall oxidative status (Bertino et al., 2013).

Human milk also contains bacteria that represent an important factor in the initiation and development of the normal and proper neonatal gut microbiota (Collado, Delgado, Maldonado, & Rodriguez, 2009). While concern about the growth of pathological bacteria in stored human milk is valid, consideration needs to be given to whether cold storage affects the natural bacterial composition of human milk. Marin et al. (2009) found that cold storage of human milk at −20°C (−4°F) for 6 weeks did not significantly affect either the quantitative or the qualitative natural bacterial composition of the milk. However, it was interesting that bacterial counts in milk expressed with a mechanical breast pump were higher than in samples obtained by manual expression. Thawing and warming of previously frozen human milk changes the integrity of the milk, but does not lead to any adverse effects (Handa et al., 2014).

Human milk contains two lipases (enzymes that digest fat) that do not change the lipid structure during refrigeration. However, when milk is frozen, lipolysis can occur. As this process continues, soaps form that can change the taste and smell of the milk. It is speculated that some mothers have more lipase activity than others, which can lead to a more rancid milk that some infants reject. If this occurs, these mothers can be advised to heat their milk to a scald (not boiling) after expressing it, then immediately cool and freeze it to stop the fat from being broken down (Jones, 2011). Hamosh, Ellis, Pollock, Henderson, and Hamosh (1996) studied the stability of protein and lipids as well as bacterial growth in expressed milk of employed mothers and generated the recommendations for short-term milk storage included in **Box 1-3**.

Box 1-3 Storage of Breastmilk for Healthy Infants

38°C (100°F) room air: Safe storage for less than 4 hours
25°C (77°F) room air: Safe storage for as long as 4 hours
15°C (59°F): Safe storage for 24 hours (equivalent to a Styrofoam box with blue ice)
4°C (39°F): In a refrigerator 72 hours and probably longer
Previously thawed milk in a refrigerator: 24 hours
Freezer inside refrigerator compartment: 2 weeks
−20°C (−4°F) freezer separate from refrigerator: 3 to 6 months, or up to 12 months (Milk should not be stored in shelves on the door but in the back of the freezer. Storage containers should be placed on a rack above the floor of the freezer to avoid warming during the automatic defrost cycle in freezers above the refrigerator compartment.)
−70°C (−94°F) deep freezer: Longer than 12 months

Modified from Hamosh, M., Ellis, L. A., Pollock, D. R., Henderson, T. R., & Hamosh, P. (1996). Breastfeeding and the working mother: Effect of time and temperature of short-term storage on proteolysis, lipolysis, and bacterial growth in milk. *Pediatrics, 97*(4), 492–498; Williams-Arnold, L. D. (2000). *Human milk storage for healthy infants and children.* Sandwich, MA: Health Education Associates.

STORAGE

Breastmilk can be stored in a number of ways (**Table 1-6**) (Arnold, 1995; Manohar, Williamson, & Koppikar, 1997; Tully, 2000; Williamson & Murti, 1996). Because human milk is a live fluid, capable of engaging in biological processes, it is important to understand that it remains active during storage. The defense agents in human milk allow it to be stored under a number of conditions and in a variety of containers. When discussing storage conditions and containers, it is also important to differentiate between the needs and tolerances of a healthy full-term infant and those of a sick or preterm infant (Jones, 2011).

Parents may wish to use glass or polypropylene bottles for feeding and storage of breastmilk (Environmental Working Group, 2007). Polycarbonate bottles have been shown to contain bisphenol A (BPA), a plasticizer that mimics estrogen. BPA has been shown to be a developmental, neural, and reproductive toxicant that can interfere with healthy growth and body function (Gibson, 2007). The amount of BPA that leaches from heated baby bottles is within the range shown to cause harm in animal studies (Workgroup for Safe Markets, 2008). Patients should avoid clear, hard plastic bottles marked with a 7 or "PC." Infant formula itself can be contaminated with BPA. Liquid formula packaged in metal cans often contains BPA from the BPA-based epoxy resin coating that lines the formula cans (Cao et al., 2008).

