Biological Effects of Novel Bovine Milk Fractions

Bo Lönnerdal

Department of Nutrition, University of California, Davis, CA, USA

Abstract

Novel dairy fractions have been isolated and are now commercially available. Several of them have been shown to have biological activities in various test systems. α-Lactalbumin was first isolated to provide a good source of tryptophan, often the first limiting amino acid in infant formulas, but has then been shown to be digested into smaller peptides with antimicrobial and prebiotic activities, immunostimulatory effect and acting as enhancers of mineral absorption. Lactoferrin bioactivities include antibacterial and antiviral effects, regulation of immune function, stimulation of intestinal proliferation and differentiation and facilitating iron absorption, but these activities may have been limited due to earlier contamination with LPS. Lactoferrin free of lipopolysaccharide may prove to be more effective with regard to exerting these activities. Osteopontin is a heavily phosphorylated and glycosylated protein that modulates immune function and stimulates Th1/Th2 switching, and, possibly, also affects bone mineralization and growth. Biological activities of lactoferrin may be facilitated by osteopontin. Milk fat globule membranes are a fraction that has previously been excluded from infant formulas, but components of this fraction have been shown to exhibit antimicrobial activities and to prevent infection. Further clinical studies are needed on infants fed formulas with these components incorporated.

Introduction

Human milk is known to contain a wide range of proteins providing various biological activities in the newborn infant [1]. These bioactivities range from enhancing immune function, antibacterial and antiviral activities, facilitating nutrient absorption, promoting bone growth, and supporting infant development. Many of these protein components have been believed to be unique to human milk, and in a sense they are, but it is possible that some bovine
counterparts may exert some of the biological activities provided by human milk proteins. In recent years, dairy technology has improved considerably, and bovine milk protein fractions are now available on a commercial scale. While some of these protein fractions have only been tested in laboratory scale experiments and in animal models, several of them have been evaluated in clinical trials on infants. In this article, some of these novel bovine milk protein fractions, namely α-lactalbumin, lactoferrin, milk fat globule membrane (MFGM) proteins and osteopontin, will be reviewed with regard to their functionality and possible use as ingredients in infant formula.

**α-Lactalbumin**

One of the major whey proteins in human milk is α-lactalbumin, which constitutes 10–20% of the total protein content of mature breast milk [1], and even more in colostrum and preterm milk. The proportion of α-lactalbumin in bovine whey is considerably less than that in human whey, and since cow’s milk protein consist of ~82% casein, α-lactalbumin is only 2–5% of the total protein content. α-Lactalbumin has a molecular weight of about 14,100 Da, and the sequence homology between human and bovine α-lactalbumin is 74% [2]. It has an amino acid composition that corresponds very well to the amino acid requirements of infants, and it is relatively high in tryptophan (5%), lysine (11%) and cysteine (6%) and can therefore complement other proteins that have lower contents of these essential amino acids. The protein content of infant formula is often considered to be higher than needed, as reflected by higher blood urea nitrogen, plasma amino acids (particularly the insulinogenic branched-chain amino acids) and insulin in formula-fed as compared to breastfed infants. However, when the protein content of infant formula is lowered, tryptophan becomes a limiting amino acid. Therefore, several studies have evaluated the effects of adding bovine α-lactalbumin or α-lactalbumin-enriched fractions when lowering the protein content of the formula [2]. We found that plasma tryptophan levels were similar to breastfed infants and significantly higher than in infants fed control formula [3].

α-Lactalbumin binds one calcium ion tightly, which aids to render the molecule a tight, compact globular structure; removal of this calcium ion results in unfolding and the molecule becomes considerably larger and more flexible. This may affect the digestibility of α-lactalbumin (see below). α-Lactalbumin also has a second calcium-binding site, which may not be occupied in vivo [2]. This site is capable of binding other divalent cations, e.g. zinc, iron and manganese, but native α-lactalbumin isolated from human milk does not contain any significant quantities of these ions. It is possible, however, that during digestion, smaller peptides with capacity of forming complexes with cations are formed. We have found increased zinc and iron absorption from formulas enriched with bovine α-lactalbumin in infant rhesus monkeys [4], suggest-
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Fig. 1. Digestion and biological functions of α-lactalbumin.

