Bioactive Proteins in Human Milk: Health, Nutrition, and Implications for Infant Formulas

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Breast milk confers many benefits to the newborn and developing infant. There is substantial support for better long-term outcomes, such as less obesity, diabetes, and cardiovascular disease, in breastfed compared with formula-fed infants. More short-term outcomes, such as incidence and duration of illness, nutrient status, and cognitive development during the first year of life also demonstrate benefits of breastfeeding. Several proteins in breast milk, including lactoferrin, α-lactalbumin, milk fat globule membrane proteins, and osteopontin, have been shown to have bioactivities that range from involvement in the protection against infection to the acquisition of nutrients from breast milk. In some cases, bovine counterparts of these proteins exert similar bioactivities. It is possible by dairy technology to add protein fractions highly enriched in these proteins to infant formula. *(J Pediatr 2016;173S:S4-9)*.

Infant formulas have undergone many modifications in previous decades so that the performance of formula-fed infants more closely resembles that of breastfed infants. These alterations, some of which are relatively recent, include: modifications of whey:casein; addition of taurine, nucleotides, docosahexanoic acid, and arachidonic acid; prebiotics (fructo-oligosaccharides/galacto-oligosaccharides); and lutein. Despite these modifications, it is clear that differences remain between breastfed infants and formula-fed infants with regard to both short-term (eg, illnesses, cognitive development) and long-term (eg, obesity, diabetes, cardiovascular disease) outcomes. It is, therefore, evident that further alterations in the composition of infant formula need to be considered.

Several components of breast milk have been shown to have bioactivities in vitro, but there is not yet enough evidence from clinical trials to incorporate them into infant formula. It also should be recognized that, with few exceptions, these components (complex oligosaccharides, proteins, and lipid components, such as gangliosides) are not commercially available.

Various bioactive proteins are the components with the most support from clinical trials to date.[1] It should be recognized that the proteins studied are largely of bovine origin and are not identical to their human counterparts. However, in many cases, the structure of the bovine milk proteins share a high degree of homology with the human milk proteins, and because in vitro studies have shown equivalence of the human and bovine proteins with regard to bioactivity(-ies), it is reasonable to study the effects that supplementation with these bovine proteins have on infants.

Bioactive Proteins Involved in Health Outcomes

It is well recognized that breastfed infants have fewer infections than formula-fed infants. This is more pronounced in less developed countries but is significant in affluent countries as well.[2] Several breast milk proteins have been shown to be involved in protecting against infection (**Figure 1**).

Lactoferrin

Breast milk was found early on to have bacteriostatic activity against *Escherichia coli*.[3] Investigators discovered that this activity was due to the presence of the protein, lactoferrin, an iron-binding protein that is, present in largely unsaturated form in breast milk. Its high-binding affinity for iron enables it to withhold iron from iron-requiring pathogens. Lactoferrin subsequently was found to be bactericidal, able to kill pathogens, such as *Vibrio cholera* and *Streptococcus mutans*.[4] Furthermore, lactoferrin can act in a synergistic fashion with lysozyme, another protein present in comparatively high concentration in breast milk, to kill Gram-negative bacteria, which are normally resistant to bactericidal action.[5] Lactoferrin, which is highly positively charged, can form a strong complex with bacterial lipopolysaccharide, which is negatively charged, and create holes in the outer membrane of Gram-negative bacteria. Following this attack, lysozyme penetrates the outer

BSSL	Bile salt-stimulated lipase
CPP	Casein phosphopeptide
GMP	Glycomacropeptide
MFGM	Milk fat globule membrane
ORS	Oral rehydration solution
RCT	Randomized controlled trial
sIgA	Secretory IgA
WHO	World Health Organization