Polyethylene bags are a popular container for storing expressed breastmilk, as they take up less space than bottles and can be connected directly to a breast pump. A recent study showed that the bactericidal activity against *E. coli* in refrigerated human milk stored in polyethylene bags decreased significantly compared with that in expressed milk stored in Pyrex bottles at 24 and 48 hours (Takci, Gulmez, Yigit, Dogan, & Hascelik, 2013). This effect could be due to selective binding of antibodies to the container's walls or surface. Fat loss from breastmilk stored in 9 different containers was reported to range from 8.2% to 9.4%, with the highest loss from a bag with an outer layer of polyester and an inner layer of polyethylene (Chang, Chen, & Lin, 2012). This fat loss was most likely due to the adherence of milk to

Table 1-6 Selected Characteristics of Various Breastmilk Storage Containers

Container Type	Description	Advantages	Disadvantages	Effect on Milk	Healthy Full Term	Child in Day Care	Preterm, Ill, Hospitalized
Polyethylene bags	Thin bottle liner, freezer bags, bags for storage, doubled bags	Inexpensive, do not require washing	Fragile, compromised by expansion of milk during freezing, easily punctured, leak, hard to pour, need support, volume marks inaccurate	Fat loss, easily contaminated, photodegradation of nutrients, loss of sIgA	OK	No	No
Hard plastic (bottles, urine specimen cups, graduated feeders, centrifuge tubes)	Polypropylene (cloudy, semi-flexible)	Good for short-term storage of colostrum in a refrigerator (retains good cell count and viability)	Can become scratched if frequently reused, increasing chance of bacterial buildup in scratches in stored milk	Small loss of cellular components	OK	OK	OK with tight-fitting lids, short storage times
	Polycarbonate (clear, hard plastic)	More durable, less prone to scratching					
Glass	Bottles, canning jars, sterile water bottles	Best for preserving immune components of milk	Can break or chip	Possible photodegradation, cellular components adhere to walls of container but drop back into the milk sooner than with plastic	OK	OK	OK with tight-fitting lids, better for longer storage, less loss of immune components
Stainless steel	Not typically used in the United States						
Other containers	Ice cube trays, popsicle molds	Handy items found around the house		No data	OK	No	No

Data from Arnold, L. D. W. (1995). Storage containers for human milk: An issue revisited. *Journal of Human Lactation, 11*, 325–328; Human Milk Banking Association of North America. (2006). *Best practice for expressing, storing and handling of mother's own milk in hospital and at home.* Raleigh, NC: Author; Manohar, A. A., Williamson, M., & Koppikar, G. V. (1997). Effect of storage of colostrum in various containers. *Indian Pediatrics, 34,* 293–295; Tully, M. R. (2000). Recommendations for handling of mother's own milk. *Journal of Human Lactation, 16,* 149–151; Williamson, M. T., & Murti, P. K. (1996). Effects of storage, time, temperature, and composition of containers on biologic components of human milk. *Journal of Human Lactation, 12,* 31–35.

the container wall, and less likely due to lipolysis, or lipid peroxidation. It is unknown what the clinical impact of this loss would be on an infant (up to 2.7 kcal/dl).

Many hospital neonatal care units use polypropylene sterile specimen containers to store expressed breastmilk. Such containers are used for collecting tissues or body fluids, not food. Most of these containers are made of polypropylene but may have polyethylene caps. They have many of the characteristics recommended in clinical guidelines, but there are few data on their chemical safety or the potential effect on the milk or infants' health from the use of such containers (Blouin, Coulombe, & Rhainds, 2014).