During the digestion of α-lactalbumin in the gastrointestinal tract, smaller peptides are formed which may have physiological activity in the small intestine, or in other tissues of the body, if they are absorbed intact (fig. 1). Several of these peptides (amino acids 50–52, 99–103, and 104–108) have been shown to inhibit angiotensin I-converting enzyme in vitro [2]. It is not known, though, whether these peptides are physiologically active in vivo. An immune-stimulating peptide, GLF, consisting of Gly-Leu-Phe (amino acids 51–53, symbols GLF), has been shown to be formed, and at concentrations that may be of physiological relevance [5]. GLF increases phagocytosis by human macrophages and stimulates polymorphonuclear neutrophil oxidative metabolism, which are important for killing pathogens [6]. Specific binding sites for GLF have been found on human phagocytic cells [7]. In vitro digestion of bovine α-lactalbumin by human neonatal gastric fluid resulted in formation of GLF, and it has also been found in gastric aspirates from young infants, showing that it can also be formed in vivo [6]. It is possible that GLF stimulates macrophages in epithelial villi of the small intestine, or that it acts upon macrophages in breast milk. Phagocytes in colostrum (~80% macrophages) have been shown to kill enteropathogenic Escherichia coli (EPEC), possibly explaining our observation that infant formula enriched with bovine α-lactalbumin protected against induced EPEC infection in infant rhesus monkeys [8].

Antibacterial peptides have also been shown to be formed during digestion of α-lactalbumin. In vitro digestion of α-lactalbumin with trypsin and chymotrypsin, or chymotrypsin alone, resulted in three different peptides which were active against Gram-positive bacteria [9]. It is not yet known whether these peptides are formed in vivo. Brück et al. [10] used batch culture and a
two-stage continuous culture system to evaluate the effect of α-lactalbumin-enriched formula on mixed populations of human gut bacteria. α-Lactalbumin supplementation resulted in a significant reduction in potentially pathogenic bacteria (*Bacteroides, Clostridia, E. coli*) and making the microflora more similar to that of breastfed infants. It is not known, however, if this was due to peptides formed from α-lactalbumin. It is also conceivable that the shift in microflora, at least in part, was due to formation of peptides stimulating host-friendly bacteria. Kee et al. [11] have characterized peptide fractions from pepsin-hydrolyzed α-lactalbumin that stimulated the growth of bifidobacteria in vitro. The relative significance of these two types of peptides in vivo needs to be investigated further.

**Lactoferrin**

In principle, lactoferrin cannot be called a ‘novel’ milk protein fraction, as it was relatively early isolated from cow milk and marketed for a variety of potential biological effects. However, it was recently noted that previously marketed bovine lactoferrin contained significant concentrations of lipopolysaccharide (LPS), which possibly could have affected the bioactivity of lactoferrin. LPS forms strong complexes with lactoferrin and at a part of the molecule that is involved in several of its activities [12]. Similarly, purified bovine and human lactoferrin that was commercially available for laboratory scale experiments was contaminated with LPS; however, most investigators isolated their own preparations and may have been more successful in avoiding LPS contamination. This may potentially explain why many of the early clinical studies (~20 years ago) yielded very disappointing results, showing little effect on gut microflora, iron status and infections. It is therefore important to re-evaluate the effects of bovine lactoferrin prepared with better techniques and without any significant contamination with LPS.