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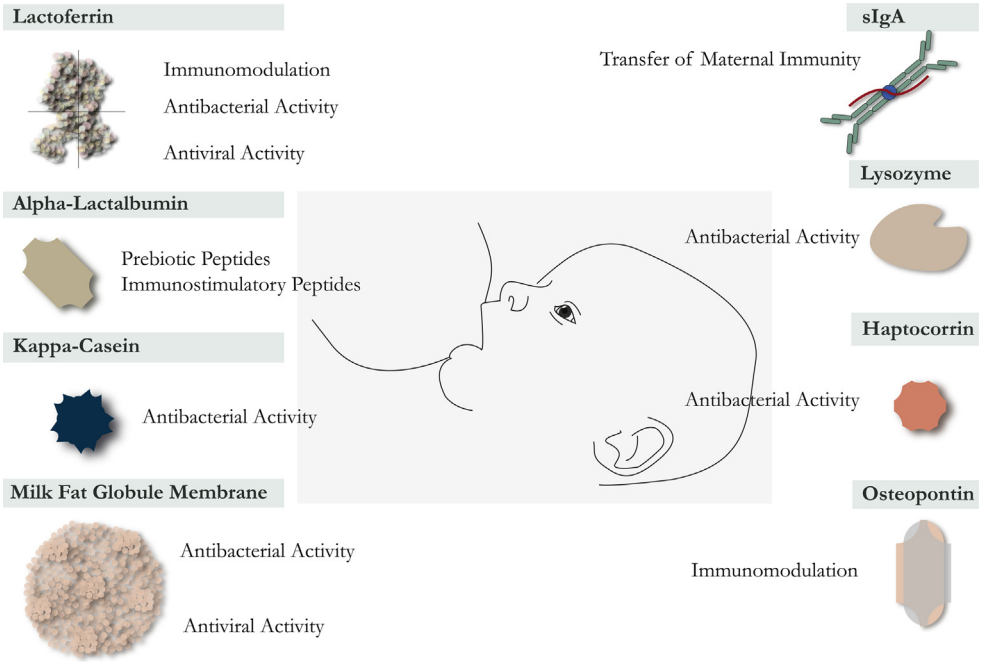

Figure 1. Bioactive proteins involved in health outcomes.

membrane, thereby obtaining access to and degrading the proteoglycan matrix, resulting in bactericidal action.

Lactoferrin also can modulate immune function.[6] This may occur through several mechanisms, one of which is mediated by an intestinal receptor for lactoferrin.[7] Lactoferrin has a structure that makes it comparatively resistant to digestion and intact lactoferrin is found in significant amounts in exclusively breastfed infants.[8] Following binding of lactoferrin to its receptor, the protein is internalized by an endocytotic process and subsequently binds to the nucleus.[9] Inside the nucleus, lactoferrin binds to specific sites on DNA and, thus, acts as a transcription factor.[10] Among the genes that are affected by lactoferrin are those for several cytokines (eg, interleukin-1β and transforming growth factor-β). Thus, lactoferrin can affect and modulate immune function in the infant, thereby affecting health outcomes.

A clinical study on bovine lactoferrin added to infant formula showed a reduction in upper respiratory disease in infants 6-12 months of age.[11] Manzoni et al[12] have shown a significant reduction in sepsis in premature infants given oral supplements of bovine lactoferrin. Further, Ochoa et al[13] showed that infants given bovine lactoferrin had significantly lower prevalence of *Giardia* species and better growth than infants not receiving the supplement.

Lysozyme

In addition to acting together with lactoferrin to kill Gram-negative bacteria (as described above), lysozyme can independently kill Gram-positive bacteria by degrading its outer membrane. Lysozyme, like lactoferrin, is found intact in the stool of both preterm and term infants[8,14] and may, therefore, exert antimicrobial activity in the gut of breastfed infants. Our group explored the possibility that recombinant forms of both human lactoferrin and human lysozyme have a beneficial effect in children hospitalized with acute diarrhea.[15] The 2 human milk proteins were added to the World Health Organization (WHO) rice-based oral rehydration solution (ORS), because both proteins were produced in rice, so that any contaminant in the proteins would be rice protein or starch. The hospital-based study was a double-blind randomized controlled trial (RCT). The other 2 groups received either regular WHO glucose-based ORS or the WHO rice-based ORS (without the recombinant proteins). All children were followed for 2 weeks. A significant reduction in diarrhea was found in the group receiving the human milk proteins, suggesting that they may serve a similar function in breastfed infants. Because of the study design, it is not known whether the activity was due to the individual components or the combination of the two.

Secretory IgA

By linking 2 molecules of IgA together with a joining chain and a secretory component, the resulting secretory IgA (sIgA) becomes resistant to proteolytic degradation,[16] and intact sIgA is found in the stool of both preterm[14] and term[8] breastfed infants. Although the intestinal mucosa is capable of producing sIgA to some extent in all infants, the amount of sIgA in breastfed infants far outweighs that of formula-fed infants. Through the so-called enteromammary link, maternal immunity can