Chapter

4

The Biological Specificity of Breastmilk

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BREASTMILK IS SOMETIMES REFERRED to as *white blood* because it is considered similar to the placental blood of intrauterine life. Indeed, human milk is similar to unstructured living tissue, such as blood, and is capable of transporting nutrients, affecting biochemical systems, enhancing immunity, and destroying pathogens. With the use of sophisticated laboratory techniques, many scientific investigators have substantiated the life-sustaining properties of breastmilk. Organs themselves provide evidence of the profound influence of breastfeeding. For example, the thymus plays a role in the development of the immune system by providing the environment for T-cell differentiation and maturation. At age 4 months, the thymus is about twice as large in exclusively breastfed infants as in infants fed only infant formula. This difference in size continues until the child is at least 10 months old (Hasselbalch et al., 1999). Although thymus size can be influenced by many factors, it would not be unreasonable to generate a variety of hypothetical mechanisms whereby breastfeeding might influence thymic size (Prentice & Collinson, 2000).

Breastmilk, like all other animal milks, is species specific. It has been adapted throughout human existence to meet nutritional and anti-infective requirements of the human infant to ensure optimal growth, development, and survival. At birth the baby's immune system is small but complete. It expands in response to exposure of newly acquired bacteria but takes time before the infant develops full capacity to defend itself (Larsson, 2004). National and international health organizations consistently recommend that mothers breastfeed for the entire first year of life and thereafter as long as it is beneficial to the mother and infant (US Department of Health and Human Services, 2000).

Because an infant's birth weight normally requires about 4 to 6 months to double, the nutritional needs of the human baby must be substantially different from those of other mammals whose birth weight doubles much more rapidly. In addition, breastmilk enhances brain development: breastfed children may be more intelligent than children not breastfed. A meta-analysis of 11 studies in which confounding variables were adjusted showed an average 3.2-point higher cognitive development score among breastfed infants. This advantage was seen early on and continued through childhood (Anderson, Johnstone, & Remley, 1999). Chapter 18 presents a detailed discussion of this topic.

This chapter breaks down the general properties of human milk into specific components and describes for each component species-specific "biochemical

messages" that contribute to the well-being of the baby and mother. The chapter also explores the concept that these "messages" can be nutritional programming, triggering an early stimulus or insult during a critical or sensitive period with long-term effects on health and disease (Nommsen-Rivers, 2003; Lucas, 1998). Knowledge of biological constructs of lactation is critical to the clinician because it forms the rationale for effective practice in the clinical setting.

Milk Synthesis and Maturational Changes

Major components of human milk (protein, fat, lactose) are synthesized and secreted by the mammary secretory epithelial cells. Cregan (1999) labeled these cells "lactocytes." During pregnancy these cells further develop under the influence of prolactin. Four of the five milk-secretion pathways necessary for milk secretion are synchronized in the alveolar cell of the mammary gland. In the fifth pathway, the passage of components is between epithelial cells, rather than through them, and is known as the paracellular pathway (Neville, 2001).

Factors that influence milk composition include stage of lactation, gestational age of the infant, stage (beginning or end) of the feeding, frequency of the baby's demand for milk, and degree of fullness or emptiness of the breasts. As discussed in Chapter 3, lactogenesis occurs in two stages. Stage I refers to the development, during late pregnancy, of the mammary gland's capacity to synthesize milk. Stage II, traditionally based on postpartum day, refers to the onset of copious milk secretion or the time at which the mother feels her milk "coming in."

Arthur, Smith, and Hartmann (1989) and Humenick (1987) proposed two different biological markers as objective measures to define stages of breastmilk maturation. Arthur, Smith, and Hartmann hold that in the first stage of lactogenesis, average concentrations of lactose, citrate, and glucose are low. A sudden and rapid increase in concentrations of these components between 24 to 48 hours after birth heralds the transition from stage I to stage II lactogenesis. Stage II lactogenesis markers (lactose, citrate, and total nitrogen) take an additional 24 hours to attain concentration in women who have insulin-dependent diabetes compared with women who do not (Hartmann & Cregan, 2001).

Humenick et al. (1994), on the other hand, consider the breakdown of an emulsion dependent on the ratio of sterols plus phospholipids to fat content of milk (maturation index of colostrum and milk [MICAM]) as the biological marker for breastmilk maturation (Figure 4–1). Both of these methods

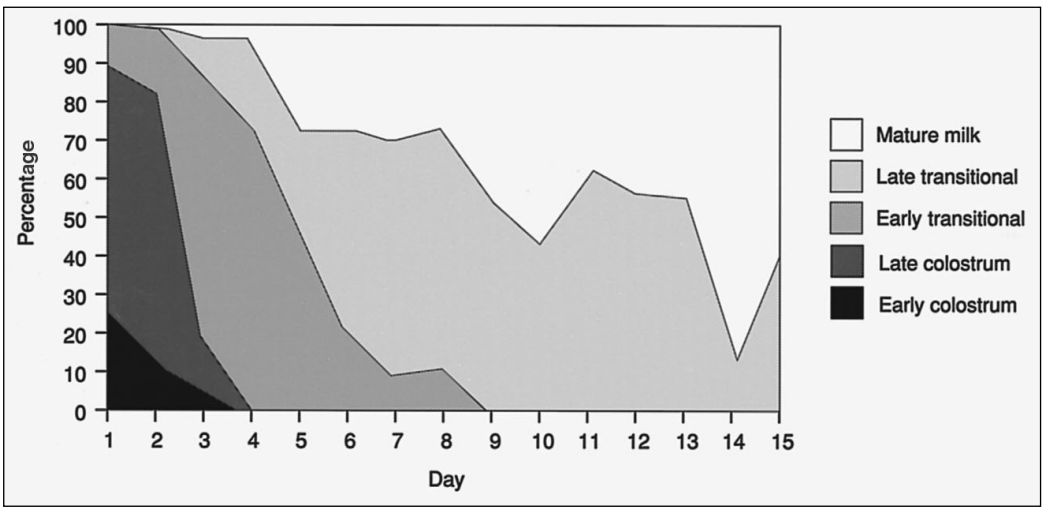

FIGURE 4–1 Milk type by day.

Source: Used with permission from Humenick SS. The clinical significance of breastmilk maturation rates. *Birth*. 1987;14:174-179.

appear to be valid in that they were positively related to greater milk yield (Casey, Hambridge, & Neville, 1985; Saint, Smith, & Hartmann, 1984), infant weight gain, and lower transcutaneous bilimeter readings (Humenick, 1987). These studies also show that breastmilk maturation during lactogenesis proceeds more rapidly in some mothers than in others and is not consistent with the coming in of the milk. Neville (2001) believes that the terms *colostrum* and *transitional milk* used to describe breastmilk during the early postpartum do not define clear-cut changes in milk composition and are not a useful distinction. Instead, they should be viewed as part of a continuum of events where changes in breastmilk occur rapidly during the first few days after birth and are followed by slow changes. The time at which mothers report that their milk comes in is highly variable and ranges from 38 to 98 hours after birth, with an average of 50 to 59 hours (Arthur, Smith, & Hartmann, 1989; Kulski & Hartmann, 1981; Hildebrandt, 1999).

Compared with mature milk, colostrum is richer in protein and minerals and lower in carbohydrates, fat, and some vitamins. This high concentration of total protein and total ash (minerals) and whey in colostrum and early milk gradually changes to reflect the infant's needs over the first two to three weeks as lactation becomes established. The total dose of such key components as immunoglobulin, which the infant receives from breastmilk, remains relatively constant throughout lactation, regardless of the amount of breastmilk provided by the mother. This happens because concentrations decrease as total volume increases as lactation is established; and, at weaning, concentration increases as total volume decreases.

At birth the neonate's intestine is sterile but it is rapidly colonized thereafter. Breastfed infants have an intestinal ecosystem that has a prevalence of bifidobacteria and lactobacilli. A symbiotic substance, human milk has the properties of both a pro- and prebiotic.

Energy, Volume, and Growth

Human milk is rich in nutrient proteins, nonprotein nitrogen compounds, lipids, oligosaccharides, vitamins, and certain minerals. In addition, it contains hormones, enzymes, growth factors, and many types of protective agents. Human milk contains about 10 percent solids for energy and growth; the rest is water, which is vital for maintaining hydration. The pH of early colostrum is 7.45; it falls to a low of 7.0 during the second week of lactation. Thereafter, the pH of milk remains at 7.0 and then rises gradually to 7.4 by 10 months. The significance of these changes is not known (Morriss et al., 1986). Infants can digest breastmilk much more rapidly than formula. The average gastric half-emptying time for breastmilk is substantially less (48 minutes) than for infant formula (78 minutes) (Cavell, 1981).

Healthy infants, even preterm infants, who consume enough breastmilk to satisfy their energy needs receive enough fluid to satisfy their requirements even in hot and dry environments (Almroth & Bidinger, 1990; Brown et al., 1986b; Cohen et al., 2000). Exclusive and prolonged breastfeeding in healthy infants enhances infant growth during the first 3 months of life and growth and does not affect the normal growth pattern during the first year (Kramer et al., 2002).

Caloric Density

The caloric content or energy density of human milk is generally considered to be 65 kcal/dl, although published values differ. Garza et al. (1983) reported 57.7 kcal/dl, Lepage et al. (1984) reported 66.6 kcal/dl, and Lemons et al. (1982) reported 72.2 kcal/dl. Using breastmilk as the "gold standard," the American Academy of Pediatrics (AAP, 1976) recommended a calorie content of 67 kcal/dl for commercial formulas.

Nature abhors waste, and breastmilk is efficiently utilized. During their first 4 months, exclusively breastfed infants attain adequate growth with nutrient intakes substantially less than the current dietary recommendation (Butte, Smith, & Garza, 1990). Energy requirement of breastfed infants is about 20 percent below recommended levels (Butte et al., 2000; Stuff & Nichols, 1989). Breastmilk of women who have been lactating for over 1 year has significantly increased fat and energy compared with breastmilk of women who have been lactating for shorter periods (Mandel et al., 2005). Caloric intake does not increase after solid foods are added to the baby's diet, strongly suggesting that the calorie value of breastmilk feeds is sufficient for the infants' needs.

Kilocalories of breastmilk ingested per kilogram by exclusively breastfed babies decrease significantly during the first few months of life (Table 4–1).

The energy intakes of breastfed and formula-fed infants differ significantly because their energy expenditure differs greatly. Total daily energy expenditure, minimal rates of energy expenditure, metabolic rates during sleep, rectal temperature, and heart rates are all lower in breastfed infants. Total body water and fat-free mass is lower, and body fat is higher in breastfed infants at 4 months of age (Butte et al., 1995). By 8 months, breastfed infants have consumed about 30,000 kcal less than bottle-fed infants (Butte, Smith, & Garza, 1990).

Although this difference in energy intake should result in about a 2.7-kg mean difference of weight, such is not the case. To explain this discrepancy, Garza, Stuff, and Butte (1986) suggested that (1) differences in intake in the general population are not as great as those found in the babies studied; (2) energy expenditure differs substantially between breastfed and bottle-fed infants; or (3) composition of newly acquired tissue differs between these two groups. A possibility is that the energy density of milk taken by a 4-month-old is higher on the average than that taken by the same baby 3 months earlier. The 4-month-old baby's suckle is more active, leading to a higher fat intake that more than compensates for the volumes needed, because breastmilk is used more completely and with less waste than is artificial milk. Differences in energy density among expressed breastmilk, preterm milk, foremilk, and hindmilk can be seen in Figure 4–2.

TABLE 4–1 Kilocalories of Breastmilk Ingested per Kilogram According to Infant Age

Time Post Birth	kcal per kg
14 days	128
3rd month	70–75
5th month	62.5

Source: Garza, Stuff, & Butte, 1986; Wood et al., 1988.

Milk Volume and Storage Capacity

The volume of milk must provide sufficient caloric energy to permit normal growth and development. Small amounts of colostrum—averaging about 37 ml (range, 7–123)—are yielded in the first 24 hours postpartum (Hartmann, 1987; Hartmann & Prosser, 1984); the infant ingests approximately 7 to 14 ml at each feeding (Houston, Howie, & McNeilly, 1983). This milk yield gradually increases for the first 36 hours, followed by a dramatic increase during the next 49 to 96 hours. By day 5, volume is about

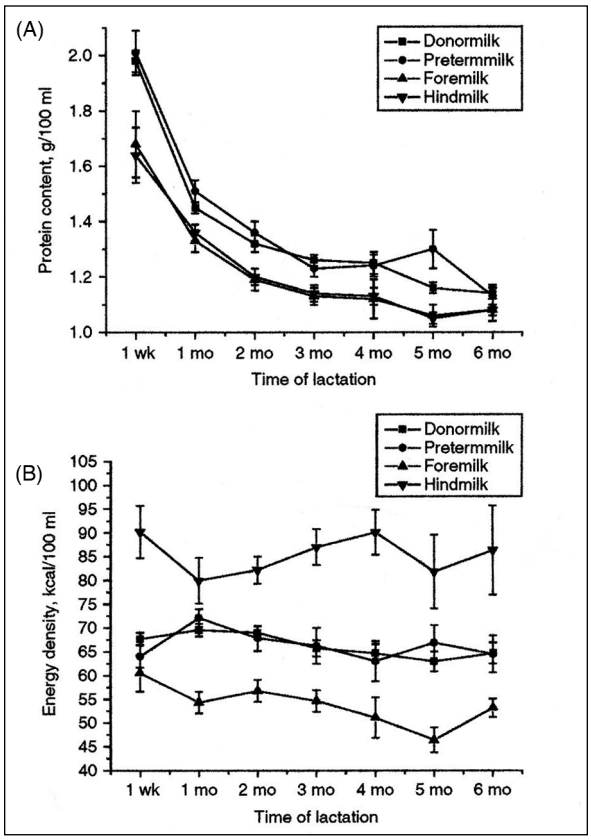

FIGURE 4–2 Mean protein content (A) and energy density (B) of full-term donor and preterm milk and full-term fore- and hindmilk during the first 6 months of lactation. Vertical lines show standard errors of means.

Source: Used with permission from Saarela AT, Kokkonen J, Koivisto M. Macronutrient and energy contents of human milk fractions during the first six months of lactation. *Acta Paediatrica*. 2005;94:1176-1181.

500 ml/day; it increases more slowly to about 800 ml/day at month 6 of full breastfeeding, with a range between 550 and 1150 (Daly, Owens, & Hartmann, 1993; Cox, Owens, & Hartmann, 1996; Cregan, Mitoulas, & Hartmann, 2002; Neville et al., 1988). These volumes are similar to others established by test-weighing the infant (using prefeeding and postfeeding infant weighings). As seen in Figure 4–3, the volume of milk taken by thriving breastfed infants varies little from 1 to 4 months. Breastmilk intake slowly declines as other foods are added to the baby's diet.

Even if a mother feels that she had insufficient milk to feed her first baby, health professionals should reassure women that it is well worth trying a second time. Multiparous mothers produce more breastmilk (about 140 ml) at 1 week than primiparous women (those giving birth for the first time) (Ingram, Woolridge, & Greenwood, 2001). Breastmilk of adolescent mothers is not different from breastmilk of adult women although adolescents breastfeed fewer times per day (Motil, Krtz, & Thotathuchery, 1997).

It is well established that breastmilk production and intake are related to infant demand. Infants have the capacity to self-regulate their own milk intake. This important concept of lactation has been extensively studied. Australian researchers measured the short-term rates of milk synthesis using a computerized system in which a camera relays video images to a computer that produces a model of the chest by active triangulation (Daly, Owens, & Hartmann, 1993). Their findings and practical applications are summarized in Box 4–1 (Cregan & Hartmann, 1999; Daly & Hartmann, 1995a,b). Breast storage capacity is important in determining how the mother meets the infant's demand for milk. Further discussion on how these research-based principles are used in lactation practice is found in later chapters.

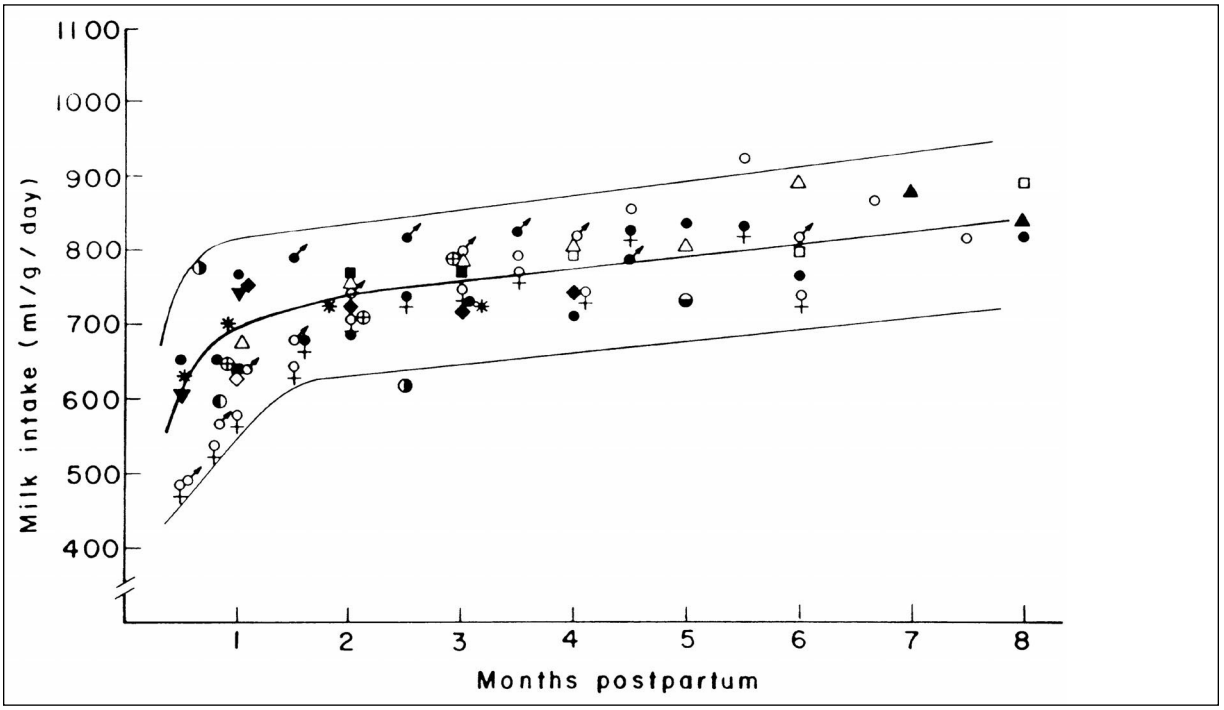

FIGURE 4–3 Milk intakes during established lactation. The lines show the smoothed mean from this study and ± 1 SD. Points are data from the literature obtained by test-weighing of fully breastfed infants.

Source: Used with permission from Neville MC et al. Studies in human lactation: milk volumes in lactating women during the onset of lactation and full lactation. *Am J Clin Nutr.* 1988;48:1375-1386.

BOX 4–1

Application of Physiological Principles

Principle from Physiological Research	Application in Practice
The breast does balance supply to meet the infant's demand for breastmilk.	Watch the baby for hunger cues.
The breast can rapidly change its rate of milk synthesis from one feed to the next.	Encourage the mother when she thinks she has "run out of milk."
The breasts have the capacity to synthesize more milk than the infant usually requires.	As above.
Breast production varies from one breast to the other; breasts operate independently of each other.	Reassure the mother that infant preference for one breast is normal.
The larger the breasts, the greater the milk storage capacity (i.e., the difference between maximum and minimum breast volumes during a 24-hour period).	Women with large breasts have more flexibility in feeding intervals.
There is no relationship between total milk storage capacity and total 24-hour milk production.	Women with smaller breasts can produce as much milk as women with larger breasts but they must breastfeed more often.
The greater the degree of emptying at a breastfeed, the greater the rate of milk synthesis after that feed.	Advise the mother to avoid fast "switching" from one breast to another and to try to empty one breast as much as possible.
The length of time between feeds (up to 6 hours) does not appear to decrease milk synthesis.	Feeding interval can be flexible once lactation is established.

Differences in Milk Volume Between Breasts

Milk output is more often greater from the right breast versus the left breast (Cox, Owens, & Hartmann, 1996; Daly, Owens, & Hartmann, 1993; Engstrom et al., 2007; Kent et al., 1999; Ramsay et al., 2005) even though right-handed mothers instinctively use the right hand to position the baby and breast, thereby making it easier to feed from the left breast. In addition, in all cultures there is a preference of mothers to hold their babies in their left arm, thus facilitating feeding at the left breast (Engstrom et al., 2007). A likely explanation is that the right breast receives more blood flow than the left breast (Aljazaf, 2004).

Daly, Owens, and Hartmann (1993) were able to determine the rate of synthesis of human milk. Figure 4–4a shows the volume of milk produced by a small-breasted woman who had a storage capacity of 111 ml for her right breast and a capacity of 81 ml for her left breast. Thus the maximum amount of milk that this woman appeared to be able to store

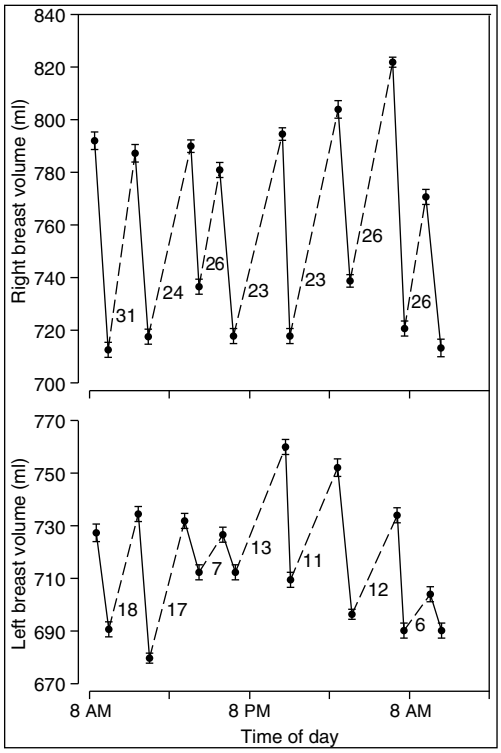

FIGURE 4–4A The right and left breast volume changes of one subject over a period of 24 hours.