Lactoferrin was first discovered to be an iron-binding protein [13]. It binds two atoms of ferric iron and its structure has similarities to that of transferrin. Lactoferrin, however, has a much stronger affinity towards iron (\(K_{\text{ass}} \approx 10^{24}\)) than transferrin, and also holds on to iron to a considerably lower pH (~3). Both these properties are likely of significance with regard to some of its bioactivities. A major part of iron in breast milk is bound to lactoferrin, and a specific receptor for lactoferrin has been found in the infant small intestine [14]. This receptor will take up lactoferrin and iron into the enterocyte by clathrin-mediated endocytosis. Thus, this is a way for iron to be taken up by the apical membrane and enter the body, either to be stored in mucosal ferritin and eventually sloughed if iron status is satisfactory, or enter the systemic circulation if iron status is low. This may explain why the iron status of exclusively breastfed infants generally is satisfactory, in spite of the comparatively low iron concentration of human milk.
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In addition, lactoferrin can enter the cell and can thus affect cell signaling and gene transcription and, consequently, affect cell proliferation and immune function. A recent double-blind clinical trial by King et al. [15] showed that iron status (as evaluated by hematocrit) of infants fed infant formula with bovine lactoferrin was better than that of infants fed formula with the same amount of iron as ferrous sulfate, but with no lactoferrin. A recent study has shown that bovine lactoferrin can also bind to a recombinant human lactoferrin receptor [16], suggesting that some forms of bovine lactoferrin may also bind to the receptor in vivo. In our early studies, bovine lactoferrin did not bind to the lactoferrin receptor, but it is possible that at that time, the lactoferrin may have contained LPS (see above) and/or possibly have been more intensely heat-treated/processed.

Lactoferrin is also known to have antimicrobial activity. Certainly, LPS contamination of commercial lactoferrin may affect this activity. Lactoferrin was early shown to have bacteriostatic activity against iron-requiring pathogens, e.g. E. coli [13]. This activity was exerted only by apo-lactoferrin – addition of iron abolished the effect – and it was shown that lactoferrin effectively can compete with these bacteria for iron, due to its high affinity. Recently, lactoferrin was shown to inhibit the growth of Enterobacter sakazakii, a food-borne pathogen that can cause diarrhea in young infants [17]. This effect was also only observed by apo- and not holo-lactoferrin. Since human milk lactoferrin is primarily in the apo-form (only saturated to 6–9%), lactoferrin in breast milk is likely to contribute to the defense against infection. It was subsequently shown that lactoferrin also can have bactericidal activity and kill pathogens such as Vibrio cholerae and Staphylococcus aureus and this activity is not affected by the iron saturation of lactoferrin [18]. It is quite possible that this anti-bacterial activity is exerted by peptides formed during digestion of lactoferrin. Lactoferricin and lactoferrampin are two such peptides that are strongly cationic and have been shown to have strong anti-microbial activity in cell and animal models [19]. Lactoferrin has also been shown to have antiviral activity [20]. In a randomized controlled double-blind study on young Peruvian children hospitalized with acute diarrhea, we found that oral rehydration solution (ORS) with recombinant human lactoferrin and lysozyme resulted in significantly reduced diarrhea duration, diarrhea volume, and recurrence of diarrhea as compared to ORS without these proteins [21]. Since the ORS contained both lactoferrin and lysozyme, it cannot be ascertained that lactoferrin was responsible for the effect observed. Ellison and Giehl [22] have shown that lactoferrin and lysozyme can act synergistically to kill bacteria, which may have occurred in our study.

Recent clinical studies support that lactoferrin can prevent infections in children. Infants fed formula supplemented with bovine lactoferrin (0.85 g/l) had significantly fewer episodes of lower respiratory illness than the placebo group, but there was no difference in diarrhea. In a study on Japanese children [23], daily supplementation with bovine lactoferrin (100 mg) was shown
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to result in significantly lower frequency and duration of vomiting and dia-
rhea than in the placebo group, although no difference in rotavirus infection
was found. Finally, Ochoa et al. [24] have shown that Peruvian children given
bovine lactoferrin had lower prevalence of colonization of Giardia than pla-
cebo controls.