Clinical Implications

Raw cow's milk contains numerous antimicrobial agents, primarily in colostrum, that are beneficial to the calf. These, however, are of little significance to the human infant consumer of a cow's milk product. Bovine colostrum is not used in the milk supply, and the effectiveness of any defense agent is neutralized by the removal of all cells, pasteurization, and homogenization. Bovine milk is intended to be consumed unaltered by the calf and conveys no disease defense to the human infant. In contrast, the immunological composition of human milk has evolved and adapted to offset the postnatal delays in the development of the human immune system. The thymus is the central organ of the immune system in an infant. Within this organ, T lymphocytes mature and multiply, including killer cells that are important for defense and regulatory T cells that are important for the prevention of autoimmune diseases (Wing, Ekmark, Karlsson, Rudin, & Suri-Payer, 2002). The thymus of a fully breastfed infant is twice the size of a formula-fed infant's thymus (Ngom et al., 2004). The thymic index has been correlated to the percentage of CD8+ cells, with a higher CD8 percentage seen in breastfed compared with formula-fed infants at 8 months of age (Jeppesen, Hasselbalch, Lisse, Ersboll, & Engelmann, 2004). The infant is exposed to microbial immunogens while receiving protective agents in human milk. This process creates an attenuated immunization, in that the pathogenicity of the microbial agent is reduced by the accompanying immune factors. Human milk also prepares the recipient infant to resist certain immune-mediated diseases and a host of other acute and chronic diseases and conditions.

Because formula-fed infants have higher rates of illness, artificial feeding results in higher costs to the healthcare system. Ball and Wright (1999) calculated the costs to the healthcare system associated with three common childhood diseases: otitis media, lower respiratory tract illness (bronchiolitis, croup, bronchitis, pneumonia), and GI illness. The excess total direct medical costs incurred by never-breastfed infants during the first year of life for these three illnesses alone ranged between $331 and $475 per infant, more than the costs incurred by breastfed infants. The authors reported 2,033 excess office visits, 212 excess days of hospitalization, and 609 excess prescriptions for these illnesses per 1,000 never-breastfed infants compared with 1,000 infants exclusively breastfed for at least 3 months. If 90% of U.S. families could comply with medical recommendations to breastfeed exclusively for 6 months, the United States would save $13 billion per year and prevent an excess 911 deaths, nearly all of which would be in infants (or $10.5 billion and 741 deaths at 80% compliance) (Bartick & Reinhold, 2010). The state of Louisiana would save $216,103,368 if 90% of newborns in that state were exclusively breastfed for the first 6 months of life, and 18 infant deaths would be prevented (Ma, Brewer-Asling, & Magnus, 2013). At 80% compliance, Louisiana would save $186,371,125 and prevent 16 infant deaths. The family of a formula-fed infant incurs direct costs for care if uninsured or for copayments if insured, as well as nonmedical costs such as family care and transportation to and from the physician's office and/or hospital. Missed days of work are costly

to both employee and employer. If a parent misses 2 hours of work for the excess illnesses attributable to formula feeding, then more than 2,000 hours, the equivalent of 1 year of employment, are lost per 1,000 never-breastfed infants.

It is recommended that clinicians take the following steps to help ensure optimal breastfeeding outcomes:

1. Ensure that infants receive colostrum and human milk as the preferred food and first immunization.
2. Educate mothers/caregivers to avoid actions that dilute the anti-infective properties of human milk such as maternal smoking, mixing human milk with formula in the same container, and microwaving expressed milk.
3. Become familiar with data on the effects of heat treatment, refrigeration, freezing, and storage containers on the defense agents in human milk, and share this information with caregivers (**Table 1-7**).

Table 1-7 Results of Various Treatments on Human Milk

Treatment	Results
Refrigerated Storage	
4°C (39°F), 72 hours	Creaming, decrease in bacterial growth, possible lipolysis
−20°C (−4°F), 12 months	Lipolysis, possible demulsification and protein denaturation when thawed
−70°C (−94°F), indefinite	Possible demulsification and protein denaturation when thawed
Pasteurization	
56°C (132.8°F), 30 minutes	Inactivation of enzymes and antimicrobial proteins, partial loss of some vitamins, destruction of microorganisms
62.5°C (149.36°F), 30 minutes	Inactivation of enzymes and antimicrobial proteins, partial loss of some vitamins, destruction of microorganisms
70°C (158°F), 15 seconds	Inactivation of enzymes and antimicrobial proteins, partial loss of some vitamins, destruction of microorganisms
Microwave treatment	Decrease in IgA and lysozyme, substantial increase in coliforms
Sonication	Homogenization of milk-fat globules
Shaking vs. stirring	Vigorous shaking of breastmilk breaks the MFGM allowing fat to flow together into clumps irreversibly separating the fat from the milk. Stored breastmilk should be gently swirled to distribute the fat layer that has risen to the top of the container.
Selection/fractionization	Selection of high-protein milks, use of high-fat hindmilk
Supplementation	Addition of nutrients for preterm infants
Processing	Treatment of milk to isolate fats, proteins, and other components; fractions then added to milk
Centrifuge	Production of skim (fat-free) milk for infant with chylothorax; performance of creamatocrit
Manipulation of mother's diet	Change the fatty acid profile
Freeze-drying	Increases shelf life of human milk