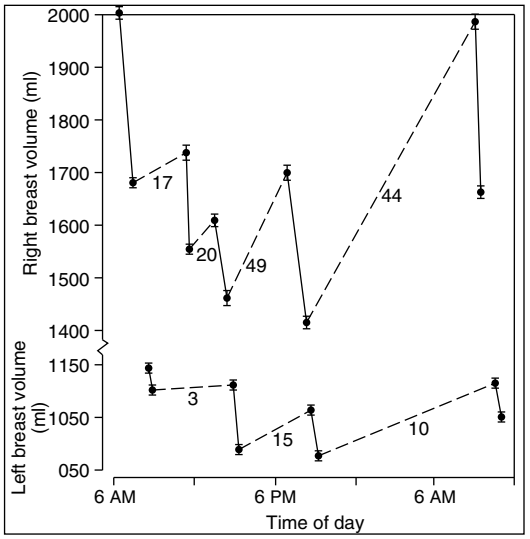

FIGURE 4–4B Breast-volume changes. The right and left breast volume changes of one subject over a period of 28 hours. Each point represents the mean plus or minus the standard error of the mean of replicate breast-volume measurement. Lines link prefeeding and postfeeding mean breast volumes. Dashed lines link postfeeding mean breast volume of a breastfeeding to the prefeeding mean breast volume of the next breast; their slope thus indicates rate of milk synthesis between the two breastfeedings. Rate of milk synthesis also is given by the number of (in milliliters per hour) accompanying each dashed line.

Source: Used with permission from Daly SE, Owens RA, Hartmann PE. The short-term synthesis and infant-regulated removal of milk in lactating women. *Exp Physiol.* 1993;78:209-220.

was about 20 percent of her infant's 24-hour milk intake. From her breast volume changes over time, it appears that her infant met its demand for milk by breastfeeding frequently. Conversely, Figure 4–4b displays a larger-breasted woman who produced similar volumes of milk but with larger storage capacities for her breasts (right breast, 600 ml; left breast, 180 ml), allowing her to store nearly 90 percent of her infant's 24-hour milk intake. Further, there was no relationship between total milk storage capacity and 24-hour milk production. Thus we can conclude that small breast size does not restrict a woman's ability to provide milk for her infant. On the other hand, mothers with a greater storage capacity do have more flexibility with patterns of breastfeeding.

There appear to be wide differences among women in the rate of milk synthesis, which among some women can be double or triple the rate of other women (Arthur et al., 1989; Daly et al., 1992). Milk volume between breasts also differs; left and right breasts rarely produce the same volume of milk (Kent et al., 2006). Milk yield from right breasts appear to be higher than that from left breasts, clearly demonstrating that the rate of milk synthesis within one breast is independent of the rate of milk synthesis in the other breast (Cox, Owens, & Hartmann, 1996; Daly et al., 1993).

At the same time, the amount of milk available in the breast is not necessarily an important determinant of the amount removed by the infant at feedings. Infant intake of breastmilk also varies widely. For example, at 5 months, infant intake of

breastmilk can range from 200 ml/day for partial breastfeeding to 3500 ml/day if a wet nurse is used (Neville & Oliva-Rasbach, 1987). These differences appear to be culturally based. Australian women, for example, have been reported to make more breastmilk than do US women. The average daily yield of well-nourished Australian mothers during the first 6 months of lactation was found to be in excess of 1100 ml in one study (Hartmann, 1987) and to range from 535 to 1078 ml in another (Daly et al., 1993). Mothers breastfeeding twins produce in excess of 2100 ml/day in the early months. Breast volume and production decrease in extended lactation. After 6 months of lactation, breast volume, milk production, and storage capacity all decline.

Seasonal changes in breastmilk volume may be influenced by some mothers' need to work during harvest and by their reluctance to introduce supplementary food for fear of diarrheal disease (Serdula, Seward, & Marks, 1986). The nutritional status of the mother does not appear to affect milk volume unless the mother is severely malnourished (Brown et al., 1986a; Forman et al., 1990).

A healthy, breastfeeding, full-term neonate breastfeeds an average of 4.3 times during the first 24 hours (range 0–11) and 7.4 times during the next 24 hours (range 1–22) (Yamauchi & Yamanouchi, 1990), and an overall median of 8 times per day after the first several days (see Hornell, 1999). Breastmilk intake shows little or no correlation with maternal factors, such as weight-for-height, weight gain, nursing frequency, maternal age, and parity (Dewey & Lönnerdal, 1983). Although birth weight is not a strong predictor of milk intake throughout lactation, infant weight at 1 month is. Thus lactation performance during the first 4 weeks postpartum is a strong predictor of milk output during the subsequent period of full lactation (Neville & Oliva-Rasbach, 1987).

Infant Growth

Normal human growth is greatest during infancy. The infant gains about 10 g/kg/day (about 5 to 7 oz/week) until about 4 weeks; then the gain drops to 1 g/kg/day (about 3 oz/week) by the end of the first year.

There are growth differences between breastfed and formula-fed infants. Infants breastfed exclusively have the same or somewhat greater weight gain in the first 3 to 4 months than do bottle-fed or mixed-fed infants (Fawzi et al., 1997; Juex et al., 1983; Motil et al., 1997). After this time, bottle-fed or mixed-fed infants clearly weigh more. The greatest differences are evident between 6 and 20 months of age, when breastfed infants are lighter than bottle-fed or mixed-fed infants (Dewey et al., 1993, 1995; Yoneyama, Nagata, & Asano, 1994). Increases in length and head circumference growth remain the same for both groups. Length is a reliable indicator for evaluating infant growth and the absence of significant difference in length between breastfed and nonbreastfed infants suggest that formula-fed infants are overfed. Small for gestational age infants who are breastfed show faster postnatal growth and are more likely to have significant catch-up growth than those who are fed a standard term infant formula (Lucas et al., 1997).

Color

Breastmilk comes in several colors. Normally, it is white or yellowish. It can be green if the mother is eating an unusual amount of green vegetables or taking a medication such as nifedipine—or it can be yellow from eating yellow vegetables such as carrots. The "rusty pipe syndrome" where the milk is tinged with pink or red is from old ductal bleeding. A variety of colors of breastmilk are shown in color photos in *The Breastfeeding Atlas* (Wilson-Clay & Hoover, 2005).

Nutritional Values

Around the world, breastmilk is remarkably stable, varying only within a relatively narrow range. Constituents of colostrum and breastmilk and their amounts are shown in Appendix 4-A of this chapter. A profile of lactose protein and lipid concentrations in human milk for the first 30 days of lactation is seen in Figure 4–5. Yet, because breastfeeding is an interactive process, the infant helps to determine composition of the feed. During weaning (involution phase), for example, the concentrations of sodium and protein in breastmilk progressively increase and the milk is saltier; in contrast, concentrations of potassium, glucose, and lactose gradually decrease (Prosser, Saint, & Hartmann, 1984).

Fat

The fat of human milk, which provides about one half of the milk's calories, is its most variable component. Fat varies from one mother to another and

from early to late lactation. The total fat content of human milk ranges from 22 to 62 g/L and is independent of breastfeeding frequency (Kent et al., 2006). Hindmilk contains at least twice the amount of fat compared to foremilk (Saarela, Kokkonen & Koivisto, 2005) (Figure 4–2). The energy density of preterm mother's milk is much greater than that of full-term mother's milk, owing to a 30 percent higher fat concentration (Atkinson, Anderson, & Bryan, 1980). Triglycerides, the main constituent (98–99 percent) of milk fat, are readily broken down to free fatty acids and glycerol by the enzyme lipase, which is found not only in an infant's intestine but in the breastmilk itself.

The lipid fraction of human milk provides essential fatty acids. The main concern about fatty acid intake is its effect on brain growth. The rate of brain growth is greatest in the last trimester of pregnancy and continues throughout the first year of life. Tissues of breastfed and formula-fed infants have distinctly different plasma fatty acid compositions. Breastmilk contains a wide range of long-chain polyunsaturated fatty acids (LC-PUFAs), which represent 88 percent of milk fat and are the most variable element in milk (Jensen, 1999). Interestingly, levels of fatty acids are low in lactating women, which indicates that transfer to breastmilk is at the expense of the maternal stores (Koletzko & Rodriquez-Palmero, 1999).

DHA and AA

LC-PUFAs include docosahexanoic acid (DHA) and arachidonic acid (AA), which are associated with higher visual acuity and cognitive ability of the child. An analysis of studies of human-milk feedings, DHA-supplemented, and unsupplemented formula documented advantages of DHA on visual acuity (SanGiovanni et al., 2000). But other studies show that DHA supplementation of the pregnant or breastfeeding woman has no impact on infant development nor visual function (Jensen et al., 1988; Malcolm et al., 2003). AA content in human milk is stable and does not vary widely throughout the world. The content of DHA, on the other hand, varies according to diet; therefore an increased supply of DHA may not always be beneficial. In the case of coastal populations with high intakes of fish, for example, little or no effects will be noted since infants already receive breastmilk with high DHA levels (Brenna et al., 2007).

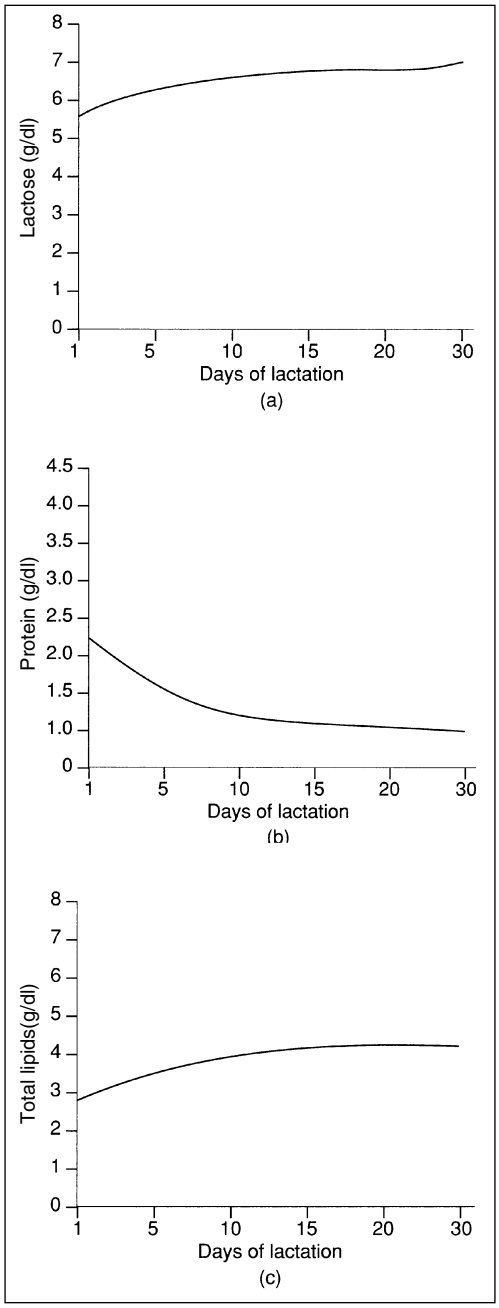

FIGURE 4–5 Lactose protein and total lipid concentration in human milk.

Another reason that not all breastfed infants have higher cognitive development and intelligence is that there are two forms of a gene known as RADS2. In infants who carry the C form of this gene, breastfeeding raises intelligence by producing an enzyme

that helps convert fatty acids in breastmilk into components that spur neurons to sprout connections which underlie intelligence, memory, and creativity. The 10 percent of infants who do not carry the C form of the gene lack the enzyme; therefore they derive no cognitive benefit from breastmilk (Caspi, Williams, & Kim-Cohen, 2007).

An essential fatty acid that enhances the developing human visual system, DHA is found in extremely high levels in the photoreceptors and the visual cortex and may ameliorate neurovisual developmental disorders such as the retinopathy of prematurity (Hylander et al., 2001). Breastfed infants accumulate DHA in the cortex, whereas formula-fed infants merely maintain the same amount of DHA present at birth. As a result, breastfed infants have higher levels of DHA than an age-matched group of formula-fed infants (Baur et al., 2000).

DHA and AA Controversy

In years past, commercial infant formula was fortified only with precursor essential fatty acids, α-linolenic acid, and linoleic acid. In an attempt to narrow the "nutrient gap" between formula and breastmilk, formula companies added DHA and AA to their infant formula after the FDA approved it as an additive in 2001. Critics charge that there is insufficient evidence that these additives are safe. DHA is extracted from fermented microalgae and AA from soil fungus. Human fatty acids are structurally different from those manufactured from plant sources and interact with each other in a special matrix that cannot be duplicated. The National Alliance for Breastfeeding Advocacy and the Cornucopia Institute filed a petition with the FDA to require a warning label on infant formula containing DHA and AA believing that parents have the right to know that these oils may be the cause of their infant's diarrhea or other gastrointestinal problems (see http://cornucopia.org/DHA/DHA_FullReport.pdf).

Breastfed infants have a higher proportion of acetic acid in the short-chain fatty acid spectra than do formula-fed infants, which, along with the monoglycerides generated by milk lipases, act against envelope viruses, bacteria, and fungus (Garza et al., 1987; Siigur, Ormission, & Tamm, 1993). The paler color, softer consistency, and milder odor of breastmilk stools, as compared with formula stools, are due in part to a higher concentration of fatty acid soaps (Quinlan et al., 1995). Fatty acid composition also differs between mothers whose babies develop atopic manifestation during the first year of life and those who remain healthy. Specifically, lower levels of α-linolenic acid and *n*-3 long-chain polyunsaturated fatty acid in mature milk of atopic mothers, especially in those with atopic babies, suggest that the low levels of this fatty acid could be associated with the development of atopy in the infants (Duchen, Yu, & Björkstén, 1999).

Although maternal dietary fat intake does not affect the total amount of fat in a mother's milk, the types of fat in the diet do influence the composition of fatty acids in milk. For example, black mothers in South Africa consuming a traditional maize diet have higher levels of monounsaturated fatty acid in their milk than do their urban counterparts who eat more animal fats (van der Westhuyzen, Chetty, & Atkinson, 1988). If the mother eats a high-carbohydrate, energy-replete diet, the proportion of triglycerides of medium-chain fatty acid increases (Garza et al., 1987).

The effects of breastfeeding can depend on the formerly breastfed individual's age. A prime example is cholesterol. Because cholesterol levels (10–20 mg/dl) in human milk are considerably higher than those of formulas derived from bovine milk (Wagner & Stockhausen, 1988), one would expect cholesterol levels in adulthood to be higher in breastfed individuals. The reverse, however, is true. Exposure to cholesterol in breastmilk may have long-term benefits for cardiovascular health. Coronary artery disease in persons up to 20 years of age is less frequent in individuals who were breastfed (Bergstrom et al., 1995). Serum total cholesterol and LDL levels (1) tend to be higher among breastfed infants compared to nonbreastfed infants, (2) are not different by infant-feeding group by 18 months of age (Demmers et al., 2005) and during childhood, and (3) tend to be lower among adults who were breastfed rather than artificially fed as infants (Owen et al., 2002). In addition to higher cholesterol concentration, adults who were bottle-fed had higher plasma glucose concentrations and impaired glucose tolerance (Ravelli et al., 2000).

Fat content of milk changes throughout a breastfeeding and, generally speaking, increases more steeply as more milk is taken. Fat content varies according to the degree to which the breast is emptied

at that breastfeeding, and that fat content increases markedly after most of the milk in the breast has been taken (Daly et al., 1993). The longer the time interval between two breastfeedings, the less likely the infant is to empty the breast and, thus, the lower the fat concentration will be in the subsequent feeding. Although the work of Daly et al. (1993) indicated that the pattern of feedings dictates the infant's fat intake, this is not necessarily the case. Woolridge, Ingram, and Baum (1990) studied mothers who fed in two patterns—either feeding at one breast or at two breasts during a feeding. The infants thus fed were able to regulate their fat intake and to achieve stable fat intakes in spite of disparate patterns of feedings. His findings support flexible "baby-led" feedings.

Lactose

Lactose, a disaccharide, accounts for most of the carbohydrates in human milk, although small quantities of oligosaccharides, galactose, and fructose are also present. Although lactose concentration is relatively constant (7.0 gm/dl) in mature milk, it is affected by maternal diet.

Lactose enhances calcium absorption and metabolizes readily to galactose and glucose, which supply energy to the rapidly growing brain of the infant. Some oligosaccharides promote the growth of *Lactobacillus bifidus*, thus increasing intestinal acidity and stemming the growth of pathogens (Dai et al., 2000).

The enzyme lactase is necessary to convert lactose into simple sugars that can be easily assimilated by the infant. The enzyme is present in the infant's intestinal mucosa from birth. Congenital or primary lactase deficiency is exceedingly rare (Montgomery et al., 1991). Lactose intolerance, however, is common in many mammals as they grow older and is the result of diminishing activity of intestinal lactase after weaning. In humans, lactose intolerance is more prevalent in adults of Asian and African heritage.

Probiotic and Prebiotic Bacteria

There is a current interest in so-called probiotic bacteria, which are certain *Lactobacillus* strains. Probiotic bacteria probably act by competing with other bacteria for nutrients thereby reducing the numbers of potentially pathogenic microbes. For example, certain *Lactobacillus* strains may ameliorate the symptoms of rotavirus infections (Larsson, 2004).

Protein

Protein content of mature human milk from well-nourished mothers is about 0.8 to 0.9 gm of protein per deciliter. Some of the protein in human milk is probably not nutritionally available to the infant; it serves immunological purposes instead. The high quality of protein in human milk and its precisely balanced quantity meet the energy needs of infants (Gaull, 1985; Raiha, 1985). As seen in Figure 4–2, the protein content decreases rapidly during the first several months (Saarela, Kokkonen, & Koivisto, 2005)

Human milk contains casein and whey protein. Casein and whey levels change as lactation progresses to meet the nutritional needs of the infant. Casein is lower in early lactation, then increases rapidly. Whey proteins are at their highest in early lactation and continue to fall. These changes result in a whey/casein ratio of about 90:10 in early lactation, 60:40 in mature milk, and 50:50 in late lactation (Kunz & Lönnerdal, 1992). Whey proteins are acidified in the stomach, forming soft, flocculent curds. These quickly digest, supplying a continuous flow of nutrients to the baby. By contrast, caseins (the primary protein in untreated bovine milk) form a tough, less digestible curd that requires high expenditure of energy for an incomplete digestive process.

Whey protein is composed of five major components: (1) α-lactalbumin, (2) serum albumin, (3) lactoferrin, (4) immunoglobulins, and (5) lysozyme. The latter three elements play important roles in immunological defense. Lactoferrin concentration of milk is higher in iron-deficient women as compared with well-nourished mothers; therefore, milk lactoferrin may also help protect the infant against iron deficiency (Raiha, 1985). A large number of other proteins (enzymes, growth modulators, and hormones) are present in low concentrations.

Nonprotein Nitrogen

Milk proteins are synthesized from amino acids derived from the bloodstream. Nonprotein nitrogen contains a number of free amino acids, including glutamic acid, glycine, alanine, valine, leucine, aspartic acid, serine, threonine, proline, and taurine. When amino acids exist singly or in free form, they are known as *free amino acids*. Of these, leucine,

valine, and threonine are essential amino acids; they must be consumed in the diet because the body does not manufacture them.

The percentage of protein in human colostrum is greater than that in mature breastmilk. This high level is due to the fact that in colostrum lactose and water haven't yet flooded the system and also because of the presence of additional amino acids and antibody-rich proteins, especially secretory IgA and lactoferrin. All 10 essential amino acids are present in colostrum and account for approximately 45 percent of its total nitrogen content.

Nucleotides

Nucleotides are low-molecular-weight compounds with a nitrogenous base. Necessary for energy metabolism, enzymatic reactions, and growth and maturation of the developing gastrointestinal tract, they also play several roles in immune function, including enhanced lymphocytic proliferation, stimulation of immunoglobulin production in lymphocytes, and increased natural killer-cell activity. Infant formula manufacturers seek to emulate the many nucleotides of breastmilk in their formulas (Cosgrove, 1998; Leach et al., 1995).

The importance to the baby of available nitrogen cannot be overstated. Atkinson, Anderson, and Bryan (1980) have shown that the concentration of nitrogen in the milk of women who deliver preterm infants is 20 percent greater than that in the milk of women delivering at term. The higher levels of available protein and fat in preterm mother's milk underscore the importance of using the milk of the preterm infant's mother rather than pooled milk from women in other stages of lactation (Table 4–2). Donated milk (not preterm milk), however, can be modified with components from other human milk to make a preterm human milk formula with none of the dangers of commercial bovine-based preterm formulas.

Vitamins and Micronutrients

The amounts of vitamins and micronutrients in human milk vary from one mother to another because of diet and genetic differences. However, it is generally true that human milk will satisfy the micronutrient requirements of a full-term healthy infant and thus can be taken as the primary yardstick of dietary recommendations, or reference values. Generally, as lactation progresses, the level of water-soluble vitamins in breastmilk increases, and the level of fat-soluble vitamins declines. The levels of fat-soluble vitamins (A, D, K, E) in human milk are minimally influenced by recent maternal diet, as these vitamins can be drawn from storage in the body.

Vitamin A

Human milk is a good source of vitamin A (200 IU/dl), which is present mainly as retinol (40–53 ng/dl). Required for vision and maintenance of epithelial structures, vitamin A is at highest levels in the first week after birth and then gradually declines. Deficiency of vitamin A is a serious health problem for young children in many developing countries, leading to blindness through damage to the corneal epithelium (xerophthalmia) and to increased morbidity from infectious diseases. The prolongation of even partial breastfeeding provides an important source of vitamin A to children in developing countries (Bates & Prentice, 1994).

Vitamin D

Human milk has very little fat-soluble vitamin D and breastfed infants can develop rickets, although it is uncommon. The risk of rickets is greatest for dark-skinned children living in inner-city areas, children whose clothing deters skin exposure to the sun, and children of mothers eating vegetarian diets that exclude meat, fish, and dairy products. For example, 10 percent of breastfed infants living in Iowa (41 degrees N) were deficient in vitamin D; most were dark skinned (Ziegler et al., 2006). The child who is adequately exposed to the sun (and thus to radiation-formed precursors of vitamin D) and whose mother consumes adequate nutrients usually does not need routine vitamin D supplements (Greer & Marshall, 1989). Concentrations in human milk range between 5 IU and 20 IU per liter. Increased vitamin D intake results in increased levels in human milk (Specker et al., 1985). Vitamin D may constitute an exception to the general rule that breastmilk micronutrient levels are protected from the effect of maternal deficiency. Scattered reports of rickets led the American Academy of Pediatrics in 2003 to recommend vitamin D supplements not only for children subject to certain conditions but to all infants (Gartner & Greer, 2003).