Lactoferrin may also have a prebiotic function in the intestine. Peptides
resulting from pepsin digestion of human lactoferrin have been shown
to stimulate the growth of bifidobacteria [25] and it is thus possible that
increased colonization by bifidobacteria may inhibit the growth of pathogens.
Treatment of these peptides with pancreatic enzymes in vitro did not destroy
the stimulatory effect on bifidobacteria, suggesting that these peptides, if
formed in vivo, may survive further digestion and exert the prebiotic activity.
It is thus possible that lactoferrin establishes a ‘beneficial’ microflora in new-
born infants by both stimulating growth of bifidobacteria and inhibiting/killing
pathogens. Such a scenario is supported by the recent study of Manzoni et al.
[26] who found that very low-birthweight infants supplemented with bovine
lactoferrin (100 mg/day) had a significantly lower incidence of late-onset sep-
sis than those receiving placebo, and that there was no significant difference
between infants given lactoferrin with or without probiotics (Lactobacillus
rhamnosus GG).

**Osteopontin**

Osteopontin is a multifunctional protein secreted by macrophages, T cells
and epithelial cells and known to induce cell-mediated responses, chemotaxis
of inflammatory cells, and anti-inflammatory responses [27]. It is an acidic,
phosphorylated glycoprotein that was first discovered in mineralized bone
matrix, but was later found in many tissues and physiological fluids, including
human milk [28]. It contains an integrin-binding RGD (Arg-Gly-Asp) motif and
is an important regulator of bone remodeling [27]. Human milk osteopontin
has 36 phosphorylation sites and 5 O-glycosylation sites [28], whereas bovine
milk osteopontin has 28 phosphorylated sites and 3 O-glycosylated sites [29].
It appears that osteopontin in milk is phosphorylated to a considerably higher
extent than in bone and other tissues, making milk osteopontin unusually
negatively charged. Osteopontin has been shown to form a strong electro-
static complex with lactoferrin [30], and each osteopontin molecule is capable
of binding three molecules of lactoferrin. It is thus possible that osteopontin
helps to protect lactoferrin against proteolysis, preserve its bioactivities in
the gut, and aid its transport to specific sites in the intestinal mucosa.

The expression of osteopontin in human milk cells was found to be very
high, and this high level of expression persists throughout the lactation period
[31]. In a microarray analysis of 240 cytokine-related genes in human milk
cells, osteopontin was most abundantly expressed [31]. The concentration of
osteopontin in human milk accounts for ~5–10% of total milk proteins, which nearly corresponds to the concentration of lactoferrin (see also above). The concentration of osteopontin in bovine milk is much lower than in human milk (<1/5th), but bovine osteopontin has recently become available commercially in larger quantities, making it possible to add this component to infant formula.

Osteopontin is considered as a key molecule inducing Th1 type immunity. Osteopontin-null mice exhibit a defective Th1 response and are more susceptible to infections than wild-type mice [32]. Growth of *Mycobacterium bovis* bacillus Calmette-Guerin (BCG) was more rapid in macrophages from osteopontin-null mice than from wild-type mice [33] and an inverse correlation was described between tissue osteopontin expression and disease progression [34]. Breastfed infants are known to have an increased response in T cell proliferation compared to formula-fed infants when vaccinated against BCG at birth, suggesting an effect of breastfeeding on Th1 stimulation [35, 36]. It is thus possible that breast milk osteopontin contributes to host resistance in infants by enhancing the Th1 response. Osteopontin-null mice were also found to be more susceptible to rotavirus infection. Since osteopontin can bind to various integrins via its RGD motif, it may prevent attachment of the virus RGD motif to integrins on target cells [37]. Both intact osteopontin and its proteolytic fragments are found in human milk throughout lactation, and one such fragment (close to the N-terminal) that contains the integrin binding motif has been found to induce IL-12 expression in macrophages [32]. Thus, osteopontin may increase host resistance against a variety of pathogens via both Th1 polarization and other mechanisms.