Data from Jensen, R. G., & Jensen, G. L. (1992). Specialty lipids for infant nutrition. I. Milks and formulas. *Journal of Pediatric Gastroenterology & Nutrition*, 15, 232–245.

SUMMARY: THE DESIGN IN NATURE

Lactation is an ancient physiological process, dating back almost 200 million years. Human lactation and human milk have evolved to meet the needs and address the vulnerabilities of the human young. Milk intended for a four-legged, cud-chewing, nonverbal species may cause human infants to grow, but their growth and development will take a different trajectory than that of their breastfed counterparts. Infant formula and human milk components are not interchangeable. Artificial diets are not the same as human milk. The immuno-nutrition provided by human milk has no equal.

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Appendix 1-1

Summary Interventions Based on the Biospecificity of Breastmilk

1. Promote breastfeeding as the normal and best way to feed an infant.
   - Educate parents regarding:
     - The components of breastmilk and formula. Remind them that the two are not the same and that health and developmental outcomes can be different.
     - Defense factors in breastmilk. Explain that these factors protect the infant from acute and chronic diseases, resulting in fewer days of missed work. Formula cannot duplicate the health-protective factors in human milk and increases the risk of acute and chronic disease, autoimmune disease, and conditions such as overweight, obesity, asthma, and diabetes.
   - Inform parents of the developmental and cognitive differences when infants are fed formula, such as lower IQ scores and poorer school performance.
   - Assist parents to critically evaluate infant formula marketing by helping them understand that formula is not equivalent to human milk.
2. Keep mother and baby together during their hospital stay.
   - If separated, have the mother spend time in the baby's environment (nursery, special care).
3. Avoid giving the baby infant formula in the hospital or before gut closure occurs.
   - In breastfed infants at risk, hypoallergenic formulas can be used to supplement breastfeeding if medically necessary and mother's own milk or banked donor milk is not available.
4. Ensure 8–12 feedings each 24 hours, starting in the hospital.
5. Teach and assess proper positioning, latch, and milk transfer.
   - Document swallowing and ensure that the mother knows when her baby is swallowing milk.
   - If a baby is latched but not swallowing adequately, have the mother use alternate massage (massage and compress the breast during pauses between sucking bursts) to sustain sucking and swallowing.
6. Avoid using sterile water or dextrose water. If used, chart the amount and reason for use.
   - Educate the parents and extended family regarding the hazard of giving young breastfed infants bottles of water, even in hot weather.

- Avoid placing water bottles in the infant's bassinet in the hospital.
- Remind mothers that babies also nurse at the breast for thirst, frequently coming off the breast after only a few minutes of nursing.