TABLE 4–2 **Composition of Term and Preterm Milk During the First Month of Lactation**

Nutrients	3–5 Days		8–11 Days		15–18 Days		26–29 Days	
	Full Term	Preterm	Full Term	Preterm	Full Term	Preterm	Full Term	Preterm
Energy (kcal/dl)	48	58	59	71	62	71	62	70
Lipid (gm/dl)	1.85	3.00	2.9	4.14	3.06	4.33	3.05	4.09
Protein (gm/dl)	1.87	2.10	1.7	1.86	1.52	1.71	1.29	1.41
Lactose (gm/dl)	5.14	5.04	5.98	5.55	6.00	5.63	6.51	5.97

Source: Adapted from Anderson, 1985.

Vitamin E

Human colostrum is particularly rich in vitamin E (tocopherol). Milk of mothers with preterm and term infants have similar levels of vitamin E (3 IU/100 kcal) and carotenoid levels, which are higher than those in bovine milk (Ostrea et al., 1986) or formula (Sommerburg et al., 2000). A deficiency of vitamin E in infancy can result in hemolytic anemia, especially in the premature infant. Because it is an antioxidant, vitamin E protects cell membranes in the retina and lungs against oxidant-induced injury. The requirement for vitamin E increases with intake of polyunsaturated fatty acids in breastmilk. Mothers who eat foods high in polyunsaturated fats and "fast foods" add to oxidant stress (Guthrie, Picciano, & Sheehe, 1977).

Vitamin K

Vitamin K, which is required for the synthesis of blood-clotting factors, is present in human milk in small amounts. A few days after birth, a baby normally produces vitamin K in sufficient quantities by enteric bacteria. However, neonates are susceptible to vitamin K deficiency until ingestion of copious amounts of breastmilk can promote gastrointestinal bacterial colonization, which enhances their low levels of vitamin K. Vitamin K supplements taken by the mother will increase breastmilk levels and infant plasma levels of the vitamin (Greer, 1999).

Insufficient vitamin K in neonates can lead to vitamin K-responsive hemorrhagic disease. To prevent hemorrhage and to raise prothrombin levels, 1 mg vitamin K is routinely given intramuscularly postpartum. Alternatively, a 1 mg oral dose of vitamin K administered at birth, at 1 to 2 weeks, and at 4 to 6 weeks is absorbed in the intestinal tract in amounts sufficient to prevent bleeding, and the infant is spared the pain of an injection and the risk of nerve damage always possible with any intramuscular injection. Formula-fed infants need not receive vitamin K routinely because formula (other than soy) contains vitamin K (Medves, 2002).

Water-soluble vitamins—ascorbic acid, nicotinic acid, B_{12}, riboflavin, and B_6—are readily influenced by the maternal diet. If maternal supplements are present, the vitamin levels in the milk increase and then plateau. Although supplementation may be beneficial for undernourished women, it is not necessary if the mother is well nourished and eating a diet that contain foods close to their natural state.

Vitamin B_{12}

Vitamin B_{12} is needed for early development of the baby's central nervous system. A mother eating a vegan diet (i.e., without meat or dairy products) may produce milk deficient in B_{12}. A deficiency of B vitamin folate during pregnancy is associated with neural tube defects. The March of Dimes' campaign to educate women on the importance of taking folic acid supplements during preconception and pregnancy has reduced neural tube deformities.

Unlike other micronutrients, folate (which is bound to a folate-binding protein) remains at the same level throughout all stages of lactation. Maternal stores of folate diminish slightly from 3 to 6 months to maintain milk folate levels (Mackey & Picciano, 1999).

Vitamin B_6

High pharmacological doses of vitamin B_6 have been reported to suppress prolactin and thus lactation. However, low nutritionally relevant doses have no effect on plasma prolactin or on breastmilk volume. Doses as high as 4.0 mg of vitamin B_6 taken as part of a vitamin B complex supplement are considered safe for both the lactating mother and the infant (Andon et al., 1985).

Minerals

The total mineral content in human milk is fairly constant. Excepting magnesium, minerals tend to have their highest concentration in human milk in the first few days after birth and decrease slightly in a consistent pattern throughout lactation, with little diurnal variation or variation within feedings. Maternal age, parity, and diet, even when supplemented, usually have minimal influence on mineral concentrations in milk, probably because of their regulation from maternal body stores (Butte et al., 1987; Casey, Neville, & Hambidge, 1989).

Sodium

Breastmilk sodium is elevated in early colostrum but falls dramatically by the third day postpartum and declines at a slower rate for 6 months. Elevated levels of sodium in human milk occur during weaning, in women with mastitis, and during the first months of gestation. A high concentration of sodium has also been found in the milk of mothers whose infants develop malnutrition, dehydration, and hypernatremia. Persistent high levels may be a marker for impaired lactation (Morton, 1994).

Zinc

Zinc is actively transported into the mammary gland. Zinc levels rise to a peak on the second day postpartum and then decline for the duration of lactation (Casey, Neville, & Hambidge, 1989). Zinc is eight times as abundant in human colostrum as in mature milk. Zinc requirements are relatively high in the very young infant and decrease with increasing age of the infant (Krachler, Rossipal, & Irgolic, 1998; Krebs & Hambidge, 1986). For fully breastfed infants, a combination of high absorption and efficient conservation of intestinal endogenous zinc retain enough zinc to meet the demands of infant growth in the face of modest intake (Abrams, Wen, & Stuff, 1996; Krebs et al., 1996). Zinc supplements when taken by women with normal zinc levels, do not affect the infant's growth, morbidity, or motor development (Heinig et al., 2006).

Zinc dramatically improves acrodermatitis enteropathica, a rare but serious congenital metabolic disorder that manifests itself in part in severe dermatitis (Evans & Johnson, 1980). While infants with this disorder continue to receive human milk, they have no symptoms. The high bioavailability of zinc in human milk is brought about by a low-molecular-weight zinc-binding ligand that facilitates zinc absorption. Abnormally low zinc levels in breastmilk are rare but can sometimes occur in mothers of infants with low birth weight or if there is an inherited genetic condition (Chowanadisai, Lönnerdal, & Kelleher, 2006). A slowing growth rate and persistent perioral or perianal rash (with or without diarrhea) in infants fed solely breastmilk may be due to zinc depletion (Atkinson et al., 1989). These infants should continue to breastfeed but they may require zinc supplementation. Maternal diet does not affect breastmilk zinc levels. In the rare case where a woman has low concentrations of breastmilk zinc, she is likely to have delivered her infant prematurely (Lönnerdal, 2000).

Iron

Although human milk has a small amount of iron (0.5–1.0 mg/L), breastfed babies rarely are iron deficient. They maintain their iron status at the same level as that of formula-fed infants receiving iron supplements for up to 9 months (Duncan et al., 1985; Salmenpera et al., 1986; Siimes et al., 1984). Breastfed infants are sustained by sufficient iron stores laid down in utero and by the high lactose and vitamin C levels in human milk, which facilitate iron absorption. Iron in human milk is absorbed five times as well as is a similar amount from cow's milk.

For the first few months of life, healthy, full-term infants draw on extensive iron reserves generally present at birth. Normally, an infant's hemoglobin level is high (16–22 gm/dl) at birth and decreases rapidly as physiological adjustment is made to extrauterine life. At 4 months of age, normal hemoglobin ranges between 10.2 and 15 gm/dl. Iron is well absorbed by older infants and is not affected by mineral intake from solid foods

in the diet or by vegetarianism (Abrams, Wen, & Stuff, 1996; Dorea, 2000; Lönnerdal, 2000). Breastmilk iron is only affected by the mother's iron intake when the mother is severely anemic, but not in those with mild to moderate anemia (Kumar et al., 2008).

Unless the infant is anemic, iron supplementation is not usually needed and may in fact be detrimental to the breastfeeding baby during the first 6 months after birth. Excess iron tends to saturate lactoferrin and thereby diminish its anti-infective properties. The authors of a randomized double-blind controlled trial concluded that routine iron supplementation of Swedish and Honduran breastfed infants with normal hemoglobin presented a greater risk of diarrhea (Dewey et al., 2002).

Calcium

Like iron, calcium appears in only small quantities in human milk (20–34 mg/dl). Yet babies absorb 67 percent of the calcium in human milk as compared to only 25 percent of that in cow's milk. Neonatal hypocalcemia and tetany are more commonly seen in the formula-fed infant, because cow's milk has a much higher concentration of phosphorus (calcium/phosphorus ratio of 1.2:1.0 versus 2:1 in human milk), which leads to decreased absorption and increased excretion of calcium. Calcium and phosphorus supplements are sometimes given to breastfed infants with low birth weight who should be monitored for hypercalcemia (calcium > 11 mg/dl) (Steichen, Krug-Wispe, & Tsang, 1987).

Magnesium

Magnesium is present in low levels in breastmilk and decreases in mature milk during 3 to 6 months (Picciano, 2001). Women who have been treated with magnesium sulfate for preeclampsia have high milk magnesium concentrations for the first day postpartum. After that time, levels return to normal (Lönnerdal, 2000).

Other Minerals

Copper levels are highest on the first few days postpartum, decrease for about 5 to 6 months, and then tend to remain stable. The mother's serum levels have no influence on milk concentration (Dorea, 2000). Selenium is usually higher in human milk than in formula (Kumpulainen et al., 1987; Smith, Picciano, & Milner, 1982). Minute amounts of aluminum, iodine, chromium, and fluorine are also found in breastmilk. Formula-fed infants ingest as much as 80 times more manganese than breastfed infants. Manganese enters the neonatal brain at a much higher rate than in the adult brain. Neonates are therefore at risk of neurotoxicity from excess manganese. High manganese levels in infant formula have been identified as being possibly related to neurocognitive deficits (Tran et al., 2002). Very little is known about the mechanisms or control of the secretion of trace elements into human milk. Table 4–3 lists the major components of human milk and their functions.

Preterm Milk

The milk of a woman who delivers a preterm infant is different from that of a woman who delivers at term, probably to meet the special needs of the low birth weight neonate. Compared with term breastmilk, preterm breastmilk has higher levels of energy, lipids, protein, nitrogen, fatty acids, some vitamins, and minerals (see Table 4–2). In addition, preterm breastmilk has higher levels of immune factors, including cells, immunoglobulins, and anti-inflammatory elements than term breastmilk. In the United States, the extra healthcare cost of not using human milk for preterm infants is estimated to be $9889 per baby (Wight, 2001). Chapter 13 also discusses preterm breastmilk.

Anti-Infective Properties

Breastmilk offers the newborn protection against disease and can reduce the risk of death for infants. When researchers compared CDC records of children who died between 28 days and 1 year, children who were breastfed had 20 percent lower risk of dying between 28 days and 1 year than children who were not breastfed. The longer the breastfeeding, the lower the risk for both black and white children (Chen & Rogan, 2004).

Only in the last few decades have investigators begun to identify the specific anti-infective components of human milk that make it a peerless substance for feeding the human infant. Breastmilk has been viewed from ancient times as living tissue and rightly so. This "white blood" contains enzymes,

TABLE 4–3 Major Components of Human Milk and Their Functions

Cells	Function
Phagocytes (macrophages)	Engulf and absorb pathogens; release IgA; polymorphonuclear and mononuclear.
Lymphocytes	T cells and B cells; essential for cell-mediated immunity; antiviral activity; memory T cells give long-term protection.
Anti-inflammatory Factors	
Prostaglandins PGE1, PGE2	Cytoprotective
Cytokines/chemokines	Immunodulating agents that bind to specific cellular receptors, activate the immune system, promote mammary growth, and move lymphocytes into breastmilk and across neonatal bowel wall. TGF-β is the dominating cytokine in colostrum.
Growth factors	Promote gut maturation, epithelial cell growth. EGF is a type of cytokine.
Enzymes	
Amylase	Facilitates infant digestion of polysaccharides.
Lipase	Hydrolizes fat in infant intestine; bacteriocidal activity.
Growth Factors/Hormones	
Human growth factors	Polypeptides that stimulate proliferation of intestinal mucosa and epithelium; strengthens mucosal barrier to antigens.
Cortisol, insulin, thyroxine cholecystokinin (CCK)	Promotes maturation of the neonate's intestine and intestinal host-defense process. Thyroxin protects against hypothyroidism; CCK enhances digestion.
Prolactin	Enhances development of B and T lymphocytes.
Lipids (Fat)	Major source of calories.
Long-chain polyunsaturated fatty acids (LC-PUFA)	DHA and AA associated with higher visual acuity and cognitive ability; breastmilk content dependent on maternal diet.
Free fatty acids (FFA)	Anti-infective effects.
Triglycerides	Largest source of calories for infant; broken down to free fatty acids and glycerol by lipase; types of fat depend on maternal diet.
Lactose	Carbohydrate, major energy source; breaks down into galactose and glucose; enhances absorption of Ca, Mg, and Mn.
Oligosaccharides	Microbial and viral ligands.
Glycoconjugates	Microbial and viral ligands.
Minerals	Regulates normal body functions; minimal influence by maternal diet.
Protein	
Whey	Contains lactoferrin, lysozyme, and immunoglobulins, alpha-lactalbumin.
Immunoglobulins (SIgA, IgM, IgG)	Immunity response to specific antigens in environment. SIgA pathways to mammary gland called GALT and BALT.

(Continues)

TABLE 4–3 Major Components of Human Milk and Their Functions (Continued)

Cells	Function
Lactoferrin	Antibacterial especially against *E. coli*; iron carrier.
Lysozyme	Bacteriocidal and anti-inflammatory; activity progressively increases starting 6 months after delivery.
Taurine	Abundant amino acid; associated with early brain maturation and retinal development.
Casein	Inhibits microbial adhesion to mucosal membranes.
Vitamins A, C, E	Anti-inflammatory action; scavenges oxygen radicals.
Water	Constitutes 87.5% of human milk volume; provides adequate hydration to infant.

immunoglobulins, and leukocytes in abundance. These components, one frequently enhancing the efficacy of another, account for most of the unique anti-infective properties of human milk. In some cultures, fresh breastmilk is used as eye drops to treat conjunctivitis; elsewhere, it is common practice to apply breastmilk on the skin to heal cracked nipples. Breastmilk provides several tiers of defense against diseases of infants that include a top tier of secretory antibodies against specific pathogens, next a tier of fatty acids and lactoferrin that provide broad-spectrum protection, followed by glyco-conjugates and oligosaccharides, each protecting against one or more specific pathogens (Newburg et al., 1998).

Recent innovations to infant formula have included probiotics as a way of making the flora of formula-fed babies more like that of the breastfed baby. Probiotics are viable nonpathogenic bacteria, so-called healthy bacteria that colonize the intestine and modify the intestinal microflora with less pathogenic bacteria than formula-fed infants. Prebiotics are nondigestible food components that stimulate the growth of bifidobacteria. Oligosaccharides, prebiotic soluble fibers, play an important role in postnatal development of intestinal flora but have not been found in infant formulas until recently.

Infant formula containing probiotics and prebiotics are new on the market as formula companies compete against each other to advertise and sell their new product. Human milk can be called a "symbiotic," a mixture of probiotics and prebiotics that is beneficial to the infant by improving the survival and implantation of live dietary microorganisms in the gastrointestinal tract.

Breastmilk provides a continuous source of microbes to the infant's gastrointestinal tract during the first few weeks after birth. Bacteria commonly isolated include staphylococci, streptococci, micrococci, lactobacilli, and enterococci. Although we tend to think of these bacteria as disease producing, some bacteria are natural microbes, and once in the gastrointestinal tract, such bacteria stimulate the neonate's immune system to grow and, thus, help protect the infant against infectious diseases (Martin et al., 2005a). Microbes that colonize the neonate's gastrointestinal tract during and after birth are safest if they are from the mother because she can provide defense against them. These microbes, especially those in the gastrointestinal tract, are a stimulus for the growth and development of the infant's immune system (Larsson, 2004).

Studies conducted in the later part of the last century measured the protectiveness of human milk to reaffirm its significance in preventing infections (Dewey, Heinig, & Nommsen-Rivers, 1995; Frank et al., 1982; Kovar et al., 1984; Kramer et al., 2001;

Pullan et al., 1980; Rosenberg, 1989; Victora et al., 1987). These studies are joined by increasing numbers of new research. The evidence is strongest for bacterial infections, gastroenteritis, and necrotizing enterocolitis but is less convincing for respiratory infections (Kramer et al., 2001).

Gastroenteritis and Diarrheal Disease

Wherever infant morbidity and mortality are high, breastfeeding conclusively helps to prevent infantile diarrhea and gastrointestinal infections (Almroth & Latham, 1982; Brown et al., 1989; Clavano, 1982; Duffy et al., 1986; Espinoza et al., 1997; Grantham-McGregor & Back, 1972; Habicht, DaVanso, & Butz, 1988; Jason, Niebury, & Marks, 1984; Koopman et al., 1985; Kovar et al., 1984; Mitra & Rabbani, 1995; Perera et al., 1999; Ravelomanana et al., 1995; Ruuska, 1992). Breastfeeding minimizes diarrhea both by providing protective factors and by reducing exposure to other foods or water that may contain enteropathogens (Van Derslice, Popkin, & Briscoe, 1994). As antibiotic resistance becomes a global problem, discoveries about the protective effect of breastfeeding become even more important (Hakansson et al., 2000).

Protection is dose dependent. In a review of field studies conducted to identify the effect of breastfeeding on childhood diarrhea in Bangladesh, children partially breastfed had a greater risk of diarrhea than had those who were exclusively breastfed (Glass & Stoll, 1989). Although breastmilk's protective effect is most easily demonstrated in areas of poverty and malnutrition, evidence of this protection is worldwide. In China, Chen, Yu, and Li (1988) showed that compared with breastfed infants, artificially fed infants are more likely to be admitted to the hospital for gastroenteritis and other conditions. In the Cebu region of the Philippines, giving water, teas, and other liquids to breastfed babies doubled or tripled the likelihood of diarrhea (Popkin et al., 1990). Young Nicaraguan children who develop rotavirus infections very early are partially protected by specific IgA antibodies in their mothers' milk. Rotavirus in stool samples correlated significantly with the concentration of antirotavirus IgA antibodies in colostrum (Espinoza et al., 1997). Canadian infants exclusively breastfed for the first 2 months had significantly fewer episodes of diarrhea than did infants bottle-fed from birth (Chandra, 1979). Breastfed children in Burma required less oral rehydration solution than did those who were not breastfed during the early acute phase of diarrhea and recovered from diarrhea more quickly (Khin-Maung-U et al., 1985).

A major methodological problem in breastfeeding research on disease is the dose-response effect—the greater the amount of breastmilk the infant receives, the greater the protection against disease; protection improves with the duration of breastfeeding. A lack of a clear consistent definition of breastfeeding is a flaw in many breastfeeding studies given the fact that there is a wide variation in feeding practices and that mothers often erroneously report supplements given to the infant (Aarts, Kylberg, Hornell et al., 2000; Zaman et al., 2002). Moreover, it is neither feasible nor ethical to randomly assign mother–infant dyads to breastfeeding or formula-feeding groups.

Kramer et al. (2001) got around this problem by looking at infant outcomes of hospitals and clinics in Belarus that introduced breastfeeding-friendly hospital initiatives and compared them with hospitals and clinics that continued their traditional practices. Results indicated that infants at the intervention site were more likely to breastfeed to any degree at 12 months and were more likely to be exclusively breastfeeding at 3 and 6 months. The risk of gastrointestinal infections and atopic eczema were significantly lower in the intervention group, but there was not significant reduction in respiratory tract infection. A follow-up study (years 2002–2005) on the children who were in the breastfeeding promotion intervention modeled on the Baby-Friendly Hospital Initiative scored higher means on the Wechsler Intelligence Test (Kramer, Aboud, Mironova et al., 2008).

Epidemiological evidence indicates that human milk continues to confer protection even with supplementation. Partial breastfeeding is better than no breastfeeding at all. This protection is specific to pathogens in the mother's and infant's environment. Moreover, the infant receives protection against the pathogens it is most likely to encounter. Table 4–4 summarizes the ameliorating and protective effects of human milk. We assume

that breastfeeding is the norm and that artificial feeding is a deviation from the norm that brings about hazards to infant health. Two infant health problems exacerbated by lack of breastfeeding—respiratory illness and otitis media—are discussed here. Others are discussed throughout this book, especially in Chapters 18 and 19.

Respiratory Illness

Studies of the protective effects of breastfeeding against respiratory tract infections are conflicting and complex because of error in parents' reports and other conditions not related to feeding. Several studies suggest that breastfeeding helps to prevent respiratory illnesses (Abdulmoneim & Al-Gamdi, 2001; Cushing et al., 1998; Lopez-Alarcon, Villalpando, & Fajardo, 1997), and others indicate little protection (Dewey, Heinig, & Nommsen-Rivers, 1995; Kramer et al., 2001). There is, however, strong evidence that breastmilk protects against respiratory syncytial virus (RSV) infection (Bell et al., 1988; Downham et al., 1976; Duffy et al., 1986; Holberg et al., 1991; Naficy et al., 1999; Newburg et al., 1998; Rahman et al., 1987). Downham et al. (1976) compared 115 infants hospitalized with RSV who were younger than 12 months with 162 control infants. Only 7 percent of the hospitalized infants were breastfed, compared with 27.5 percent of the control infants, a statistically significant difference. In the case of pneumonia caused by *Streptococcus*, researchers recently discovered a novel folding variant of α-lactalbumin that is a naturally occurring antibacterial compound in breastmilk (Hakansson et al., 2000).

As with gastroenteritis, the preventive effect of breastmilk is global. When Chen, Yu, and Li (1988) looked for an association between type of feeding and hospitalization of infants in Shanghai, they found that artificial feeding was associated with more frequent hospitalizations for respiratory infections during the first 18 months of life. About one fourth of hospitalizations of United Kingdom infants with lower respiratory tract infection could have been prevented if they had been exclusively breastfed (Quigley, 2007). In Brazil, babies who were not being breastfed were 17 times more likely than those being exclusively breastfed to be admitted to the hospital for pneumonia (Cesar, Victoria, & Barros, 1999). Similar protection has been established for *Haemophilus influenzae* bacteremia and meningitis (Cochi et al., 1986; Istre et al., 1985; Takala et al., 1989).