**Milk Fat Globule Membrane Proteins**

The lipids in milk are surrounded by a bi-layered membrane containing unique proteins, but also phospholipids and gangliosides (fig. 2). Although this MFGM protein fraction is quantitatively minor [40], it may be of significance in the protection against infections. Several of these proteins, such as lactadherin, butyrophilin, xanthine oxidase and alkaline phosphatase have been shown to have antimicrobial activity in vitro. For example, components of this fraction isolated from human milk were able to bind to some rotavirus strains and prevent their replication, and this activity was correlated with the protein lactadherin [39]. The bioactivity of lactadherin in human milk is supported by the observation that its content in breast milk was shown to be negatively correlated with symptomatic rotavirus infection in Mexican infants [33]. The phospholipids and gangliosides in the MFGM fraction may be important in providing building blocks for the brain and promoting neurodevelopment.

Many of the components of the MFGM fraction from human milk have been shown to also be present in the bovine MFGM fraction and also to have very
similar structure. Infant formula made from cow’s milk, however, is manufactured from skim milk powder and whey protein concentrate and consequently does not contain any MFGM. Recently, milk fractions enriched with MFGM have become available on a large scale commercially and they could therefore be added to infant formulas in the future, provided that support for their bioactivity can be obtained. Several proteins in the bovine MFGM have been shown to exert inhibitory activities against various pathogens, and a whey protein concentrate enriched with MFGM may therefore help to protect against diarrhea of both bacterial and viral origin [41]. The commercially available MFGM fraction contains several bioactive components including mucin (MUC1), lactadherin, lactoferrin, sialic acid, sphingomyelin, and gangliosides. A bovine milk fraction containing MUC1 has previously been shown to inhibit hemagglutination of *V. cholerae* and *E. coli* [42]. Further, mucin purified from MFGM was shown to decrease the adherence of *Yersinia enterolytica* to intestinal membranes [43]. The MFGM fraction has also been found to inhibit rotavirus in vitro [44]. Sphingolipids, particularly gangliosides, have been shown to inhibit enterotoxins both in vitro and in vivo [45]. In addition, infant formula with added sphingolipids (gangliosides) has been shown to reduce *E. coli* counts in infant feces, and to increase beneficial bifidobacteria [46].

We have tested the concept of MFGM protein fractions having an effect on infectious diseases in Peruvian infants [47]. The infants were given MFGM proteins (~6 g/day) in a milk-based meal twice daily for 6 months in a randomized controlled double-blind study. Prevalence of diarrhea was significantly lower in the group given MFGM than in the group given the same type of meal with skim milk protein instead of MFGM. Thus, it is quite possible
that addition of the MFGM fraction to infant formula may have an effect on infectious disease.

**Conclusions**

Several novel dairy fractions are now available, and have been shown to have biological activities. Further clinical studies are needed on infants fed formulas with these components incorporated.

**References**

5. Chatterton DEW: Identification and bioactivity of peptides released from alpha-lactalbumin during digestion using the neonatal model; thesis; Danish Academy of Technical Sciences, Aarhus.
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Discussion

*Dr. Hernell:* I was thinking about the fragments of α-lactalbumin that you mentioned are formed during digestion. Not only is exocrine pancreas not fully developed at birth and at least in breastfed infants the secretion of digestive enzymes is low, but human milk also contains protease inhibitors. Given that, do you think that there is a difference in how much of these peptides are formed and how long they actually stay in the intestine before they are finally digested if you compare a breastfed infant receiving human α-lactalbumin and a formula-fed infant receiving bovine α-lactalbumin?

*Dr. Lönnerdal:* It’s a very good point you bring up. Coming back to what we discussed earlier about breast milk composition, the protein composition changes a lot during lactation, but for infant formula you are stuck with one constant composition. When looking at protease inhibitors, they are in much higher concentration during early lactation. We have done studies on α1-antitrypsin, which is actually a major breast milk protein, and its digestive function. Pancreatic protease activity is lower in early infancy, but with increasing maturity there will be less inhibition of proteases, which means more effective digestion, and that will affect α-lactalbumin, lactoferrin, and many other components. I think you have a ‘gradient of survival’, and it would be interesting to follow the survival of these bioactive components with increasing maturity, possibly looking at both duodenal aspirates (which are not easy to obtain) and fecal output, and then by extrapolating what you see in the duodenum to the stool I think we will have a better idea how much of these proteins can persist in the intact form and for how long.