7. Mothers do not need excessive amounts of water and simply need to drink to quench thirst.
   - Maternal consumption of water in excess of thirst does not increase milk supply and can decrease milk production.
8. Teach mothers to allow the baby to finish the first side before switching to the second breast.
   - For infants experiencing slow weight gain, mothers can be guided to finish the first side using alternate massage before offering the second side and to shorten the intervals between daytime feeds to increase the fat content of the milk.
   - When breastmilk is stored in the refrigerator or freezer, the fat rises to the top of the container; it can be skimmed off and given to slow gaining or preterm infants for extra calories.
   - Avoid vigorously shaking stored breastmilk to redistribute the fat layer. Doing so can damage some of the cellular components and inject air into the milk.
   - Infants whose mothers smoke need to be carefully monitored and to have frequent weight checks.
9. Prenatally, help parents understand the importance of exclusive breastfeeding for about 6 months, especially those with a family history of allergies and diabetes.
10. Reassure parents that it is uncommon for infant fussiness to be related to lactose intolerance. Primary lactose intolerance (lactase deficiency) is extremely rare.
11. Lactating mothers rarely need supplemental vitamins and minerals.
    - Fluoride supplementation is no longer recommended for infants younger than 6 months of age and only thereafter for infants living in communities with suboptimally fluoridated water supplies.
    - Mothers can maintain good vitamin D status by consuming vitamin D–fortified food and through exposure to sunlight. If mothers are vitamin D deficient, vitamin D supplements can be taken to raise their levels and the vitamin D levels in their milk.
    - Child care providers should be instructed to take infants outside for short periods each day.
      - Breastfed infants require 30 minutes of exposure to sunlight each week if wearing only a diaper or 2 hours per week if fully clothed without a hat to maintain normal vitamin D levels. Darkly pigmented infants require a greater exposure to sunshine to initiate the synthesis of vitamin D in the skin. Some infants may require additional vitamin D supplements, especially if they are dark skinned, experience reduced sunlight exposure, or demonstrate signs of suboptimal vitamin D intake.
      - Vegetarian mothers may either need a vitamin B_{12} supplement or consultation regarding acceptable food sources of this vitamin in their diet.
12. Adding solid foods or infant formula before about 6 months of age may interfere with iron uptake in the breastfed infant and saturate the iron-binding capacity of lactoferrin, increasing the risk of GI disease.

13. Some commercially available bottled baby water contains added fluoride. Parents should be advised that breastfed infants do not require additional water or fluoride and to check with their primary healthcare provider about the safety of products advertised for young infants.
14. Microwaving bottles of expressed breastmilk can interfere with the disease-protective factors in breastmilk and poses an injury hazard to the infant. Advise parents to place the bottle of expressed breastmilk under warm running water or in a bowl of warm water.
15. Human milk can be safely stored under appropriate conditions.

Appendix 1-2

Human Milk Banks in North America

UNITED STATES

Alabama
Mother's Milk Bank of Alabama
107 Walter Davis Dr.
Birmingham, AL 35209
Phone: (205) 942-8911
Website: http://www.mmbal.org

California
Mothers' Milk Bank
751 South Bascom Ave.
San Jose, CA 95128
Phone: (408) 998-4550
Fax: (408) 297-9208
Website: http://www.mothersmilk.org
Email: donate@mothersmilk.org

Colorado
Mothers' Milk Bank, a program of Rocky Mountain Children's Health Foundation
394 Marshall St, Suite 400
Arvada, CO 80002
Phone: (303) 869-1888
Website: http://rmchildren.org/mothers-milk-bank
Email: mothersmilkbank@rmchildren.org

Florida
Mothers' Milk Bank of Florida
8669 Commodity Circle
Suite 490
Orlando, FL 32819
Phone: (407) 248-5050
Email: info@milkbankofflorida.org

Illinois
Mothers' Milk Bank of the Western Great Lakes
1691 Elmhurst Road
Elk Grove Village, IL 60007
Phone: (847) 262-5134
Website: http://www.milkbankwgl.org
Email: info@milkbankwgl.org

Indiana
The Milk Bank
5060 E. 62nd Street, Suite 128
Indianapolis, IN 46220
Phone: (317) 536-1670
Toll-free: (877) 829-7470
Fax: (317) 536-1676
Website: http://www.themilkbank.org
Email: info@themilkbank.org

Iowa
Mother's Milk Bank of Iowa
Department of Food and Nutrition Services
University of Iowa Hospitals and Clinics
University of Iowa at Liberty Square

119 2nd Street, Suite 400
Coralville, IA 52241
Phone: (319) 384-9929
Website: http://www.uichildrens.org/mothers-milk-bank
Email: jean-drulis@uiowa.edu

Michigan
Bronson Mothers' Milk Bank
601 John Street, Suite N1300
Kalamazoo, MI 49007
Phone: (269) 341-6146
Fax: (269) 341-8365
Website: http://www.bronsonhealth.com
Email: Duffc@bronsonhg.org