Otitis Media

Breastfeeding protects against ear infections (otitis media) for reasons that are not completely clear. However, immunological factors, the feeding position, and lack of irritation from bovine-based formula may explain it. Saarinen et al. (1982) followed healthy term infants for 3 years. Up to 6 months of age, no infant had otitis during the period of exclusive breastfeeding, whereas 10 percent of the babies who were given any cow's milk did. These significant differences persisted up to 3 years of age. Other studies (Aniansson et al., 1994; Dewey, Heinig, & Nommsen-Rivers, 1995) support an inverse relationship between ear infections and breastfeeding.

Controversies and Claims

In contrast to global evidence that breastfeeding helps to protect infants against health problems, Bauchner, Levanthal, and Shapiro (1986), and Leventhal et al. (1986) challenged the claim that breastfeeding protects infants in developed countries, citing lack of control for potentially confounding factors, such as low birth weight, parental smoking, crowding, sanitation, and other characteristics of socioeconomic status.

Howie et al. (1990) settled this controversy by examining the effect of breastfeeding on childhood illness in Scotland in a study using an adequate sample that met the methodological criteria set by Bauchner, Levanthal, and Shapiro (1986). Howie concluded that breastfeeding during the first 13 weeks of life confers protection against gastrointestinal illness beyond the period of breastfeeding itself. A few years later, Fuchs, Victor, and Martines (1996) questioned this long-term protection for diarrhea. They found that children who stopped breastfeeding in the previous 2 months were vulnerable to developing dehydrating diarrhea. Certain supplemental foods such as herbal teas prolonged diarrheal disease in Mexican children (Long et al., 1999).

TABLE 4–4 Amelioration of Disease in Infants and Children By Human Milk

Disease in Child	Ameliorating Properties of Human Milk
Acrodermatitis enteropathica	More efficient zinc absorption (Evans & Johnson, 1980).
Appendicitis	Anti-inflammatory properties (Pisacane et al., 1995b).
Asthma	Introduction of milk other than human milk prior to four months is a risk factor for asthma at age 6 years (Dell & To, 2001; Oddy, 2000). Risk of asthma reduced 4 percent with each additional month of exclusive breastfeeding (Oddy, 2004). Breastfeeding provides protection against asthma in children with family history of atopy (Gdalevich et al., 2001), especially if the child is exposed to tobacco smoke (Chulada et al., 2003).
Atherosclerosis	Having been breastfed is inversely associated with carotid intima-media thickness, carotid plaque, and femoral plaque (Martin et al., 2005).
Bacterial infections, neonatal sepsis	Leukocytes, lactoferrin, immune properties (Ashraf et al., 1991; Fallot, Boyd & Oski, 1980; Leventhal et al., 1986).
Cardiovascular disease	Dietary cholesterol in infancy elevates plasma total cholesterol levels through direct mechanism that persists only until weaning (Demmers et al., 2005). High-adult erythrocyte sedimentation rate, a moderate risk factor for coronary heart disease among those bottle-fed compared to those breastfed (Gunnarsdottir et al., 2007).
Celiac disease	Longer duration and greater exclusivity of breastfeeding associated with later diagnosis. Protects against development of villous atrophy in intestinal mucosa. Later introduction of gluten in breastfeeders (Ascher et al., 1997; Auricchio, 1983; Bouguerra et al., 1998; Greco et al., 1988; Ivarsson et al., 2000; Kelly et al., 1989; Logan, 1990).
Childhood cancer (lymphoma, leukemia neuroblastoma)	Modulates and strengthens defenses against carcinogenic insult by enhancing long-term development of infant immune system (Davis, 1998). Cancer cells undergo apoptosis (destruction) in human milk (Bener, Denic, & Galadari, 2001; Daniels et al., 2002; Davis, Savitz, & Graubard, 1988; Franke, Custer, & Tanaka, 1998; Gimeno & de Suza, 1997; Grufferman et al., 1998; Hakansson et al., 1995; Kwan et al., 2004; Martin et al., 2005; Mathur et al., 1993; Shu et al., 1995; Smulevich et al., 1999; Svanborg et al., 2003); Swartzbaum et al., 1991).
Colitis	Less exposure to cow's milk proteins (Anveden-Hertzberg, 1996; Jenkins et al., 1984; Rigas et al., 1993).
Crohn's disease	Uncertain (Bergstrand & Hellers, 1983; Koletzko et al., 1989; Rigas et al., 1993).
Diabetes, type 1 (IDDM)	Lack of antigenic peptides helps protect against autoimmune disease. Lessens risk 2–26%. Short duration of breastfeeding associated with induction of beta-cell autoantibodies (Borch-Johnson et al., 1984; Gimeno & de Suza, 1997; Kostraba et al., 1993; Mayer et al., 1988; Perez-Bravolt et al., 1996; Rosenbauer, Herzig, & Giani, 2008; Verge et al., 1994; Virtanen et al., 1992; Wahlberg, Vaarala, & Ludvigsson, 2006; Wasmuth & Kolb, 2000) and many more articles.
Dental caries	Less occurrence of dental caries (Erickson, 1999).

(Continues)

TABLE 4–4 Amelioration of Disease in Infants and Children By Human Milk (Continued)

Disease in Child	Ameliorating Properties of Human Milk
Gastrointestinal infection/ Diarrheal disease	Humoral and cellular anti-infectious factors (Dewey et al., 1995; Espinoza et al., 1997; Howie et al., 1990; Long et al., 1999; Sadeharju et al., 2007). Numerous other studies discussed throughout this text.
Gastroesophageal reflux	More rapid gastric emptying; lower esophageal pH (Heacock et al., 1992).
Hypertrophic pyloric stenosis	Uncertain; breastfeeding may prevent pyloric spasm and edema (Habbick, Kahnna, & To, 1989).
Hypertension	Children breastfed until at least 6 months have lower systolic blood pressure than those breastfed for a shorter duration (Lawlor, 2004).
Inguinal hernia	Hormones in breastmilk might stimulate neonatal testicular function to close inguinal canal and promote descent of testes. One-fourth incidence (Pisacane et al., 1995a).
Juvenile rheumatoid arthritis	Anti-inflammatory properties protect against autoimmune disease (Mason et al., 1995).
Liver disease	Protease inhibitors (including antitrypsin) protect children with alpha-antitrypsin deficiency (Udall et al., 1985).
Malocclusion	Physiological suckling patterns (Labbok & Hendershot, 1987).
Multiple sclerosis	Protects against autoimmune disease (Pisacane et al., 1994).
Necrotizing entercolitis	Immunological factors, macrophages, osmolarity of human milk, high levels of platelet-activating acetyl-hydrolase; suppression of (IL)-8 (Akisu et al., 1998; Lucas & Cole, 1990; Minekawa et al., 2004).
Otitis media	Antibody, T- and B-cell protection; lack of irritation from cow's milk; upright feeding position (Aniansson et al., 1994; Duncan et al., 1993; Sassen, Brand, & Grote, 1994).
Oral development	Fewer malocclusions and reduced need for orthodontic intervention because breastfed children have well-rounded, U-shaped dental arch. Fewer problems with snoring and sleep apnea in later life (Palmer, 1998).
Respiratory syncytial virus	IgA, IgG antibody transmitted to breastmilk and infant through gut-associated or bronchus-associated lymphoid tissue (GALT & BALT). Lactadherin, a glycoprotein binds to rotavirus and inhibits activity (Bell, 1988; Duffy et al., 1986; Holberg et al., 1991; Naficy et al., 1999; Newburg, et al., 1998; Rahman et al., 1987).
Lower respiratory tract disease	Meta-analysis of 33 studies on healthy infants in developed nations. Severe respiratory tract illnesses with hospitalization were tripled for infants who were not breastfed compared with those who were exclusively breastfed for 4 months (Bachrach, Schwarz, & Bachrach, 2003).
Retinopathy of prematurity	Antioxidants (inositol, vitamin E, beta-carotene) and DHA may protect against the development of retinopathy of prematurity (Hylander et al., 2001).
Sudden infant death syndrome	Uncertain; possibly anti-infectious, antiallergic (Ford et al., 1993; Gilbert et al., 1995; Kum-Nji, 2001).
Urinary tract infections	Antibacterial properties (sIgA) bind to bacteria and prevent them from reaching the urinary tract. Protection is greatest in girls (Marild et al., 2004; Pisacane et al., 1990).

In a prospective multicenter study on the effect of breastmilk in preventing necrotizing enterocolitis in premature infants, Lucas and Cole (1990) found that the disease was 6 to 10 times more common in exclusively formula-fed babies than in exclusively breastfed babies. This held true even though the human milk received was often pooled and not derived from the baby's mother. These findings support the contention that breastfeeding is more than a lifestyle choice; it has profound implications for the health of the child. Parents sometimes ask how long breastmilk protective effects last. Table 4–5 presents research on the length of breastfeeding and expected protection.

Chronic Disease Protection

The protection offered by breastmilk against illness extends beyond infancy to childhood and adulthood. Breastfeeding contributes to prevention of celiac disease, diabetes, multiple sclerosis, sudden infant death syndrome, childhood cancer, and many other health problems that are discussed throughout this book. The longer the duration of breastfeeding and the more complete exclusivity of breastmilk, the greater its protective effect.

Childhood Cancer

Does a mother's milk modulate the interaction between the developing infant immune system and infectious agents that helps protect an infant against carcinogenic insults? The evidence is conflicting. When Davis (1998) reviewed nine case-control studies on the association between infant feeding and childhood cancer, she confirmed that children who are never breastfed or are breastfed for a short term have a higher risk of developing Hodgkin's disease than those breastfed for at least 6 months. It is possible that a type of human α-lactalbumin found in breastmilk lessens the risk of childhood cancer. This alpha-lactalbumin, a protein-lipid complex called HAMLET, induces apoptosis-like death in tumor cells but leaves fully differentiated cells unaffected (Hakansson et al., 1995; Svanborg et al., 2003). A meta-analysis (Martin et al., 2005b) concluded that breastfeeding reduces the risk of childhood acute lymphoblastic leukemia (ALL) and acute myeloblastic leukemia (AML); however, the public health importance may be small. Increasing breastfeeding from 50 percent to 100 percent would prevent at most 5 percent of cases of childhood acute leukemia or lymphoma. In another meta-analysis, Kwan et al. (2004), found that long-term breastfeeding was linked to a 24 percent lower risk of ALL. Breastfeeding for 6 months or less appeared to reduce ALL risk by 12 percent; the next year this same researcher concluded that there was no evidence that breastfeeding affects the occurrence of childhood ALL (Kwan et al., 2005). Evidence showing that breastfeeding is protective against childhood cancer is inconsistent.

TABLE 4–5 Minimum Length of Breastfeeding for Protection Against Infectious Diseases

Health Problem	Minimum Length of Breastfeeding	Length of Protection	Source
Gastroenteritis/diarrheal disease	13 weeks	7 years	Howie, 1990
Otitis media	4 months	3 years	Duncan et al., 1993
Respiratory infections	15 weeks	7 years	Wilson et al., 1998
Wheezing bronchitis	—	6–7 years	Burr et al., 1993; Porro et al., 1993
Haemophilus influenzae, type b	—	10 years	Silfverdal et al., 1997
Hodgkin's disease	6 months	Not specified	Davis, 1998

Allergies and Atopic Disease

The incidence of food-induced allergic disease in children has been estimated to be between 0.3 to 7.5 percent (Metcalfe, 1984). Heredity is a significant predictor of allergic disease, even when the mother is on a milk-free diet during late pregnancy and lactation (Lovegrove, Hampton, & Morgan, 1994). Sixty percent of all those who will develop atopic eczema do so within the first year of life, and 90 percent do so within the first 5 years. Before 6 to 9 months of age, the infant intestinal mucosa is permeable to proteins; moreover, secretory IgA, which will later "paint" the mucosa and bind sensitizing proteins to itself, is not yet functioning effectively. After following 150 infants from birth to 17 years of age, Saarinen and Kajosaari (1995) concluded that breastfeeding is prophylactic against allergies—including eczema, food allergy, and respiratory allergy—throughout childhood and adolescence.

Cow's milk is the most common single allergen affecting infants. Proteins in cow's milk known to act as allergens include lactoglobulin, casein, bovine serum albumin, and lactalbumin. Modern heat treatment of formula has reduced—but certainly not eliminated—the allergic potential of these proteins. The problem is probably increased by the sizable dose of allergens in formula and by the large volume of formula ingested. At 2 to 4 months of age, for example, infants consume their body weight in milk each week. This is the equivalent of nearly 7 quarts per day for an adult—truly a macrodose!

Vomiting, diarrhea, colic, and occult bleeding are symptoms of allergy. It also affects the respiratory tract (runny nose, cough, asthma) and the skin (dermatitis, urticaria). Because the symptoms are varied and nonspecific, the diagnosis is often mistaken or missed.

At birth, the IgE system is defective in the potentially allergic infant, and problems arise if this system is activated by allergens. When the introduction of foreign proteins is delayed for 4 to 6 months, the baby's own IgA system is permitted to become more fully functional; thus allergic responses may be minimized or entirely avoided. Exclusive consumption of breastmilk facilitates the early maturation of the intestinal barrier and provides a passive barrier to potentially antigenic molecules until the baby's own natural barriers develop.

Chemokines IL-8 are higher in the breastmilk of allergic women who also have significantly more IL-4 in their milk, needed for the production of IgE. High levels of neonatal blood IgE are thought to predict later development of atopic symptoms. When the relationship between fecal IgE levels (a reliable indicator of serum IgE levels) was compared in infants 1 month old, formula-fed babies showed a higher incidence of high fecal IgE levels than did the breastfed infants (Furukawa et al., 1994).

A few breastfed infants develop atopic eczema. Of those who do, the culprit is often foods ingested by the mother—especially cow's milk. Cow's milk antigen can be detected in breastmilk (Axelsson et al., 1986; Cavagni et al., 1988; Odze et al., 1995; Paganelli, Cavagni, & Pallone, 1986). Early and occasional exposure to cow's milk protein sensitizes neonates so that even minute amounts of bovine milk protein in human milk may later act as booster doses that elicit allergic reactions (Host, Husby, & Osterballe, 1988). Prolonged breastfeeding exclusively or combined with infrequent exposure to small amounts of cow's milk during the first 8 weeks induces the development of IgE-mediated cow's milk allergy (Saarinen et al., 2000). By almost completely excluding milk, other dairy products, eggs, fish, beef, and peanuts throughout pregnancy and lactation, Chandra et al. (1986) documented a significant reduction in the incidence and severity of atopic eczema among breastfed infants of these mothers.

The "hygiene hypothesis" maintains that early exposure to microbes helps prevent allergies in children. As Larsson explains it, normal bacteria in the gut is important for the maturation of the immune system so that it learns to react against microbes rather than its own tissue. The infant develops an immunological tolerance to food, pollen, mites and other structures instead of reacting against them causing allergic reactions. Thus, having pets such as cats or dogs and ingestion or inhalation of endotoxin-containing material appears to help prevent allergies (Larsson, 2004).

Problems in conducting research on allergies and breastfeeding are manifold. Because it is not possible to classify mothers randomly into breastfeeding and nonbreastfeeding groups, are those infants with a family history of atopic eczema more likely to be breastfed because the parents are aware that it has a protective effect? When the infant is identified as

breastfed, does that mean that the baby received no other nutriments? If so, for how long was breastfeeding continued? After conducting a meta-analysis of 22 original research reports on infant feeding and atopic disease, Kramer (1988) decided that errors in research methods are conflicting and seriously flawed, which thus, precludes definitive conclusions.

Asthma

Outcomes of epidemiological and clinical studies on asthma and breastfeeding are inconsistent, and the longstanding question of whether breastfeeding prevents or reduces the incidence of asthma has been controversial. To help settle the question Gdalevich, Mimouni, and Mimouni (2001) conducted a meta-analysis of research on the effect of breastfeeding on bronchial asthma. They found 41 studies that showed a protective effect, five studies that had no association, and two studies that had a positive association. Twelve of these studies were prospective and met the standards for study methodology as determined by Kramer (1988). Meta-analysis of these 12 showed that exclusive breastfeeding during the first months after birth is associated with lower asthma rates during childhood (OR 0.70, 95% CI 0.60 to 0.81). Oddy (2000, 2004) has carefully researched the effect of breastfeeding on asthma in children in Western Australia and found an association with less exclusive breastfeeding and asthma.

Finally, in regard to breastfeeding's protective effect against chronic disease, Palmer (1998) makes a convincing case that artificial feedings alter normal early oral cavity development so much that it can cause later problems such as snoring, sleep apnea, and malocclusion. For the mother herself, breastfeeding promotes health because it helps to prevent breast and ovarian cancer (see Chapter 16).

The Immune System

The notion of a separate immune system as an integral part of a body capable of fighting disease at all of the body's surfaces is relatively new, starting in the mid 1900s. Although it had long appeared that breastfeeding enhances immunity, a cause-effect relationship was not acknowledged as a scientific fact until more was known about the existence of an immune system outside of the bloodstream. (Koerber, 2006). Because the human immune system is not fully developed at birth, infants are particularly vulnerable to infections and gastrointestinal illnesses. Breastmilk stimulates and supplements the infant's developing immune system.

The body's overall immune system is known as the *systemic immune system*. Another immune system, the *secretory immune system*, involves surfaces of the body (such as the breast) and acts locally. Lymphocytes in the secretory immune system are different from other lymphocytes. Sensitized to antigens found in the gastrointestinal or the respiratory tracts, these lymphocytes travel through mucosal lymphoid tissues (e.g., breasts, salivary glands, bronchi, intestines, and genitourinary tract) where they secrete antibodies.

Most antigens to which a mother has been exposed sensitize lymphocytes migrating to the breast. There they secrete immunoglobulins into the milk—hence, the term *secretory IgA* or *sIgA*. These components are described later in this chapter where immunoglobulins are discussed. Lawrence and Pane (2007) have presented a recent extensive review of the immunology of human milk.

Active Versus Passive Immunity

Immunity occurs actively and passively. Maternal antibodies passed to the fetus through the placenta before birth present an example of passive immunity. Passive immunological protection is only temporary, as the infant's immune system has not itself responded. Breastfeeding can also confer long-term protection by stimulating an active immune response. Active immunity is a specific immunity whereby the immune system formulates a long-term memory of exposure to a certain antigen. Later exposure to the same antigen will produce an immune response. Poliovirus or rubella immunization of women or any attenuated virus immunization of the mother provides active immunity to the infant, as the virus will likely appear in her milk and thus immunize the infant. Reports indicate enhanced vaccine responses in breastfed infants compared with those not breastfeeding. After being vaccinated for measles-mumps-rubella (MMR), only breastfed children had increased production of interferon-gamma (Pabst et al., 1997). Another

example of active immunity is the breastfed infant's immune response to cytomegalovirus in human milk.

Cells

Human milk contains two main types of white cells (leukocytes): phagocytes and lymphocytes (Figures 4–6 and 4–7). Although phagocytes (mostly macrophages) are most abundant (90 percent), the lymphocyte population (10 percent) provides significant protective effects to the recipient infant. The concentration of these cells and the predominant cell type vary with the duration of lactation. After birth, the number of these cells is higher than at any other time; they decline progressively thereafter.

Phagocytes

Macrophages, a type of leukocyte, are the dominant phagocyte in human milk. They engulf and absorb pathogens. Macrophages release IgA, although they probably do not synthesize it. Macrophages are both polymorphonuclear (PMN) and mononuclear. Because PMN numbers increase dramatically during inflammation of the breast, they may function to protect the mammary tissue per se rather than to impart protection to the newborn (Buescher & Pickering, 1986). Macrophages also produce complement, lactoferrin and lysozyme (discussed later in this chapter). Neutrophils are yet another phagocytic leukocyte. Short lived but effective, they are first to arrive at an inflamed site, such as that which may occur during mastitis.

Lymphocytes

Lymphocytes are also leukocytes and include T cells, B cells, and assorted T-cell subsets. Lymphocytes compose about 4 percent of the total leukocytes in early lactation; about 83 percent of the lymphocytes are T cells that appear to transfer through human milk to infants (Wirt et al., 1992). The various ways in which lymphocytes recognize and help to destroy antigens are called *cell-mediated immunity*. Such immunity is important in the destruction of viruses because the cells within which viruses live shield them from the action of antibodies. Formula-fed and breastfed infants have different types of lymphocyte subsets (Hawkes & Gibson, 2001).

Decreasing rapidly in the first week after birth and continuing to decline steadily, T cells are a special and separate immune component that can be activated into memory T cells (Wirt et al., 1992).

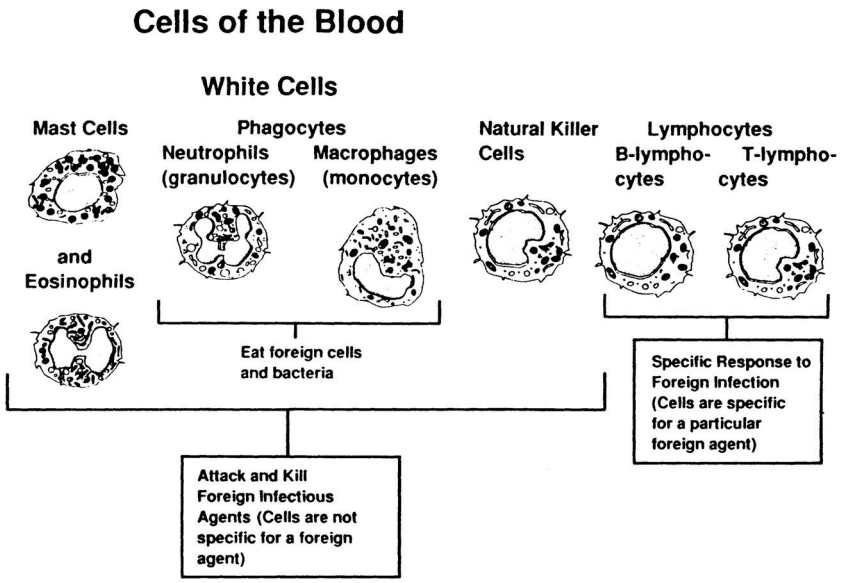

FIGURE 4–6 White cells of the blood.

Source: Fan, Conner, & Villareal, 1989.

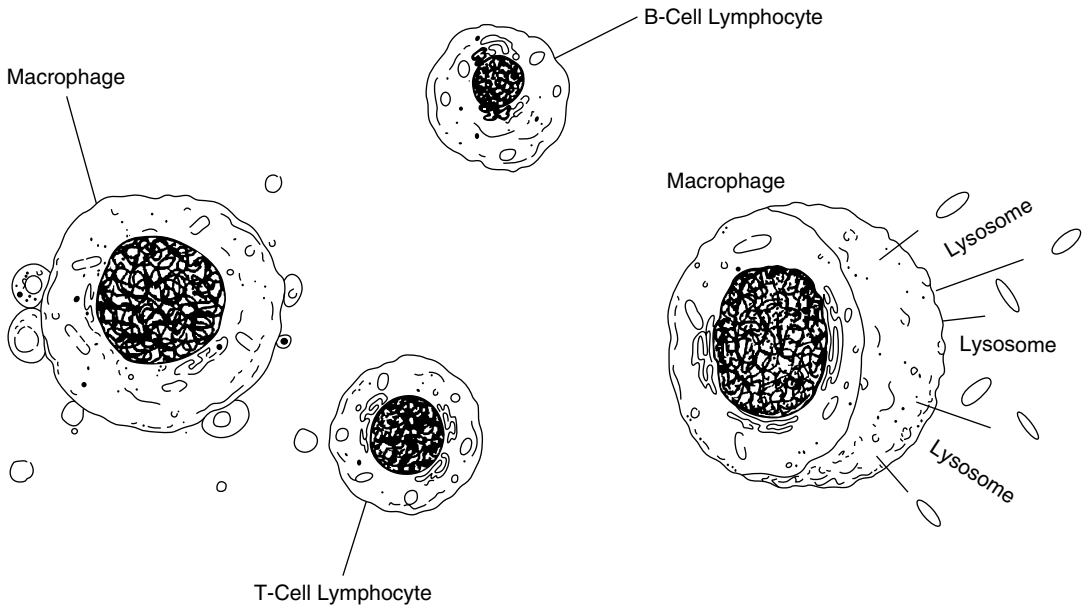

FIGURE 4–7 Microscope view of living cells in human milk. The 4,000 cells per centimeter of human milk consist mainly of macrophages and T-cell and B-cell lymphocytes. Macrophages secrete lysozyme, which help destroy the cell walls of bacteria.

These memory cells are the key to active immunity. Antibodies persist for only a few weeks before breaking down; however, memory cells can live for years, providing long-lasting protection. It is not clear whether T cells are activated in human milk or whether there is a specific homing of activated and memory T lymphocytes to the breast. B cells have functional capabilities similar to those of T cells. They mature into plasmalike cells that travel to epithelial tissues in the breast and release antibodies (Bellig, 1995; Newman, 1995) that reflect exposure to pathogens encountered in their environment. For example, milk from mothers living in Nigeria and exposed to malaria was compared with milk from a control population of mothers living in Washington, DC. The Nigerian mothers carried a high IgA level of antimalaria antibodies compared with the Washington mothers (Kassim et al., 2000).

Stem Cells

Human mammary tissue contains stem cells—an exciting new discovery. The discovery of stem cells in mammary tissue has led to research to determine whether mammary stem cells are present in expressed breastmilk (Cregan et al., 2007). The presence of nestin-positive putative mammary stem cells suggest that breastmilk is a readily available and noninvasive source of mammary stem cells that could be used to treat a multitude of health problems (see Figure 4–8). If so, they promise to provide an ethical means of harvesting stem cells.

Antibodies and Immunoglobulins

Antibodies are immunoglobulins that recognize and act on a particular antigen. Immunoglobulins are proteins produced by plasma cells in response to an immunogen. There are five types of immunoglobulins: (1) IgG, (2) IgA, (3) IgM, (4) IgE, and (5) IgD. Both IgA and IgE play a critical role in biological specificity of human milk on the recipient infant.

Secretory IgA (sIgA) is the major immunoglobulin in all human secretions. sIgA provides the initial bolus that supplements immunoglobulins transferred earlier across the placenta to the fetus. It is the immunoglobulin most frequently noted in medical literature as having immense immunological value to the neonate. sIgA, which is both synthesized and stored in the breast, reaches levels up to 5 mg/ml in colostrum; then it decreases to 1 mg/ml in mature

milk. Interleukin-6 in human milk may be partly responsible for the genesis of IgA- and IgM-producing cells in the mammary gland (Rudloff et al., 1993). As the mother yields more milk, the infant receives more sIgA so that the total dose of sIgA the baby receives throughout lactation is constant or even increases (depending on the milk intake). Mothers of infants with a systemic infection and poor suckling have higher IgA levels in their breastmilk (Feist, Berger, & Speer, 2000) as well as women with a low income who have nearly three times the milk sIgA of high-income women (Groer, Davis & Steele, 2004).

sIgA synthesis via the secretory immune system described is an elegant lymphocyte traffic pathway called *gut-associated lymphoid tissue* (GALT) or *bronchus-associated lymphoid tissue* (BALT). This pathway leads to the development of lymphoid cells in the mammary gland, which produce IgA antibodies after exposure to specific microbial or environmental antigens on the intestinal or the respiratory mucosa (Goldman et al., 1983; Okamoto & Ogra, 1989). This migration of immunological responsiveness from both BALT and GALT to the mammary glands supports the unique concept of a common mucosal immune system (see Color Plate 4).

Because the infant's own IgA is deficient and only slowly increases during the first several months after birth, sIgA in human milk provides important passive immunological protection to the digestive tract of newborn infants. sIgA protects the newborn's entire intestinal tract. It is only minimally absorbed from the intestine because it is bound to the human milk fat globule membrane, travels through the newborn's entire intestinal tract, and is found unaltered in the newborn feces (Schroten et al., 1999).

A number of IgA antibodies in human milk that act upon viruses or bacteria that cause respiratory and gastrointestinal tract infections have been reported. These infecting agents include *E. coli, V. cholerae, Clostridium difficile, Salmonella, G. lamblia, E. histolytica, Campylobacter,* rotavirus, and poliovirus (Pickering & Kohl, 1986; Ruiz-Palacios et al., 1990). As stated earlier, immunizing breastfeeding women with poliovirus or rubella creates IgA antibodies in milk that specifically target these agents. IgA4 may also play a role in host defense of mucosal surfaces; in some women IgA4 is produced locally in the mammary gland (Keller et al., 1988).

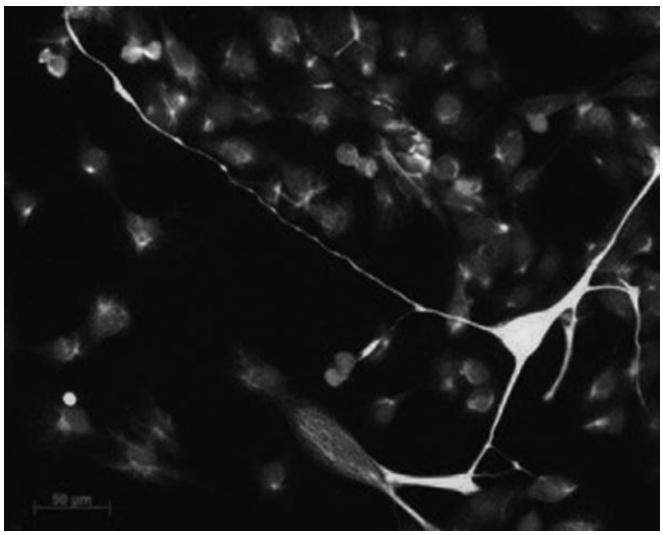

FIGURE 4–8 Mammary stem cells isolated from human breast milk.

Source: Courtesy of Mark Cregan.

In addition to IgA, other Ig classes, including IgD, may be involved in local immunity of the breast. Several investigators (Litwin, Zehr, & Insel, 1990; Steel & Leslie, 1985) have demonstrated high levels of locally produced IgD in breast tissues and breastmilk. Another immune messenger, sCD14, has been found in significant quantities in breastmilk (25,100 ng/ml) versus formula (0.6 ng/ml). sCD14 plays an important role in enabling intestinal epithelial cells to prevent gastrointestinal gram-negative infections and stimulating the newborn immune system (Blais, Harrold & Altosaar, 2006).

As shown in Figure 4–9, clear biological rhythms of protective factors predictably rise and fall as lactation progresses. The reasons for waxing and waning of various anti-infective components are not always clear but are assumed to be adapted to the needs of the infant.

Nonantibody Antibacterial Protection

Nonantibody factors in human milk make up an elegant and intricate system that protects the infant against bacterial infection. These factors include lactoferrin, the bifidus factor, lactoperoxidase, and oligosaccharides.

Lactoferrin

Lactoferrin, a potent bacteriostatic iron-binding protein, is abundant in human milk (1–6 mg/ml) but is not present in bovine milk. It is present in higher proportions relative to total protein in preterm milk (de Ferrer et al., 2000). Lactoferrin inhibits adhesion of *Escherichia coli* to cells and helps prevent diarrheal disease (de Araujo & Giugliano, 2001). In the presence of IgA antibody and bicarbonate, lactoferrin readily absorbs enteric iron and thus prevents pathogenic organisms, particularly *Escherichia coli* and *Candida albicans* (Borgnolo et al., 1996; Kirkpatrick et al., 1971), from obtaining the iron needed for survival. Because exogenous iron may well interfere with the protective effects of lactoferrin, giving iron supplements to the healthy breastfed infant must be carefully weighed. Lactoferrin also has been shown to be an essential growth factor for human B and T lymphocytes (Hashizume, Kuroda, & Murakami, 1983) and to inhibit fungal growth.

The Bifidus Factor

The intestinal flora of breastfed infants is dominated by gram-positive lactobacilli, especially *Lactobacillus bifidus*. This bifidus factor in human milk, first recognized by Gyorgy (1953), promotes the growth of these beneficial bacteria. The buffering capacities of milk (bifidus factor), together with the low protein and phosphate levels, contribute to the low pH (5–6) of stools. This acid environment, present even in the first days of life of the breastfeeding infant (Rubaltelli et al., 1998) discourages replication of enteropathogens such as *Shigella, Salmonella*, and some *E. coli*. This protection does not appear to be complete, however. Although breastmilk inhibits bacterial-cellular adhesion to intestinal epithelial cells, a sign of the beginning of the infectious process, it does not prevent loss of the epithelial barrier (Kohler, 2002). Whether the breastfed infants in the study were fed other liquids and foods was not addressed.

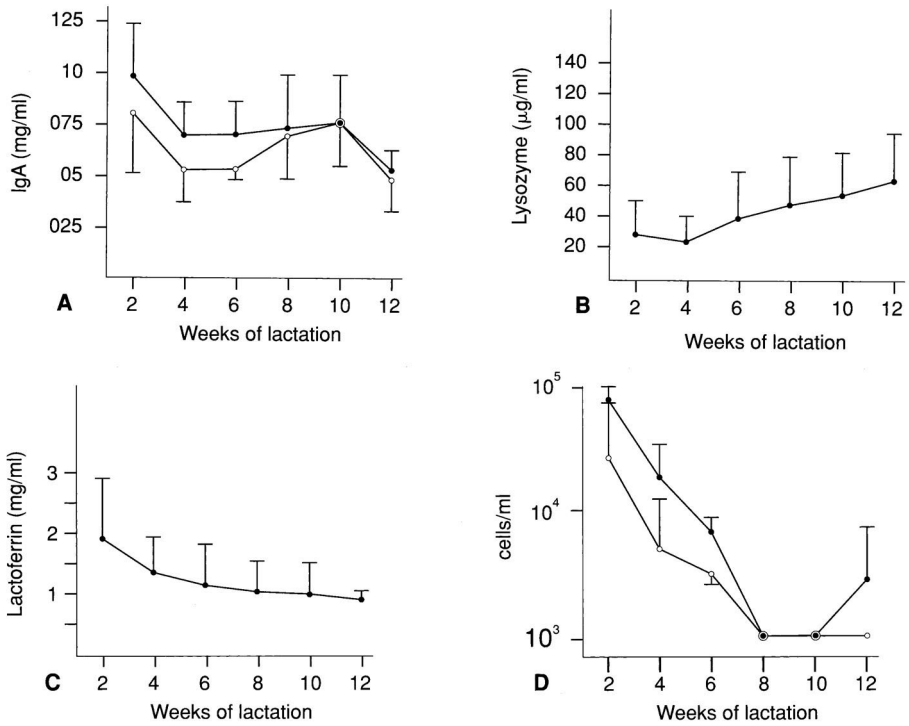

FIGURE 4–9 A longitudinal study of selected resistance factors in human milk. (A) Total (•) and secretory (◦) IgA. (B) Lysozyme. (C) Lactoferrin. (D) Macrophages-neutrophils (•-•) and lymphocytes (◦-◦).

Source: Adapted from Goldman et al., 1982.

Lactoperoxidase

Although levels of the enzyme lactoperoxidase are low, substantial amounts are present in the newborn's saliva. It is thought that IgA in milk enhances the ability of lactoperoxidase to kill streptococci.

Oligosaccharides

Oligosaccharides (carbohydrates composed of a few monosaccharides) in human milk help to block antigens from adhering to the epithelium of the gastrointestinal tract. This blocking mechanism prevents the attachment of *Pneumococcus*, which is particularly adhesive (Goldman et al., 1986). There are about 130 different oligosaccharides in human milk. Breastmilk contains many times the amount of oligosaccharides that are found in bovine milk or formula. A few formula companies are adding a limited number of simple oligosaccharides to infant formula; yet the oligosaccharides found in human milk can be thought of as 130 reasons to breastfeed (McVeagh & Miller, 1997). Because of their complexity, oligosaccharides with structures identical to human milk oligosaccharides are not yet available; instead non-milk-derived oligosaccharides are being used in formula and other foods.

Cytokines and Chemokines

Cytokines are protein signals secreted by lymphocytes, monocytes, macrophages and other cells. Proinflammatory cytokines are responsible for body responses of swelling, tenderness, and fever. Chemokines are proinflammatory cytokines that signal to bring in more phagocytic cells to an infected or inflamed site (Larsson, 2004). Chemokines are also called immunomodulators in that they operate in networks and orchestrate activation of the immune system (Goldman et al., 1996) probably by moving lymphocytes into breastmilk and across the neonatal bowel wall (Michie et al., 1998). Several different cytokines and chemokines have been discovered in human milk recently, and the list is growing rapidly (Garofalo & Goldman, 1998). In addition to activating the immune system to protect the infant against infection (Wallace et al., 1997), these biologically active molecules also appear to play a role in growth and differentiation of the mammary gland (Goldman et al., 1996). Groer (2005, 2006) measured cytokines Th1/Th2 and stress levels in breastfeeding and formula-feeding women. Formula-feeding women had decreased cytokines and other immunologic factors suggesting that breastfeeding was more protective against stress. Much has yet to be learned about cytokines.

Anti-Inflammatory and Immunomodulating Components

Many host defense agents in human milk have more than one function. Secretory IgA, lactoferrin, and lysozyme are examples. Human milk, rich in anti-inflammatory agents, supplies key protection during the vulnerable period of infancy. Major biochemical pathways of inflammations are either absent or poorly represented in breastmilk. Garofalo and Goldman (1999) have identified several anti-inflammatory factors in breastmilk, such as antioxidants (vitamins A, C, and E, and enzymes), $alpha_1$-antitrypsin, cortisol, epidermal growth factor, IgA, lysozyme, prostaglandins, and cytokines. The anti-inflammatory effects of these components have not as yet been directly demonstrated in the nursing infant, but they are thought to modulate cytokine responses to infection and facilitate defense mechanisms while minimizing tissue damage (Kelleher & Lönnerdal, 2001).

Goldman et al. (1996) suggest that the immunomodulating properties of human milk have a long-term influence on the development of the immune system and explain why the long-term risk for many diseases are lessened by breastfeeding. An immunomodulator changes the function of another defense agent and thus changes the quality or magnitude of the immune response. Fibronectin in human milk is an example of an immunomodulator that acts by augmenting the clearance of bacteria and intravascular debris. Interferon-alpha is not in human milk; yet breastfed infants with respiratory syncytial virus infection have higher blood levels of this element than nonbreastfed infants. Cytokines are also thought to immunomodulate the immune system. For example, interleukin-18, which is activated by macrophages, is higher in colostrum compared with early milk and mature milk. Interleukin-10 increases the development of IgA antibody production, thus playing an important role in host defense of neonates (Takahata et al., 2001). Other anti-inflammatory properties of human milk such as sIgA are more indirect.

Bioactive Components

Hamosh (2001) designates a special group of substances in human milk as *bioactive components*. These substances promote growth and development of the newborn by special activities that continue after the infant ingests breastmilk. Many are not available to the infant in commercial infant formula. Research on bioactive components is a growing area of investigation. These bioactive components may play a significant role in child health.

Enzymes

Mammalian milk contains a large number of enzymes, some of which appear to have a beneficial effect on the development of the newborn. The enzyme content of human milk and bovine milk differ substantially (Hamosh, 1996). For example, lysozyme activity is several thousand times greater in human milk than in bovine milk. The alkaline pH of the human infant's stomach has a limited effect on the antitrypsin activity of breastmilk, thereby protecting children with alpha$_1$-antitrypsin deficiency against severe liver disease and early death (Udall et al., 1985). Most mammal milks contain many enzymes that appear to be species specific because of their varying level of activity in different species. The enzymes discussed next serve a digestive function in the infant or may be important to neonatal development.

Lysozyme

Lysozyme, a major component of human milk whey fraction, produces both bacteriocidal and anti-inflammatory action. It acts with peroxide and ascorbate to destroy *E. coli* and some *Salmonella* strains (Pickering & Kohl, 1986). Lysozyme is much more abundant in human milk (400 ug/ml) than in bovine milk. Rather than slowly declining as lactation progresses, lysozyme activity increases progressively, beginning about 6 months after delivery (Goldman et al., 1982; Prentice et al., 1984). The lysozyme differs from other protective factors in this respect because babies begin receiving solid foods around 6 months, and high levels of lysozyme may be a teleological, practical safeguard against the greater risk from pathogens and diarrheal disease at this time.

Lipase

For human infants to digest fat, adequate lipase activity and bile salt levels must be present. The bile salt-stimulated lipase and lipoprotein lipase present in human milk compensate for immature pancreatic function and for the absence of amylase in neonates, especially in the premature infant. When human milk is frozen or refrigerated (Hamosh et al., 1997), lipase is not affected; however, heating severely reduces lipase activity. Several protozoa—*Giardia lamblia*, *Entamoeba histolytica*, and *Trichomonas vaginalis*—have been shown in vitro to be killed rapidly by exposure to salt-stimulated lipase, which is found only in the milk of humans and mountain gorillas (Blackberg et al., 1980).

Amylase

Amylase is necessary for the digestion of starch. Although amylase is synthesized and stored in the pancreas of the newborn, the infant is around 6 months old before amylase is released into the duodenum. Human milk contains about 10 to 60 times as much alpha-amylase as does normal human serum, thus providing an alternate source of this starch-digestive substance. No alpha-amylase is present in bovine, goat, or swine milk, suggesting that this enzyme appeared late in the evolutionary continuum. Breastfed infants have fewer problems digesting solid foods than do formula-fed infants, even if these foods are introduced early, because of the alpha-amylase provided by breastmilk. Amylase is stable when refrigerated (95–100 percent activity after 24 hours storage at 15 to 25°C) (Hamosh et al., 1997).

Leptin

Leptin, a hormone that regulates appetite, food intake, and energy metabolism, is present in breastmilk but not in formula. Leptin is produced mainly by the adipose tissue and varies significantly between people. Leptin concentrations in breastmilk decrease with time during lactation and show significant relationships with other maternal hormones. A recent study (Miralles et al., 2006) showed that leptin in breastmilk may regulate body weight during infancy. The higher the milk leptin concentration the lower the infants' BMI indicating that milk leptin could explain, at least partially, the greater risk of obesity in formula-fed infants compared with breastfed infants.

Growth Factors and Hormones

Human milk contains growth-promoting components also known as *growth factors* or *growth modulators*. As with the anti-infective properties of breastmilk, these substances are more pronounced in colostrum than in mature milk. Neither their biological significance nor their method of action is yet clear, but it appears that they have a greater synergistic effect when combined with each other. The source of growth factors is epithelial and stromal cells, and macrophages in the breast. These factors exert growth-promoting and protective effects on the neonatal gastrointestinal tract that cannot be provided by commercial formula (Kobata et al., 2008). Different growth factors may have overlapping functions, both stimulating cell growth and indirectly affecting the infant's defense mechanisms against disease (Morriss et al., 1986).

Epidermal Growth Factor

Epidermal growth factor (EGF), a type of cytokine, is a major growth-promoting agent in breastmilk that stimulates proliferation of intestinal mucosa and epithelium and strengthens the mucosal barrier to antigens (Carpenter, 1980; Petschow et al., 1993). A polypeptide that contains 53 amino acids, EGF is highest in human milk after delivery and decreases rapidly thereafter (Matsuoka & Idota, 1995). There is no diurnal variation or variation between preterm and term milk. EGF is also present in plasma, saliva, and amniotic fluid, but human milk contains a higher concentration. EGF may also be involved in the development of low-density lipoprotein receptors and in cholesterol metabolism.

Human Milk Growth Factors I, II, and III

Three polypeptides—called *human milk growth factors* (HMGF) I, II, and III—have been isolated (Shing & Klagsburn, 1984). HMGF III stimulates DNA synthesis and cellular proliferation, suggesting that it is an epidermal growth factor. In vivo studies (Heird, Schward, & Hansen, 1984; Widdowson, Colombo, & Artavanis, 1996) on growth factors in animal milk have shown striking increases in the mass of intestinal mucosa. Growth factors in human milk influence the growth of target tissues in the breastfed infant by provoking an endogenous hormonal response that is different from that provoked by formula—a possible stimulus of nutritional programming.

Insulin-Like Growth Factor

An insulin-like growth factor (IGF-I) in human milk is thought to have a growth-promoting role. The concentration of this factor in colostrum is about 30 times that in human serum, significantly higher than cow's milk, and very low to almost absent in formula (Shehadeh et al., 2001). These high levels (4.1 nmol/L) decrease rapidly (to 1.3 nmol/L) as colostrum alters to transitional milk (Read et al., 1984) but do not decline further. In fact, Corps et al. (1988) found that the concentration of an insulin-like growth factor in human milk increased (2.5 nmol/L) by the sixth week postpartum.

Thyroxine and Thyrotropin-Releasing Hormone

Thyroxine is present in human milk in small quantities but is not found in commercial formulas. The concentration in colostrum is low, increases by the first week postpartum, and gradually declines thereafter. It has been suggested that thyroxine may stimulate the maturation of the infant's intestine (Morriss, 1985).