Mississippi
Mothers' Milk Bank of Mississippi
2001 Airport Road, Suite 204
Flowood, MS 39232
Phone: (601) 939-6555
Website: http://www.msmilkbank.org

Missouri
Heart of America Mothers' Milk Bank at
St. Luke's Hospital
4401 Wornail Rd.
Kansas City, MO 64111
Phone: (816) 932-4888
Website: http://www.stlukeshealthsystem.org
Email: kcmilkbank@saint-lukes.org

Montana
Mothers' Milk Bank of Montana
734 Kensington Ave.
Missoula, MT 59801
Phone: (406) 529-2749
Email: mothersmilkbankofmt@yahoo.com

New England
Mothers' Milk Bank of Northeast
377 Elliot St.
Newton Upper Falls, MA 02464
Phone: (617) 527-6263
Website: http://www.milkbankne.org
Email: info@milkbankne.org

North Carolina
WakeMed Mothers' Milk Bank and
Lactation Center
3000 New Bern Ave.
Raleigh, NC 27610
Phone: (919) 350-8599
Website: http://www.wakemed.org/
Email: mothersmilkbank@wakemed.org

Ohio
Ohio Health Mothers' Milk Bank
4850 E. Main St.
Columbus, OH 43213
Phone: (614) 566-0630
Website: http://www.ohiohealth.com
Email: helen.harding@ohiohealth.com

Oklahoma
Oklahoma Mothers' Milk Bank Inc.
901 N. Lincoln Blvd., Suite #330
Oklahoma City, OK 73104
Phone: (405) 297-LOVE
Website: http://www.okmilkbank.org
Email: becky@okmilkbank.org

Oregon
Northwest Mothers Milk Bank
417 SW 117th Ave, Suite #105
Portland, OR 97225
Phone: (503) 469-0955
Email: joanne@nwmmb.org

Pennsylvania
CHOP Mothers' Milk Bank
34th and Civic Center Blvd.
Philadelphia, PA 19104
Email: CHOPmmb@email.chop.edu

South Carolina
Mothers' Milk Bank of South Carolina
Charleston, South Carolina
To find a depot site near you, please contact:
Ann Harvey Shrum, Milk Bank Coordinator
Director Email: Frampton@musc.edu
Phone: (843) 792-5415
Email: scmilkbank@musc.edu

Texas
Mothers' Milk Bank at Austin
2911 Medical Arts St., Suite 12
Austin, TX 78705
Phone: (512) 494-0800
Toll-free: (877) 813-MILK (6455)
Website: http://www.milkbank.org
Email: info@milkbank.org

Mothers' Milk Bank of North Texas
600 West Magnolia Ave
Ft. Worth, TX 76104
Phone: (817) 810-0071
Toll-free: (866) 810-0071
Website: http://www.texasmilkbank.org
Email: info@TexasMilkBank.org

Virginia
The King's Daughters Milk Bank
The Children's Hospital of the King's Daughters
400 Gresham Dr., Suite 410
Norfolk, VA 23507
Phone: (757) 668-6455
Email: Kdmilkbank@chkd.org

CANADA

Alberta
NorthernStar Mothers Milk Bank
103-10333 Southport Rd. S.W.
Calgary, Alberta T2W 3X6
Phone: (403) 475-6455
Fax: (888) 334-4372
Website: http://northernstarmilkbank.ca/
Email: contact@northernstarmilkbank.ca

British Columbia
BC Women's Provincial Milk Bank
BCW Lactation Services
1U 50-4450 Oak St.
Vancouver, BC V6H 3N1
Phone: (604) 875-2282
Website: http://www.bcwomensmilkbank.ca
Email: fjones@cw.bc.ca

Ontario
Rogers Hixon Ontario Human Milk Bank
Mount Sinai Hospital
600 University Avenue
Toronto, Ontario, Canada M5G 1X5
Phone: (416) 586-4800 x 3053
Website: http://www.milkbankontario.ca
Email: info@milkbankontario.ca