Although the thyroxine level is significantly higher in breastfed children than in formula-fed children at 1 and 2 months of age (Rovet, 1990), it is unclear whether breastfeeding protects breastfed infants against clinical evidence of congenital hypothyroidism (Latarte et al., 1980; Rovet, 1990). Some infants receive sufficient thyroxine in their mother's milk to compensate for hypothyroidism; thus the symptoms may be masked for several months. Although this does not appear to be true for all infants, the results of thyroid studies after the first week of life should be interpreted with caution in breastfed infants and should include measurements of both thyroxine and thyroid-stimulating hormone (TSH) concentrations.

Cortisol

Cortisol is present in relatively high concentrations in colostrum, declines rapidly by the second day, and remains low thereafter. Its role in infant physiology is not clear. Three theories have been presented concerning the function of cortisol in infants. The first is that it may control the transport of fluids and salts in an infant's gastrointestinal tract

(Kulski & Hartmann, 1981). Another theory is that it may play a role in the growth of an infant's pancreas (Morrisset & Jolicoeur, 1980). Or cortisol may serve as a hormone released during chronic stress. A mother's higher level of satisfaction with breastfeeding is associated with lower levels of cortisol in her milk. The amount of cortisol in milk is inversely related to sIgA, suggesting that cortisol may suppress the function of immunoglobulin-producing cells in milk (Groer, Humenick, & Hill, 1994).

Cholecystokinin

Cholecystokinin (CCK) is a gastrointestinal hormone that enhances digestion, sedation, and a feeling of satiation and well-being. During suckling, vagal stimulation causes CCK release in both mother and infant, producing a sleepy feeling. The infant's CCK level peaks twice after suckling. The first peak occurs immediately after the feeding. It peaks again 30 to 60 minutes later. The first CCK rise is probably induced by suckling; the second by the presence of milk in the gastrointestinal tract (Marchini & Linden, 1992; Uvnas-Moberg, Marchini, & Windberg, 1993).

Beta-Endorphins

Beta-endorphins are higher in the colostrum of women who delivered (1) prematurely, (2) vaginally and, (3) without epidural analgesia. It is hypothesized that elevated beta-endorphin concentrations in colostrum may contribute to postnatal fetal adaptation to overcoming birth stress of natural labor and delivery, and at the same time contribute to the postnatal development of several related biological functions of the growing newborn (Zanardo et al., 2001).

Prostaglandins

Prostaglandins, a special group of lipids, are present in most mammal cells and tissues and affect almost every biological system. Formed by numerous body tissues, prostaglandins affect many physiological functions, including local circulation, gastric and mucous secretion, electrolyte balance, zinc absorption, and the release of brush border enzymes. The protective activity of milk lipids is thought to be due to the presence of prostaglandins PGE_2 and PGF_{2a}, present both in colostrum and in mature milk. Concentrations there are about 100 times as great as their levels in adult plasma (Lucas & Mitchell, 1980). PGE_2 particularly is thought to exert a cytoprotective action (protection against inflammation and necrosis) on the gastric mucosa by promoting the accumulation of phospholipids in the neonatal stomach (Reid, Smith, & Friedman, 1980). The full extent of the beneficial effects of prostaglandins in human milk awaits future scientific investigation.

Taurine

Taurine, absent in bovine milk, is the second most abundant amino acid in human milk (Raiha, 1985). This unusual amino acid, which may function as a neurotransmitter, plays an important role in early brain maturation (Gaull, 1985). Before 1983, taurine was thought to act only in the conjugation of bile acids. Infants who do not receive taurine in their diet conjugate bile acids with glycine, which less effectively assists in absorbing dietary fats. Although deleterious effects of low taurine levels are not known in humans, deficiencies have caused retinal problems in cats and monkeys (Jensen et al., 1988). Taurine was added to most commercial formulas when formula-fed infants were found to have plasma taurine levels only half as high as those of breastfed infants.

Implications for Clinical Practice

Human milk is a species-specific fluid of diverse composition that includes both nutrient and nonnutrient substances, all of which protect the infant. Although the significance to young infants of these components is well known, the influence of their nutritional programming on the subsequent health of infants is a relatively new field.

A thorough understanding of the biological components of human and bovine milks and of manufactured formulas is essential for the healthcare specialist who is providing lactation assistance. When prenatal discussion with the parents and prenatal classes include information about the immunological protection available from breastmilk but absent from formula, parents can then make an informed choice of infant feeding method.

This chapter objectively describes human milk components—but what about mothers' views of their breastmilk? Bottorff and Morse (1990) revealed that mothers clearly recognize the difference between colostrum and mature breastmilk. Because of the relative thickness of colostrum, some mothers believe it is the "strongest" milk, significant for its "rich" supply of antibodies rather than for its nutritional properties. Breastmilk was frequently described by using fat-related terms (e.g., lean, creamy, rich) and evaluated by drawing comparisons to cow's milk and infant formula, as if some similarities should exist between the two.

Knowledge of lactation physiology and breastmilk components provides us direction for lactation practice and advice to mothers. For example, the high fat (and thus calories) in hindmilk—the milk that appears when the breast is nearly empty—imply caution in routinely recommending "switch" nursing (repeatedly switching feedings from breast to breast during a breastfeeding) (Woolridge & Fisher, 1988). On the other hand, infants whose requirements may fluctuate with time are amazingly adept at self-regulating their nutrient intake (Woolridge, Ingram, & Baum, 1990). Thus we can encourage women to be flexible about breastfeedings and to be led by infants' cues that tell mothers when to continue and when to stop a feeding.

Given the differences between the growth patterns of breastfed infants and infants fed human-milk substitutes, practitioners need to evaluate infant growth using standardized growth charts based on breastfed infants. Otherwise, breastfeeding mothers might be told that their babies are gaining too slowly and that their milk production must be insufficient, when their babies are healthy in all respects. (See Chapter 10 on slow weight gain for a more detailed discussion of this issue.)

The drop of infant CCK levels 10 minutes after a feeding implies a "window" within which the infant can be awakened to feed from the second breast or to reattach to the first side for additional fat-rich milk. Waiting 30 minutes after the feeding before laying the baby down takes advantage of the second CCK peak to help the infant to stay asleep.

The studies cited here support giving fresh, rather than heat-treated or frozen, human milk whenever possible. Some living cells are killed by both of these treatments. Pasteurization significantly decreases concentrations of IgM, IgA, IgG and lysozymes (Koenig et al., 2005). Also, due to the action of the bile salt-stimulated lipase, fat in fresh human milk is absorbed more completely than that in pasteurized milk. Mixing mother's milk with formula is acceptable. It is particularly important for premature infants, who lack digestive enzymes, and mixing fresh human milk with formula improves fat absorption. Ideally, preterm infants will be receiving high volumes of their own mother's milk or mother's milk enriched with human milk components, both of which sustain excellent growth without the risks of bovine milk.

During the assessment phase of working with a breastfeeding family, the practitioner needs to ask if there is a family history of allergies. If so, the mother should be encouraged to breastfeed for a minimum of 12 months and to delay feeding the infant solid foods until the baby shows signs of readiness. Because of the risk of sensitization to allergenic proteins, particularly in babies who have a family history of allergies, even occasional formula supplements can trigger an allergic reaction and should be avoided as long as possible. In addition to preventing allergies, infant malabsorption problems, such as celiac disease, are lessened when the baby is breastfed and solid foods are delayed. Solid foods are usually started around 6 months of age as a baby's intestinal enzymes mature and become increasingly capable of digesting complex proteins and starches. After 6 months, babies can eat whatever they like and in any order they want.

In the maternal diet, dairy products are particularly potential allergens to the breastfeeding baby. If the mother notices that a particular food seems to cause an allergic response in her infant, she needs to consider eliminating it from her diet. A case report (Wilson, Self, & Hamburger, 1990) describes rectal bleeding in a 4-day-old infant who was exclusively breastfed: Her mother was drinking four to five glasses of cow's milk per day. Although this case is extreme and rare, it demonstrates the potential for problems when a breastfeeding mother drinks large quantities of cow's milk. Discussion of diet and appropriate substitution should be part of the care provided the mother by the healthcare worker offering lactation consultation and support.

Summary

The nutritional components of human milk, combined with its immune and antiallergic properties, make it the ideal foundation for optimal infant health. Immunological and allergy protection are obvious, but it is more difficult to substantiate the protection by breastfeeding against inflammatory and immunologically determined disorders that emerge later in life. There appears to be a threshold level for passive immunity conferred by breastmilk that is related to the amount of breastmilk a baby receives—a dose-response effect where exclusively breastfed infants benefit far more than infants who receive minimum amounts of breastmilk (Raisler, 1999).

Allowed to breastfeed at will in response to their own needs, infants generally obtain milk in amounts that satisfy their energy needs and maintain normal growth. Practical experience clearly supports the benefits of breastfeeding. In recent years, scientific data from all parts of the world confirm what the practitioner has long observed. It is ironic that many of the complex properties of human milk described in this chapter have been identified through research funded by formula companies, which stand to make large sums of money if they can develop products for which they can claim a close resemblance to human milk.

With the advent of managed care that rewards prevention of health problems and avoidance of using health services, healthcare corporations look for cost-effective ways to keep their insured clients healthy. Studies discussed in Chapter 2 show additional billions of dollars in healthcare costs for not breastfeeding.

Human milk has a remarkable fitness in terms of the demands and needs of the infant. The configuration of elements in breastmilk is nutritional programming with a reciprocal fitness between the mother and the infant. In special cases, such as the accelerated energy needs of the premature infant, this adaptability is seen in the greater availability of energy in preterm milk. Human milk is a carrier of important physiological messages to the recipient infant.

Key Concepts

- Human milk—the gold standard for infant nutriment—has between 57 to 65 kcal per deciliter.
- Breastfed infants ingest less volume than formula-fed infants because human milk is more energy efficient.
- Babies do not usually remove all the milk available in the breast during a single feeding.
- Small amounts of colostrum are produced in the first day or two after delivery followed by rapid increases to about 500 ml at five days postpartum.
- Differences in milk output from the right and left breasts are common. Milk output is often greater from the right breast.
- Milk storage capacity differs among women. Women with larger breasts have a greater milk storage capacity and may breastfeed less often; women with small breasts may need to breastfeed more often; otherwise, breast size does not affect the ability to breastfeed.
- Milk synthesis and volume differs between breasts.
- The nutritional status of a lactating mother has a minimal effect on milk volume unless she is malnourished.
- Multiparous women produce more breastmilk than primiparous women; mothers produce significantly more breastmilk with their second baby.
- Breastfed infants grow at about the same rate as those not breastfed for the first 3 to 4 months.
- Fat in human milk varies according to the degree to which the breast is emptied; high volume is associated with low milk fat content; accordingly, fat content progressively increases during a single feeding.
- Breastfeeding should be early and frequent; the longer the interval between feedings, the lower the fat content.
- The type of fat the mother eats affects the type of fatty acids present in her milk.
- Primary or congenital lactose deficiency or intolerance in infants is rare or nonexistent.

- Lactose in human milk supplies quick energy to the infant's rapidly growing brain.
- Human milk contains two main proteins: casein and whey. Casein is tough and less digestible curd; whey is soft and flocculent, and it digests rapidly.
- The amount of protein in colostrum is greater than that in mature milk because of the immune factors (IgA, lactoferrin) present in colostrum.
- Preterm mother's milk contains high levels of protein and fat compared with nonpreterm milk; thus using the milk of the preterm infant's mother is preferred.
- Generally speaking, human milk contains sufficient amounts of vitamins and minerals to meet the needs of full-term infants. Exceptions are premature infants and vitamin D supplementation for dark-skinned babies living in northern climates.
- Mineral content in human milk is fairly constant, tending to be highest right after birth and decreasing slightly throughout lactation.
- Healthy infants who consume enough breastmilk to satisfy their energy needs receive enough fluid to satisfy their requirements even in hot and dry environments.
- In the first 1 to 2 days after birth, the infant ingests small amounts, approximately 7 to 14 ml of colostrum at each feeding. Milk yield gradually increases for the first 36 hours, then rapidly increases. By day 5, volume is about 500 ml/day and 800 ml/day (range 550 and 1150) during months 1 and 6 of full breastfeeding.
- Immunity occurs actively and passively. Colostrum is densely packed with antibodies and immunoglobulins.
- Human milk contains two types of white cells: phagocytes and lymphocytes. Phagocytes (1) engulf and absorb pathogens and (2) release IgA. Lymphocytes (83 percent are T cells) protect an infant by destroying cell walls of viruses in a process called cell-mediated immunity.
- Antibodies are immunoglobulins that act against specific antigens or pathogens. Secretory IgA is the major immunoglobulin. Total sIgA remains relatively constant throughout lactation.
- sIgA passes from the mother's mucosa (intestinal, respiratory) to the mammary gland/breastmilk through lymphocyte traffic pathways (GALT and BALT).
- Immunity has a dose-response effect—the more breastmilk the infant ingests, the greater the immunity.

Internet Resources

CDC growth charts based on both breastfed and formula-fed infants:
http://www.cdc.gov/growthcharts

Report on DHA and AA in infant formula:
http://cornucopia.org/DHA/DHA_FullReport.pdf

References

Aarts E, Kylberg A, Hornell A, et al. How exclusive is breastfeeding? A comparison data since birth with current status data. *Int J Epidemiol*. 2000;29: 1041–1046.

Abrams SA, Wen H, Stuff JE. Absorption of calcium, zinc, and iron from breast milk by five to seven-month-old infants. *Pediatr Res*. 1996;39:384–390.

Abdulmoneim I, Al-Gamdi SA. Relationship between breastfeeding duration and acute respiratory infections in infant. *Saudi Med J*. 2001;22:347–350.

Akisu M, Kultursay N, Ozkayin N, et al. Platelet-activating factor levels in term and pre-term milk. *Biol Neonate*. 1998;74:289–283.

Aljazaf KMNH. Ultrasound imaging in the analysis of the blood supply and blood flow in the human lactating breast [dissertation]. Perth, Australia, Medical Imaging Science, Curtin University of Technology; 2004.

Almroth S, Bidinger PD. No need for water supplementation for exclusively breast-fed infants under hot and arid conditions. *Trans Roy Soc Trop Med Hyg*. 1990;84:602–604.

Almroth SG, Latham MC. Breast feeding practices in rural Jamaica. *J Trop Pediatr*. 1982;28:103–109.

American Academy of Pediatrics, Committee on Nutrition. Commentary on breastfeeding and infant

formulas, including standards for formulas. *Pediatrics*. 1976;57:278–285.
Anderson CH. Human milk feeding. *Pediatr Clin No Amer.* 1985;32:335–352.
Anderson JW, Johnstone BM, Remley DT. Breastfeeding and cognitive development: a meta-analysis. *Am J Clin Nutr.* 1999;70:25–35.
Andon MB et al. Nutritionally relevant supplementation of vitamin B_6 in lactating women: effect on plasma prolactin. *Pediatrics.* 1985;76:769–773.
Aniansson G et al. A prospective cohort study on breast-feeding and otitis media in Swedish infants. *Pediatr Infect Dis J.* 1994;13:183–188.
Anveden-Hertzberg L. Proctocolitis in exclusively breast-fed infants. *Eur J Pediatr.* 1996;155:464–467.
Arthur PG et al. Measuring short-term rates of milk synthesis in breast-feeding mothers. *Q J Exp Physiology.* 1989;47:419–428.
Arthur PG, Smith M, Hartmann PE. Milk lactose, citrate, and glucose as markers of lactogenesis in normal and diabetic women. *J Pediatr Gastroenterol Nutr.* 1989;9:488–496.
Ascher H et al. Influence of infant feeding and gluten intake on celiac disease. *Arch Dis Child.* 1997;76:113.
Ashraf RN et al. Breast feeding and protection against neonatal sepsis in a high-risk population. *Arch Dis Child.* 1991;66:488–490.
Atkinson SA, Anderson G, Bryan MH. Human milk: comparison of the nitrogen composition of milk from mothers of premature infants. *Am J Clin Nutr.* 1980;33:811–815.
Atkinson SA et al. Abnormal zinc content in human milk: risk for development of nutritional zinc deficiency in infants. *Am J Dis Child.* 1989;143:608–611.
Auricchio S et al. Does breast feeding protect against the development of clinical symptoms of celiac disease in children? *J Pediatr Gastroenterol Nutr.* 1983;2:428–433.
Axelsson I et al. Bovine beta-lactoglobulin in the human milk. *Acta Pediatr Scand.* 1986;75:702.
Bachrach VR, Schwarz E, Bachrach LR. Breastfeeding and the risk of hospitalization for respiratory disease in infancy: a meta-analysis. *Arch Pediatr Adolesc Med.* 2003;157:237–243.
Bates CJ, Prentice A. Breast milk as a source of vitamins, essential minerals and trace elements. *Pharmacol Ther.* 1994;62:193–220.
Bauchner J, Levanthal JM, Shapiro ED. Studies of breastfeeding and infections: how good is the evidence? *JAMA.* 1986;256:887–892.
Baur LA et al. Relationships between the fatty acid composition of muscle and erythrocyte membrane phospholipid in young children and the effect of type of infant feeding. *Lipids.* 2000;35:77–82.
Bell LM et al. Rotavirus serotype-specific neutralizing activity in human milk. *Am J Dis Child.* 1988; 142:275–278.
Bellig LL. Immunization and the prevention of childhood diseases. *J Obstet Gynecol Neonatal Nurs.* 1995;24:469–477.
Bener A, Denic S, Galadari S. Longer breast-feeding and protection against childhood leukaemia and lymphomas. *European J Cancer.* 2001;37:234–238.
Bergstrand O, Hellers G. Breast-feeding during infancy in patients who develop Crohn's disease. *Scand J Gastroenterol.* 1983;18:903–906.
Bergstrom O et al. Serum lipid values in adolescents are related to family history, infant feeding, and physical growth. *Atherosclerosis.* 1995;17:1–13.
Blackberg LD et al. The bile salt-stimulated lipase in human milk is an evolutionary newcomer derived from a non-milk protein. *FEBS Lett.* 1980;112:151.
Blais DR, Harrold J, Altosaar I. Killing the messenger in the nick of time: persistence of breastmilk sCD14 in the neonatal gastrointestinal tract. *Pediatric Res.* 2006;59:371–376.
Borch-Johnson K et al. Relation between breast-feeding and incidence rates of insulin-dependent diabetes mellitus. *Lancet.* 1984;2:1083–1086.
Borgnolo G et al. A case-control study of Salmonella gastrointestinal infection in Italian children. *Acta Paediatr.* 1996;85:804–808.
Bottorff JL, Morse JM. Mother's perceptions of breast milk. *JOGN Nursing.* 1990;19:518–527.
Bouguerra F et al. Effect of breastfeeding relative to the age at onset of celiac disease. *Arch Pediatr.* 1998;5:621.
Brenna JT et al. Docosahexaenoic and arachidonic acid concentrations in human breastmilk worldwide. *Am J Clin Nutr.* 2007;85:1457–1464.
Brown KH et al. Lactational capacity of marginally nourished mothers: relationships between maternal nutritional status and quantity and proximate composition of milk. *Pediatrics.* 1986a;78:909–919.
Brown KH et al. Milk consumption and hydration status of exclusively breast-fed infants in a warm climate. *J Pediatr.* 1986b;108:677–680.
Brown KH et al. Infant-feeding practices and their relationship with diarrheal and other diseases in Huascar (Lima), Peru. *Pediatrics.* 1989;83:31–40.
Buescher ES, Pickering LK. Polymorphonuclear leukocytes in human colostrum and milk. In: Howell RR, Morriss FH, Pickering LK, eds. *Human milk in infant nutrition and health.* Springfield, Ill: Thomas; 1986:160–173.
Burr ML et al. Infant feeding, wheezing, and allergy: a prospective study. *Arch Dis Child.* 1993;68:724–728.
Butte NF, Smith EO, Garza C. Energy utilization of breast-fed and formula-fed infants. *Am J Clin Nutr.* 1990;51:350–358.
Butte NF et al. Macro- and trace-mineral intakes of exclusively breast-fed infants. *Am J Clin Nutr.* 1987; 45:42–47.
Butte NF et al. Influence of early feeding mode on body composition of infants. *Biol Neonate.* 1995;67: 414–424.
Butte NF et al. Energy requirements derived from total energy expenditure and energy deposition during the first 2 years of life. *Am J Clin Nutr.* 2000;72: 1558–1569.

Carpenter G. Epidermal growth factor is a major growth-promoting agent in human milk. *Science*. 1980;210:198–199.
Casey CE, Hambidge KM, Neville MC. Studies in human lactation: zinc, copper, manganese and chromium in human milk in the first month of lactation. *Am J Clin Nutr.* 1985;41:1193–1200.
Casey CE, Neville MC, Hambidge KM. Studies in human lactation: secretion of zinc, copper, and manganese in human milk. *Am J Clin Nutr.* 1989;49: 773–785.
Caspi A, Williams B, Kim-Cohen J. Moderation of breastfeeding effect on the IQ by genetic variation in fatty acid metabolism. *Proc Natl Acad Sci USA*. 2007;104(47):188860–188865.
Cavagni G et al. Passage of food antigens into circulation of breast-fed infants with atopic dermatitis. *Ann Allergy.* 1988;61:361–365.
Cavell B. Gastric emptying in infants fed human or infant formula. *Acta Paediatr Scand.* 1981;70: 639–641.
Cesar JA, Victoria CG, Barros FC. Impact of breastfeeding on admission for pneumonia during postneonatal period in Brazil: nested case-control study. *Br Med J.* 1999;318:1316–1320.
Chandra RK. Prospective studies of the effect of breastfeeding on incidence of infection and allergy. *Acta Paediatr Scand.* 1979;68:691–694.
Chandra RK et al. Influence of maternal food antigen avoidance during pregnancy and lactation on incidence of atopic eczema in infants. *Clin Allergy.* 1986;16:563–569.
Chen A, Rogan WJ. Breastfeeding and the risk of post neonatal death in the United States. *Pediatrics.* 2004;113:435–439.
Chen Y, Yu S, Li W. Artificial feeding and hospitalization in the first 18 months of life. *Pediatrics.* 1988; 81:58–62.
Chowanadisai W, Lönnerdal B, Kelleher SL. Identification of a mutation in SLC30A2 (ZnT-2) in women with low milk zinc concentration that results in transient neonatal zinc deficiency. *J Biol Chem.* 2006;281: 39699–39707.
Chulada PC et al. Breast-feeding and the prevalence of asthma and wheeze in children: analyses from the third national health and nutrition examination survey, 1988–1994. *J Allergy Clin Immunol.* 2003;111: 328–336.
Clavano NR. Mode of feeding and its effect on infant mortality and morbidity. *J Trop Pediatr.* 1982;28: 287–293.
Cochi SL et al. Primary invasive *Haemophilus influenzae* type b disease: a population-based assessment of risk factors. *J Pediatr.* 1986;108:87–96.
Cohen RJ et al. Exclusively breastfed, low birth weight term infants do not need supplemental water. *Acta Paediatr.* 2000;89:550–552.
Corps AN et al. The insulin-like growth factor I content in human milk increases between early and full lactation. *J Clin Endocrinol Metab.* 1988;67:25–29.
Cosgrove M. Perinatal and infant nutrition: nucleotides. *Nutrition.* 1998;14:748.
Cox DB, Owens RA, Hartmann PE. Blood and milk prolactin and the rate of milk synthesis in women. *Exp Physiol.* 1996;81:1007–1020.
Cregan MD, Hartmann PE. Computerized breast measurement from conception to weaning: clinical implications. *J Hum Lact.* 1999;15:89–95.
Cregan MD et al. Identification of nestin-positive putative mammary stem cells in human breastmilk. *Cell Tissue Res.* 2007;329:129.
Cregan MD, Mitoulas LR, Hartmann PE. Milk prolactin, feed volume, and duration between feeds in women breastfeeding their full-term infants over a 24-hour period. *Exp Physiol.* 2002;87:207–214.
Cushing AH et al. Breastfeeding reduces the risk of respiratory illness in infants. *Am J Epidemiol.* 1998; 147:863–870.
Dai D et al. Role of oligosaccharides and glycoconjugates in intestinal host defense. *J Pediatr Gastroenterol Nutr.* 2000;30(suppl):23.
Daly SE, Hartmann PE. Infant demand and milk supply. Part 1: Infant demand and milk production in lactating women. *J Hum Lact.* 1995a;11:21–26.
Daly SE, Hartmann PE. Infant demand and milk supply. Part 2: The short-term control of milk synthesis in lactating women. *J Hum Lact.* 1995b;11:27–36.
Daly SE, Owens RA, Hartmann PE. The short-term synthesis and infant-regulated removal of milk in lactating women. *Exp Physiol.* 1993;78:209–220.
Daly SE et al. The determination of short-term breast volume changes and the rate of synthesis of human milk using computerized breast measurement. *Exp Physiol.* 1992;77:79–87.
Daly SE et al. Degree of breast emptying explains changes in the fat content, but not fatty acid composition of human milk. *Exp Physiol.* 1993;78:741–755.
Daniels JL et al. Breast-feeding and neuroblastoma, USA and Canada. *Cancer Causes Control.* 2002;13:401–405.
Davis MK. Review of the evidence for an association between infant feeding and childhood cancer. *Int J Cancer.* 1998;S11:29–33.
Davis MK, Savitz DA, Graubard B. Infant feeding and childhood cancer. *Lancet.* 1988;2(8607):365–368.
de Araujo AN, Giugliano LG. Lactoferrin and free secretory component of human milk inhibits the adhesion of enteropathic *Escherichia coli* to HeLa cells. *BMC Microbiol.* 2001;1:25.
de Ferrer PA et al. Lactoferrin levels in term and preterm milk. *J Amer College Nutr.* 2000;19:370–373.
Dell S, To T. Breastfeeding and asthma in young children: findings from a population-based study. *Arch Pediatr Adolesc Med.* 2001;155:1261–1265.
Demmers TA et al. Effects of early cholesterol intake on cholesterol biosynthesis and plasma lipids among infants until 28 months of age. *Pediatrics.* 2005;115: 1594–1601.
Dewey KG, Heinig J, Nommsen-Rivers LA. Differences in morbidity between breast-fed and formula-fed infants. *J Pediatr.* 1995;126:697–702.

Dewey KG, Lönnerdal B. Milk and nutrient intake of breast-fed infants from 1 to 6 months: relation to growth and fatness. *J Pediatr Gastroenterol Nutr.* 1983; 2:497–506.

Dewey KG et al. Breast-fed infants are leaner than formula-fed infants at 1 year of age: the DARLING study. *Am J Clin Nutr.* 1993;57:140–145.

Dewey KG et al. Growth of breast-fed infants deviates from current reference data: a pooled analysis of US, Canadian, and European data sets. *Pediatrics*. 1995; 96:495–503.

Dewey KG et al. Iron supplementation affects growth and morbidity of breast-fed infants: results of a randomized trial in Sweden and Honduras. *J Nutr.* 2002; 132:3249–3255.

Dorea JG. Iron and copper in human milk. *Nutrition*. 2000;16:209–220.

Downham MA et al. Breast-feeding protects against respiratory syncytial virus infection. *Br Med J.* 1976; 2:274–276.

Duchen K, Yu G, Björkstén B. Polyunsaturated fatty acids in breast milk in relation to atopy in the mother and her child. *Int Arch Allergy Immunol*. 1999;118:321–323.

Duffy LC et al. The effects of infant feeding on rotavirus-induced gastroenteritis: a prospective study. *Am J Public Health*. 1986;76:259–263.

Duncan B et al. Iron and the exclusively breast-fed infant from birth to six months. *J Pediatr Gastroenterol Nutr.* 1985;4:412–425.

Duncan J et al. Exclusive breast-feeding for at least 4 months protects against otitis media. *Pediatrics*. 1993;91:867–872.

Engstrom JL et al. Comparison of milk output from the right and left breasts during simultaneous pumping in mothers of very low birth weight infants. *Breastfeeding Medicine*. 2007;2:83–91.

Erickson PR, Mazhari E. Investigation of the role of human breast milk in caries development. *Pediatr Dent*. 1999;21:86–90.

Espinoza E et al. Rotavirus infections in young Nicaraguan children. *Pediatr Infect Dis*. 1997;16:564–571.

Evans GS, Johnson PE. Characterization and quantitation of a zinc-binding ligand and human milk. *Pediatr Res*. 1980;14:876–880.

Fallot MB et al. Breast-feeding reduces incidence of hospital admissions for infections in infants. *Pediatrics*. 1980;65:1121–1124.

Fan H, Conner R, Villareal L. *The Biology of AIDS*. Boston, Mass: Jones and Bartlett; 1989:28.

Fawzi WW et al. Maternal anthropometry and infant feeding practices in Israel in relation to growth in infancy: the North African Infant Feeding Study. *Am J Clin Nutr.* 1997;65:1731–1737.

Feist N, Berger D, Speer CP. Anti-endotoxin antibodies in human milk: correlation with infection of the newborn. *Acta Paediatr.* 2000;89:1087–1092.

Ford RPK et al. Breastfeeding and the risk of sudden infant death syndrome. *Int J Epidemiol*. 1993;22: 885–890.

Forman MR et al. Undernutrition among Bedouin Arab infants: the Bedouin Infant Feeding Study. *Am J Clin Nutr.* 1990;51:339–343.

Frank AL et al. Breast-feeding and respiratory virus infection. *Pediatrics*. 1982;70:239–245.

Franke AA, Custer LJ, Tanaka Y. Isoflavones in human breast milk and other biological fluids. *Am J Clin Nutr.* 1998;68(suppl):1466S–1473S.

Fuchs SC, Victor CG, Martines J. Case-control study of risk of dehydrating diarrhoea in infants in vulnerable period after full weaning. *BMJ*. 1996;313:391–394.

Furukawa SK et al. Fecal IgE in infants at 1 month of age as indicator of atopic disease. *Allergy.* 1994;49: 791–794.

Garofalo RP, Goldman AS. Cytokines, chemokines, and colony-stimulating factors in human milk. *Biol Neonate*. 1998;74:134–142.

Garofalo RP, Goldman AS. Expression of functional immunomodulating and anti-inflammatory factors in human milk. *Clin Perinatol*. 1999;26:361.

Gartner LM, Greer FR. Prevention of rickets and vitamin D deficiency: new guidelines for vitamin D intake. *Pediatrics*. 2003;111(Part 1):908–910.

Garza C, Stuff J, Butte N. Growth of the breast-fed infant. In: Goldman AS, Atkinson SA, Hanson LA, eds. *Human Lactation: The Effects of Human Milk on the Recipient Infant*. New York, NY: Plenum; 1986: 109–121.

Garza C et al. Changes in the nutrient composition of human milk during gradual weaning. *Am J Clin Nutr.* 1983;37:61–65.

Garza C et al. Special properties of human milk. *Clin Perinatol*. 1987;14:11–31.

Gaull GE. Significance of growth modulators in human milk. *Pediatrics*. 1985;75(suppl):142–145.

Gdalevich M, Mimouni D, Mimouni M. Breast-feeding and the risk of bronchial asthma in childhood: a systematic review with meta-analysis of prospective studies. *J Pediatr.* 2001;139:261–266.

Gilbert RE et al. Bottle-feeding and the sudden infant death syndrome. *BMJ*. 1995;310:88–90.

Gimeno SGA, de Suza JMP. IDDM and milk consumption. *Diabetes Care*. 1997;20:1256–1260.

Glass RI, Stoll BJ. The protective effect of human milk against diarrhea: a review of studies from Bangladesh. *Acta Paediatr Scand*. 1989;351(suppl):131–136.

Goldman AS et al. Immunologic factors in human milk during the first year of lactation. *J Pediatr.* 1982; 100:563–567.

Goldman AS et al. Immunologic components in human milk during gradual weaning. *Acta Paediatr Scand.* 1983;72:133–134.

Goldman AS et al. Anti-inflammatory properties of human milk. *Acta Paediatr Scand.* 1986;75:689–695.

Goldman AS et al. Cytokines in human milk: properties and potential effects upon the mammary gland and the neonate. *J Mammary Gland Biol Neoplasia*. 1996;1:251–258.

Grantham-McGregor SM, Back EH. Breast feeding in Kingston, Jamaica. *Arch Dis Child.* 1972;45:404–409.

Greco L et al. Case control study on nutritional risk factors in celiac disease. *J Pediar Gastroenterol Nutr.* 1983;7:395–399.
Greer FR. Vitamin K status of lactating mothers and their infants. *Acta Paediatr.* 1999;88(suppl):95.
Greer FR Marshall S. Bone mineral content, serum vitamin D metabolite concentrations and ultraviolet B light exposure in infants fed human milk with and without vitamin D2 supplements. *J Pediatr.* 1989;114: 204–212.
Groer M, Davis M, Steele K. Associations between human milk sIgA and maternal immune, infections, endocrine, and stress variables. *J Hum Lact.* 2004;20:153–158.
Groer MW, Humenick S, Hill P. Characterizations and psychoneuroimmunologic implications of secretory immunoglobulin A and cortisol in preterm and term breast milk. *J Perinat Neonatal Nurs.* 1994;7:42–51.
Gulick EE. The effect of breast-feeding on toddler health. *Pediatr Nurs.* 1986;12:51–54.
Gunnarsdottir I et al. Infant feeding patterns and midlife erythrocyte sedimentation rate. *Acta Paediatrica.* 2007;96:852–856.
Guthrie HA, Picciano MF, Sheehe D. Fatty acid patterns of human milk. *J Pediatr.* 1977;90:39–41.
Gyorgy P. A hitherto unrecognized biochemical difference between human milk and cow's milk. *Pediatrics.* 1953;11:98–104.
Habbick BF, Kahnna C, To T. Infantile hypertropic pyloric stenosis: a study of feeding practices and other possible causes. *Can Med Assoc J.* 1989;140:401–404.
Habicht JP, DaVanso J, Butz WP. Mother's milk and sewage: their interactive effects on infant mortality. *Pediatrics.* 1988;81:456–460.
Hakansson A, Svensson M, Mossberg A-K et al. A folding variant of α-lactalbumin with bactericidal activity against *Streptococcus pneumoniae*. *Molec Microbiol.* 2000;35:589–600.
Hakansson A et al. Apoptosis induced by a human protein. *Proc Natl Acad Sci.* 1995;92:8064.
Hamosh M. Human milk. Digestion in the neonate. *Clin Perinatol.* 1996;23:191.
Hamosh M. Bioactive factors in human milk. In: Schanler, RJ, ed. *The Evidence for Breastfeeding.* Breastfeeding 2001, Part I. *Ped Clin N Amer.* 2001;48:69–86.
Hamosh M et al. Digestive enzymes in human milk: stability at suboptimal storage temperatures. *J Pediatr Gastroenterol Nutr.* 1997;24:38–43.
Hartmann PE. Lactation and reproduction in Western Australian women. *J Reprod Med.* 1987;32:543–557.
Hartmann PE, Cregan M. Lactogenesis and the effects of insulin-dependent diabetes mellitus and prematurity. *J Nutrition.* 2001;131:3016S–3020S.
Hartmann PE, Prosser CG. Physiological basis of longitudinal changes in human milk yield and composition. *Fed Proc.* 1984;43:2448–2453.
Hashizume S, Kuroda K, Murakami H. Identification of lactoferrin as an essential growth factor for human lymphocytic cell lines in serum-free medium. *Biochem Biophys Acta.* 1983;763:377.
Hasselbalch H et al. Breastfeeding influences thymic size in late infancy. *Eur J Pediatr.* 1999;158:964–967.
Hawkes JS, Gibson RA. Lymphocyte subpopulations in breast-fed and formula-fed infants at six months of age. *Adv Exp Med Biol.* 2001;501:497–504.
Heacock H et al. Influence of breast versus formula milk on physiological gastroesophageal reflux in healthy, newborn infants. *J Pediatr Gastroenterol.* 1992;14:41–46.
Heinig J et al. Zinc supplementation does not affect growth, morbidity, or motor development of US term breastfed infants at 4–10 mo of age. *Am J Clin Nutr.* 2006;84:594–601.
Heird WC, Schward SM, Hansen IH. Colostrum-induced enteric mucosal growth in beagle puppies. *Pediatr Res.* 1984;18:512.
Hildebrandt HM. Maternal perception of lactogenesis time: a clinical report. *J Hum Lact.* 1999;15:317–323.
Holberg CJ et al. Risk factors for respiratory syncytial virus-associated lower respiratory illnesses in the first year of life. *Am J Epidemiol.* 1991;133:1135–1151.
Host A, Husby S, Osterballe O. A prospective study of cow's milk allergy in exclusively breast-fed infants. *Acta Paediatr Scand.* 1988;77:663–670.
Hornell A et al. Breastfeeding patterns in exclusively breastfed infants: a longitudinal prospective study in Uppsala, Sweden. *Acta Paediatr.* 1999;88:203–211.
Houston MJ, Howie PW, McNeilly AS. Factors affecting the duration of breast feeding: 1. measurement of breast milk intake in the first week of life. *Early Hum Dev.* 1983;8:49–54.
Howie PW et al. Protective effect of breast feeding against infection. *Br Med J.* 1990;300:11–16.
Humenick SS. The clinical significance of breastmilk maturation rates. *Birth.* 1987;14:174–179.
Humenick SS et al. The maturation index of colostrum and milk (MICAM): a measurement of breast milk maturation. *J Nurs Measurement.* 1994;2:16-86.
Hylander MA et al. Association of human milk feedings with a reduction in retinopathy of prematurity among very low birth weight infants. *J Perinatology.* 2001;21:356–362.
Ingram JC, Woolridge MS, Greenwood RJ. Breastfeeding: it is worth trying with the second baby. *Lancet.* 2001;358:986–987.
Istre GR et al. Risk factors for primary *Haemophilus influenzae* disease: increased risk from day care attendance and school-aged household members. *J Pediatr.* 1985;106:190–195.
Ivarsson A et al. Epidemic of celiac disease in Swedish children. *Acta Pediatr.* 2000;89:165.
Jason JM, Niebury P, Marks JS. Mortality and infectious disease associated with infant-feeding practices in developing countries. *Pediatrics.* 1984;74(suppl): 702–727.
Jenkins HR et al. Food allergy: the major cause of infantile colitis. *Arch Dis Child.* 1984;59:326–329.
Jensen CL. Effects of maternal docosahexaenoic acid intake on visual function and neurodevelopment in breastfed term infants. *Am J Clin Nutr.* 2005;82: 125–132.

Jensen RG. Lipids in human milk. *Lipids*. 1999;34:1243.
Jensen RG et al. Human milk as a carrier of messages to the nursing infant. *Nutr Today*. 1988;23:20–25.
Juex G et al. Growth pattern of selected urban Chilean infants during exclusive breast feeding. *Am J Clin Nutr*. 1983;38:462–468.
Kassim OO et al. Inhibitory factors in breast milk, maternal and infant sera against in vitro growth of *Plasmodium falciparum* malaria parasite. *J Trop Pediatr*. 2000;46:92–96.
Kelleher SL, Lönnerdal B. Immunological activities associated with milk. *Adv Nutr Res*. 2001;10:39–65.
Keller MA et al. $IgAG_4$ in human colostrum and human milk: continued local production or selective transport form serum. *Acta Paediatr Scand*. 1988;77:24–29.
Kelly DW et al. Rise and fall of coeliac disease 1960–1985. *Arch Dis Child*. 1989;64:1157–1160.
Kent JC et al. Volume and frequency of breastfeedings and fat content of breastmilk throughout the day. *Pediatrics*. 2006;117:387–395.
Kent et al. Breast volume and milk production during extended lactation in women. *Exp Physiol*. 1999; 82:435–447.
Khin-Maung-U J et al. Effect of clinical outcome of breastfeeding during acute diarrhea. *Br Med J*. 1985; 290:587–589.
Kirkpatrick CH et al. Inhibition of growth of *Candida albicans* by iron-unsaturated lactoferrin: relation to host defense mechanisms in chronic mucocutaneous candidiasis. *J Infect Dis*. 1971;124:539.
Kobata R et al. High levels of growth factors in human breast milk. *Early Hum Dev*. 2008;84:67–9.
Koenig A et al. Immunologic factors in human milk: the effects of gestational age and pasteurization. *J Hum Lact*. 2005;21(4):439–443.
Kohler H et al. Antibacterial characteristics in the feces of breast-fed and formula-fed infants during the first year of life. *J Pediatr Gastroenterol Nutr*. 2002;34: 188–193.
Koletzko B, Rodriquez-Palmero M. Polyunsaturated fatty acids in human milk and their role in early human development. *J Mammary Gland Biol Neoplasia*. 1999;4:269.
Koletzko S et al. Role of infant feeding practices in development of Crohn's disease in childhood. *Br Med J*. 1989;298:1617–1618.
Koopman JS et al. Infant formulas and gastrointestinal illness. *Am J Public Health*. 1985;75:477–480.
Koerber A. From folklore to fact: the rhetorical history of breastfeeding immunity, 1950–1997. *J Med Humanity*. 2006;27:151–166.
Kostraba JN et al. Early exposure to cow's milk and solid foods in infancy, genetic predisposition and risk of IDDM. *Diabetes*. 1993;42:288–295.
Kovar MG et al. Review of the epidemiologic evidence for an association between infant feeding and infant health. *Pediatrics*. 1984;74(suppl):615–638.
Krachler M, Rossipal SE, Irgolic KJ. Changes in the concentrations of trace elements in human milk during lactation. *J Trace Elements Biol*. 1998;12: 159–176.
Kramer MS, Aboud F, Mironova E, et al. Breastfeeding and child cognitive development: new evidence from a large randomized trial. *Arch Gen Psychiatry*. 2008;65:578–584.
Kramer MS. Infant feeding, infection, and public health. *Pediatrics*. 1988;81:164–166.
Kramer MS et al. Promotion of breastfeeding intervention trial (PROBIT): a randomized trial in the Republic of Belarus. *JAMA*. 2001;285:413–420.
Kramer MS et al. Breastfeeding and infant growth: biology or bias? *Pediatrics*. 2002;110:343–347.
Krebs NF, Hambidge KM. Zinc requirements and zinc intakes of breast-fed infants. *Am J Clin Nutr*. 1986;43:288–292.
Krebs NF et al. Zinc homeostasis in breast-fed infant. *Pediatr Res*. 1996;39:661–665.
Kulski JK, Hartmann PE. Changes in the concentration of cortisol in milk during different stages of human lactation. *Aust J Exp Biol Med Sci*. 1981;59:769.
Kumar A et al. Cord blood and breast milk iron status in maternal anemia. *Pediatrics*. 2008;121:e673–e677.
Kum-Nji et al. Reducing the incidence of sudden infant death syndrome in the delta region of Mississippi: a three-pronged approach. *South Med J*. 2001;94: 704–710.
Kumpulainen J et al. Formula feeding results in lower selenium status than breast-feeding or selenium-supplemented formula feeding: a longitudinal study. *Am J Clin Nutr*. 1987;45:49–53.
Kunz C, Lönnerdal B. Re–evaluation of the whey protein/casein ratio of human milk. *Acta Paediatr*. 1992;81:107–112.
Kwan ML et al. Breastfeeding patterns and risk of childhood acute lymphoblastic leukaemia. Br J *Cancer*. 2005;93:379–384.
Kwan ML et al. Breastfeeding and the risk of childhood leukemia: a meta-analysis. *Public Health Rep*. 2004;119:521–535.
Labbok M, Hendershot GE. Does breast-feeding protect against malocclusion? An analysis of the 1981 Child Health Supplement to the National Health Interview survey. *Am J Priv Med*. 1987;3:227–232.
Larsson LA. *Immunobiology of Human Milk*. Amarillo, Tex: Pharmasoft; 2004:32.
Latarte J et al. Lack of protective effect of breast-feeding in congenital hypothyroidism: report of 12 cases. *Pediatrics*. 1980;65:703–705.
Lawlor DA et al. Associations of parental, birth, and early life characteristics with systolic blood pressure at 5 years of age. *Circulation*. 2004;110:2417–2423.
Lawrence RM, Pane CA. Human breast milk: current concepts of immunology and infectious diseases. *Curr Probl Pediatr Adolesc Health Care*. 2007;37:7–36.

Leach JL et al. Total potentially available nucleotides of human milk by stage of lactation. *Am J Clin Nutr.* 1995;61:1224–1230.
Lemons JA et al. Differences in the composition of preterm and term human milk during early lactation. *Pediatr Res.* 1982;16:113–117.
Lepage G et al. The composition of preterm milk in relation to the degree of prematurity. *Am J Clin Nutr.* 1984;40:1042–1049.
Leventhal JM et al. Does breastfeeding protect against infection in infants less than 3 months of age? *Pediatrics.* 1986;78:896–903.
Litwin SD, Zehr BD, Insel RA. Selective concentration of IgD class-specific antibodies in human milk. *Clin Exp Immunol.* 1990;80:262–267.
Long K et al. The impact of infant feeding patterns on infection and diarrheal disease due to enterotoxigenic *Escherichia coli. Salud Publica Mex.* 1999;41:263–270.
Lönnerdal B. Regulation of mineral and trace elements in human milk: exogenous and endogenous factors. *Nutr Review.* 2000;58:223–229.
Lopez-Alarcon M, Villalpando S, Fajardo A. Breastfeeding lowers the frequency and duration of acute respiratory infection and diarrhea in infants under six months of age. *J Nutr.* 1997;127:436–443.
Lovegrove JA, Hampton SM, Morgan JB. The immunological and long-term atopic outcome of infants born to women following a milk-free diet during late pregnancy and lactation: a pilot study. *Br J Nutr.* 1994;71:223–238.
Lucas A. Programming by early nutrition: an experimental approach. *J Nutr.* 1998;128:401S–406S.
Lucas A, Cole TJ. Breast milk and neonatal necrotizing enterocolitis. *Lancet.* 1990;336:1519–1523.
Lucas A, Mitchell MD. Prostaglandins in human milk. *Arch Dis Child.* 1980;55:950.
Lucas A et al. Breastfeeding and catch-up growth in infants born small for gestational age. *Acta Paediatr.* 1997;86:564–569.
Mackey AD, Picciano MF. Maternal folate status during extended lactation and the effect of supplemental folic acid. *Am J Clin Nutr.* 1999;69:285.
Malcolm CA et al. Maternal docosahexaenoic acid supplementation during pregnancy and visual evoked potential development in term infants: a double blind, prospective, randomised trial. *Arch Dis Child.* 2003;88:F383.
Mandel D et al. Fat and energy contents of expressed human breast milk in prolonged lactation. *Pediatrics.* 2005;116:e432–e435.
Marchini G, Linden A. Cholecystokinin, a satiety signal in newborn infants? *J Dev Physiol.* 1992;17:215–219.
Marild S et al. Protective effect of breastfeeding against urinary tract infection. *Acta Paediatr.* 2004;93:164–167.
Martin R et al. Probiotic potential of 3 lactobacilli strains isolated from breast milk. *J Hum Lact.* 2005a;21:8–17.
Martin RM et al. Breast-feeding and childhood cancer: a systematic review with meta-analysis. *Int. J Cancer.* 2005b;117:1020–1031.
Martin RM et al. Breastfeeding and atherosclerosis: intima-media thickness and plaques at 65-year follow-up of the Boyd Orr Cohort. *Arterioscler Thrombo Vasc Biol.* 2005c;25:1482–1488.
Mason T et al. Breast feeding and the development of juvenile rheumatoid arthritis. *J Rheumatol.* 1995;22: 1166–1170.
Mathur GP et al. Breastfeeding and childhood cancer. *Indian Pediatr.* 1993;30:651–657.
Matsuoka Y, Idota T. The concentration of epidermal growth factor in Japanese mother's milk. *J Nutr Sci Vitaminol.* 1995;41:24–51.
Mayer EJ et al. Reduced risk of IDDM among breast-fed children. *Diabetes.* 1988;37:1625–1632.
McVeagh P, Miller JB. Human milk oligosaccharides: only the breast. *J Paediatr Child Health.* 1997;33: 281–286.
Medves JM. Three infant care interventions: reconsidering the evidence. *JOGNN.* 2002;31:563–569.
Metcalfe DD. Food hypersensitivity. *J Allergy Clin Immunol.* 1984;73:749–762.
Michie et al. Physiological secretion of chemokines in human breast milk. *Eur Cytokine Netw.* 1998;9: 123–129.
Minekawa R et al. Human breast milk suppresses the transcriptional regulation of IL-1(beta)-induced NF-(kappa)B signaling in human intestinal cells. *Am J Physiol.* 2004;287:C1404–C1411.
Miralles O et al. A physiological role of breast milk leptin in body weight control in developing infants. *Obesity.* 2006;14:1371–1377.
Mitra AK, Rabbani F. The importance of breastfeeding in minimizing mortality and morbidity from diarrhoeal diseases: the Bangladesh perspective. *J Diarrhoeal Dis Res.* 1995;13:1–7.
Montgomery RK et al. Lactose intolerance and the genetic regulation of intestinal lactose-phlorizin hydrotase. *Fed Am Soc Exp Biol J.* 1991;5:2824–2832.
Morriss FH. Method for investigating the presence and physiologic role of growth factors in milk. In: Jensen RG, Neville MC, eds. *Human Lactation: Milk Components and Methodologies.* New York, NY: Plenum; 1985:193–200.
Morriss FH et al. Relationship of human milk pH during course of lactation to concentrations of citrate and fatty acids. *Pediatrics.* 1986;78:458–464.
Morrisset J, Jolicoeur L. Effect of hydrocortisone on pancreatic growth in rats. *Am J Physiol.* 1980; 239:295.
Morton JA. The clinical usefulness of breast milk sodium in the assessment of lactogenesis. *Pediatrics.* 1994;93:802–806.
Motil KJ, Krtz B, Thotathuchery M. Lactation performance of adolescent mothers show preliminary

differences from that of adult women. *J Adolescent Med.* 1997;20:442–449.
Motil KJ et al. Human milk protein does not limit growth of breast-fed infants. *J Pediatr Gastroenterol Nutr.* 1997;24:10–17.
Naficy AB et al. Epidemiology of rotavirus diarrhea in Egyptian children and implications for disease control. *Am J Epidemiol.* 1999;150:770–777.
Neville MC. Lactogenesis. In: Schanler RJ, ed. *Breastfeeding 2001. Part I. The Evidence for Breastfeeding. Ped Clin N Amer.* 2001;48:69–86.
Neville MC, Oliva-Rasbach J. Is maternal milk production limiting for infant growth during the first year of life in breast-fed infants? In: Goldman AS, Atkinson SA, Hanson LA, eds. *Human Lactation.* Vol. 3. New York, NY: Plenum; 1987:123–133.
Neville MC et al. Studies in human lactation: milk volumes in lactating women during the onset of lactation and full lactation. *Am J Clin Nutr.* 1988;48: 1375–1386.
Newburg DS et al. Role of human-milk lactadherin in protection against symptomatic rotavirus infection. *Lancet.* 1998;351:1160.
Newman J. How breast milk protects newborns. *Scientific American.* December 1995:76–79.
Nommsen-Rivers L. The long-term effects of early nutrition: the role of breastfeeding on cholesterol levels. *J Hum Lact.* 2003;19:103–104.
Oddy WH et al. The relation of breastfeeding and body mass index to asthma and atopy in children: a prospective cohort study to age 6 years. *Am J Public Health.* 2004;94:1531–1537.
Oddy WH. Breastfeeding and asthma in children: findings from a West Australian study. *Breastfeeding Rev.* 2000;8:5–11.
Odze RD et al. Allergic colitis in infants. *J Pediatr.* 1995;126:163–170.
Okamoto Y, Ogra P. Antiviral factors in human milk: implications in respiratory syncytial virus infection. *Acta Paediatr Scand.* 1989;351(suppl):137–143.
Ostrea EM et al. Influence of breast-feeding on the restoration of the low serum concentration of vitamin E and beta-carotene in the newborn infant. *Am J Obstet Gyncecol.* 1986;154:1014–1017.
Owen CD et al. Infant feeding and blood cholesterol: a study in adolescents and a systematic review. *Pediatrics.* 2002;110:597–608.
Pabst HE et al. Differential modulation of the immune response to breast- or formula-feeding of infants. *Acta Paediatr.* 1997;86:1291–1297.
Paganelli R, Cavagni G, Pallone F. The role of antigenic absorption and circulating immune complexes in food allergy. *Ann Allergy.* 1986;57:330–336.
Palmer B. The influence of breastfeeding on the development of the oral cavity: a commentary. *J Hum Lact.* 1998;14:93–99.
Perera BJ et al. The impact of breastfeeding practices on respiratory and diarrhoeal disease in infants: a study from Sri Lanka. *J Trop Pediatr.* 1999;45:115–118.
Perez-Bravolt F et al. Genetic predisposition and environmental factors leading to the development of insulin-dependent diabetes mellitus in Chilean children. *J Mol Med.* 1996;74:105–109.
Petschow B et al. Influence of orally administered epidermal growth factor on normal and damaged intestinal mucosa in rats. *J Pediatr Gastroenterol Nutr.* 1993;17:49–57.
Picciano MF. Nutrient composition of human milk. In: Schanler RJ, ed. *Breastfeeding 2001, Part I. The evidence for breastfeeding. Ped Clin N Amer.* 2001;48:69–86.
Pickering LK, Kohl S. Human milk humoral immunity and infant defense mechanisms. In: Howell RR, Morriss FH, Pickering LK, eds. *Human Milk in Infant Nutrition and Health.* Springfield, Ill: Thomas; 1986:123–140.
Pisacane A et al. Breast feeding and urinary tract infection. *Lancet.* 1990;336:50.
Pisacane A et al. Breast feeding and multiple sclerosis. *Br Med J.* 1994;308:1411–1412.
Pisacane A et al. Breast-feeding and inguinal hernia. *J Pediatr.* 1995a;127:109–111.
Pisacane A et al. Breast feeding and acute appendicitis. *BMJ.* 1995b;310:836–837.
Popkin BM et al. Breast-feeding and diarrheal morbidity. *Pediatrics.* 1990;86:874–882.
Porro E et al. Early wheezing and breast feeeding. *Asthma.* 1993;30:23–28.
Prentice AM, Collinson AC. Does breastfeeding increase thymus size? *Acta Paediatr.* 2000;89:8–10.
Prentice AM et al. Breast-milk antimicrobial factors of rural Gambian mothers. *Acta Paediatr Scand.* 1984;73: 796–812.
Prosser CG, Saint L, Hartmann PE. Mammary gland function during gradual weaning and early gestation in women. *Aust J Exp Biol Med Sci.* 1984; 62:215–228.
Pullan CR et al. Breast-feeding and respiratory syncytial virus infection. *Br Med J.* 1980;281(6247):1034–1036.
Quigley MA, Kelly YJ, Sacker A. Breastfeeding and hospitalization for diarrheal and respiratory infection in the United Kingdom Millennium Cohort Study. *Pediatrics.* 2007;119:e837–e842.
Quinlan PT et al. The relationship between stool hardness and stool composition in breast- and formula-fed infants. *J Pediatr Gastroenterol Nutr.* 1995;20: 81–90.
Rahman MM et al. Local production of rotavirus specific IgA in breast tissue and transfer to neonates. *Arch Dis Child.* 1987;62:401–405.
Raiha NCR. Nutritional proteins in milk and the protein requirement of normal infants. *Pediatrics.* 1985;75(suppl):136–141.
Raisler J, Alexander C, Campo P. Breastfeeding and infant illness: a dose-response relationship? *Am J Public Health.* 1999;89:25–30.
Ramsay DT et al. Anatomy of the lactating human breast redefined with ultrasound imaging. *J Anat.* 2005;206:525–534.

Ravelli A et al. Infant feeding and adult glucose tolerance, lipid profile, blood pressure, and obesity. *Arch Dis Child.* 2000;82:248–252.

Ravelomanana N et al. Risk factors for fatal diarrhoea among dehydrated malnourished children in a Madagascar hospital. *Eur J Clin Nutr.* 1995;49:91–97.

Read L et al. Changes in the growth-promoting activity of human milk during lactation. *Pediatr Res.* 1984;18:133–138.

Reid B, Smith H, Friedman Z. Prostaglandins in human milk. *Pediatrics.* 1980;66:870–872.

Rigas A et al. Breast-feeding and maternal smoking in the etiology of Crohn's disease and ulcerative colitis in childhood. *Ann Epidemiol.* 1993;3:387–392.

Rosenbauer J, Herzig P, Giani G. Early infant feeding and risk of type 1 diabetes mellitus—a nationwide population-based case-control study in pre-school children. *Diabetes Metab Res Rev.* 2008;24:211–222.

Rosenberg M. Breast-feeding and infant mortality in Norway 1860–1930. *J Biosoc Sci.* 1989;21:335–348.

Rovet JF. Does breast-feeding protect the hypothyroid infant whose condition is diagnosed by newborn screening? *Am J Dis Child.* 1990;144:319–323.

Rubaltelli FR et al. Intestinal flora in breast- and bottle-fed infants. *J Perinat Med.* 1998;26:186–191.

Rudloff EH et al. Interleukin-6 in human milk. *J Reprod Immunol.* 1993;23:13–20.

Ruiz-Palacios GM et al. Protection of breast-fed infants against *Campylobacter* diarrhea by antibodies in human milk. *J Pediatr.* 1990;116:707–713.

Ruuska R. Occurrence of acute diarrhea in atopic and nonatopic infant: the role of prolonged breast-feeding. *J Pediatr Gastroenterol Nutr.* 1992;14:27–33.

Saarela AT, Kokkonen J, Koivisto M. Macronutrient and energy contents of human milk fractions during the first six months of lactation. *Acta Paediatrica.* 2005;94:1176–1181.

Saarinen KM et al. Breast-feeding and the development of cow's milk protein allergy. *Adv Exp Med Biol.* 2000;478:121–130.

Saarinen UM, Kajosaari M. Breastfeeding as prophylaxis against atopic disease: prospective follow-up study until 17 years old. *Lancet.* 1995;346:1065–1069.

Saarinen UM et al. Prolonged breast feeding as prophylaxis for recurrent otitis media. *Acta Paediatr Scand.* 1982;71:567–571.

Sadeharju K et al. Maternal antibodies in breast milk protect the child from enterovirus infections. *Pediatrics.* 2007;119:941–996.

Saint L, Smith M, Hartmann PE. The yield and nutrient content of colostrum and milk of women giving birth to 1 month postpartum. *Br J Nutr.* 1984;52:87–95.

Salmenpera L et al. Folate nutrition is optimal in exclusively breast-fed infants but inadequate in some of their mothers and in formula-fed infants. *J Pediatr Gastroenterol Nutr.* 1986;5:283–289.

SanGiovanni JP et al. Meta-analysis of dietary essential acuity in healthy preterm infants. *Pediatrics.* 2000; 105:1292–1298.

Sassen ML, Brand R, Grote JJ. Breast-feeding and acute otitis media. *Amer J Otolaryngol.* 1994;15:351–357.

Schroten H et al. Secretory immunoglobulin A is a component of the human milk fat globule membrane. *Pediatr Res.* 1999;45:82–86.

Serdula MK, Seward J, Marks JS. Seasonal differences in breast-feeding in rural Egypt. *Am J Clin Nutr.* 1986;44: 405–409.

Shehadeh N et al. Importance of insulin content in infant diet: suggestion for a new infant formula. *Acta Paediatr.* 2001;90:93–95.

Shing YW, Klagsburn M. Human and bovine milk contain different sets of growth factors. *Endocrinology.* 1984;115:273.

Shu XO et al. Infant breastfeeding and the risk of childhood lymphoma and leukaemia. *Int J Epidemiol.* 1995;24:27–34.

Siigur U, Ormission A, Tamm A. Faecal short-chain fatty acids in breast-fed and bottle-fed infants. *Acta Paediatr.* 1993;82:536–538.

Siimes MA et al. Exclusive breast-feeding for nine months: risk of iron deficiency. *J Pediatr.* 1984;104: 196–199.

Smith AM, Picciano MF, Milner JA. Selenium intakes and status of human milk and formula fed infants. *Am J Clin Nutr.* 1982;35:521.

Smulevich et al. Parental occupation and other factors and cancer risk in children: 1. Study methodology and non-occupational factors. *Int J Cancer.* 1999; 83:712.

Sommerburg O et al. Carotenoid supply in breast-fed and formula-fed neonates. *Eur J Pediatr.* 2000; 159:86–90.

Specker BL et al. Sunshine exposure and serum 25-hydroxyvitamin D concentrations in exclusively breast-fed infant. *J Pediatr.* 1985;107:372–376.

Steichen JJ, Krug-Wispe SK, Tsang RC. Breastfeeding the low birth weight preterm infant. *Clin Perinatol.* 1987;14:131–171.

Steel MG, Leslie GA. Immunoglobulin D in rat serum, saliva and milk. *Immunology.* 1985;55:571–577.

Stuff JE, Nichols GL. Nutrient intake and growth performance of older infants fed human milk. *J Pediatr.* 1989;115:959–968.

Svanborg C et al. HAMLET fills tumor cells by an apoptosis-like mechanism—cellular, molecular, and therapeutic aspects. *Adv Cancer Res.* 2003;8:1–29.

Swartzbaum JA et al. An exploratory study of environmental and medical factors potentially related to childhood cancer. *Med Pediatr Oncol.* 1991;19: 115–121.

Takahata Y et al. Interleukin-18 in human milk. *Pediatr Res.* 2001;50:268–272.

Takala AK et al. Risk factors of invasive *Haemophilus influenzae* type b disease among children in Finland. *J Pediatr.* 1989;115:694–701.

Tran TT et al. Effects of neonatal dietary manganese exposure on brain dopamine levels and neurocognitive functions. *Neurotoxicology.* 2002;145:1–7.

Udall JN et al. Liver disease in a1-antitrypsin deficiency. *JAMA*. 1985;253:2679–2682.

US Department of Health and Human Services. *Healthy People 2010*. Washington, DC: USDHHS; 2000.

Uvnas-Moberg K, Marchini G, Windberg J. Plasma cholecystokinin concentrations after breastfeeding in healthy 4 day old infants. *Arch Dis Child*. 1993;68:46–48.

Van Derslice J, Popkin B, Briscoe J. Drinking-water quality, sanitation, and breast-feeding: their interactive effects on infant health. *Bull WHO*. 1994;72: 589–601.

van der Westhuyzen, Chetty M, Atkinson PM. Fatty acid composition of human milk from South African black mother consuming a traditional maize diet. *Eur J Clin Nutr*. 1988;42:213–220.

Verge CF et al. Environmental factors in childhood IDDM: a population-based case-control study. *Diabetes Care*. 1994;17:1381.

Victora CG et al. Evidence for protection by breastfeeding against infant deaths from infectious diseases in Brazil. *Lancet*. 1987;2:319–321.

Virtanen SM, Rasanen L, Aro A et al. Childhood diabetes in Finland Study Group: feeding in infancy and the risk of type 1 diabetes mellitus in Finnish children. *Diabet Med*. 1992;9:815.

Wagner V, Stockhausen JG. The effect of feeding human milk and adapted milk formulae on serum lipid and lipoprotein levels in young infants. *Eur J Pediatr*. 1988;147:292–295.

Wahlberg J, Vaarala O, Ludvigsson J. Dietary risk factors of the emergence of type 1 diabetes-related autoantibodies in 2 1/2 year-old Swedish children. *Br J Nutr*. 2006;95:603–608.

Wallace JM et al. Cytokines in human milk. *Br J Biomed Sci*. 1997;54:85–87.

Wasmuth HE, Kolb H. Cow's milk and immune-mediated diabetes. *Proc Nutr Soc*. 2000;59:573–579.

Widdowson EM, Colombo VE, Artavanis CA. Changes in the organs of pigs in response to feeding for the first 24 hours after birth: II. The digestive tract. *Biol Neonate*. 1996;28:272.

Wight NE. Donor human milk for preterm infants. *J Perinatol*. 2001;21:249–254.

Wilson JV, Self TW, Hamburger R. Severe cow's milk-induced colitis in an exclusively breast-fed neonate. *Clin Pediatr*. 1990;29:77–80.

Wilson-Clay B, Hoover K. *The breastfeeding atlas*, 4th ed. Manchaca, TX: LactNews Press, 2008.

Wirt DP et al. Activated and memory T lymphocytes in human milk. *Cytometry*. 1992;13:282–290.

Woolridge MW, Fisher C. Colic, "overfeeding," and symptoms of lactose malabsorption in the breast-fed baby: a possible artifact of feed management? *Lancet*. 1988;2:382–384.

Woolridge MW, Ingram JC, Baum JD. Do changes in pattern of breast usage alter the baby's nutrient intake? *Lancet*. 1990;336:395–397.

Yamauchi Y, Yamanouchi I. Breast-feeding frequency during the first 24 hours after birth in full-term neonates. *Pediatrics*. 1990;86:171–175.

Yoneyama K, Nagata H, Asano H. Growth of Japanese breast-fed and bottle-fed infants from birth to 20 months. *Ann Hum Biol*. 1994;21:597–608.

Zaman K et al. Children's fluid intake during diarrhoea: a comparison of questionnaire responses with data from observations. *Acta Paediatr*. 2002;91:376–382.

Zanardo V et al. Beta endorphin concentrations in human milk. *J Pediatr Gastroenterol Nutr*. 2001; 33:160–164.

Ziegler EE et al. The vitamin deficiency in breastfed infants in Iowa. *Pediatrics*. 2006;118:603–610.

Appendix 4-A

Composition of Human Colostrum and Mature Breastmilk

Constituent (per 100 mL)	Colostrum 1–5 days	Mature Milk > 30 days
Energy, kcal	58	70
Lactose, g	5.3	7.3
Total nitrogen, mg	360	171
Protein nitrogen, mg	313	129
Nonprotein nitrogen, mg	47	42
Total protein, g	2.3	0.9
Casein, mg	140	187
α-lactalbumin, mg	218	161
Lactoferrin, mg	330	167
IgA, mg	364	142
Urea, mg	10	30
Creatine, mg	—	3.3
Total fat, g	2.9	4.2
Cholesterol, mg	27	16
Vitamins (Fat Soluble)		
Vitamin A, µg	89	47
Beta-carotene, µg	112	23
Vitamin D, µg	—	0.04
Vitamin E, µg	1280	315
Vitamin K, µg	0.2	0.21
Vitamins (Water Soluble)		
Thiamine, µg	15	16
Riboflavin, µg	25	35
Niacin, µg	75	200
Folic acid, µg	—	5.2
Vitamin B_6, µg	12	28
Vitamin B_{12}, ng	200	26
Vitamin C, mg	4.4	4.0
Minerals and Trace Elements		
Calcium, mg	23	28
Sodium, mg	48	15
Potassium, mg	74	58
Iron, µg	45	40
Zinc, µg	540	166

Source: Used with permission from Casey CE, Hambidge KM. Nutritional aspects of human lactation. In: Neville MC, Neifert MR, eds. *Lactation: physiology, nutrition and breastfeeding*. New York: Plenum, 1983:203–204.