*Dr. Gibson:* I was wondering, can you help us put this into context? Some of the effects that we have seen from these trace fractions that you have been examining like lactoferrin and osteopontin and so forth, they have benefits and they have effects, but in well-nourished communities such as in the US or in Sweden the difference in the infection rate between the breast and the formula fed infant is relatively small. Is it going to make much difference if we put all these compounds into an infant formula and what sort of a difference are we expecting, and how important do you think it is?

*Dr. Lönnerdal:* We are touching socioeconomic and family factors here, but I would like to amplify on that. Kay Dewey did a study in Davis, where she looked at formula-fed infants and breastfed infants. In Davis, we have excellent sanitary conditions, mothers’ average education is 16 years, they are of upper-to-middle class, and still there was a significant difference in illness, both in prevalence of illness and in duration. I think it matters if you are a parent, if you during a period of, for example,
3 months have three episodes of diarrhea, and you have to stay home from work to take care of your ill infant during 3 days instead of 1 day. We also know from studies in developing countries that each day of illness will have an effect on appetite and growth. I am not sure how to measure this, but the more ill you are, the more responsibilities for the parents in developed countries because they need to take time off from work, cost for the family and so on. Otherwise, I think that, in general, if we can prevent illness it will be beneficial for any infant, whether in an industrialized or in a developing country.

**Dr. Haschke:** You are studying very interesting bioactive molecules in formula. We are far away from putting them in infant formula because of regulatory aspects and costs. If you added the five bioactive molecules that you have presented in your talk to an infant formula, its price would be too high. Taking a stepwise approach, only the \( \alpha \)-lactalbumin is realistic. I would see lactoferrin as the next step. Would you agree?

**Dr. Lönnerdal:** I am not in the commercial business, but it’s also going to be a question of demand and production – the greater the demand for an ingredient, the higher the production, greater the competition, and lower the prices. But I think with these findings for lactoferrin and osteopontin we may not need to copy everything in breast milk to make infant formula functionally more similar. The difficulty will be to do the kind of studies where you would add one component, another one, another one, and then add all of them; it’s very costly, requires a lot of subjects, and so on. We talked at previous workshops about the fact that federal agencies, the European Community, etc. don’t want to fund such studies because they basically say this is the responsibility of infant formula manufacturers. I respect that infant formula manufacturers cannot study all these permutations that I am talking about but that we as scientists would like to see. Therefore, we need to home in on some key components.

**Dr. Hernell:** Perhaps a comment to that; there are various groups of infants, for instance preterm infants, it may be a small fraction of all infants, for whom a benefit is greater while the cost less critical because the cost of care of a preterm is still quite high.

**Dr. Haschke:** It depends, the formula for premature infants which is delivered to hospitals usually is the most complex one; but the hospitals want to have discount prices, which slows down innovation in that important segment. Industry needs to prove safety and efficacy of any new bioactive molecule that is added to those formulas. Huge sample sizes are needed to prove a positive health outcome, and the likelihood that an industry-sponsored study is challenged is high.

**Dr. Lönnerdal:** Yes, I agree.

**Dr. Johansson:** You talked about the proteases and the release of bioactive peptides. I think we should not restrict our thinking to proteases in the milk as such but also have microbiota-produced proteases in mind, and how this could be beneficial or not depending on feeding.

**Dr. Lönnerdal:** That’s a very good point.

**Dr. Jongpiputvanich:** I would like to know whether \( \alpha \)-lactalbumin in cow’s milk has a different amino acid sequence and function than \( \alpha \)-lactalbumin in breast milk.

**Dr. Lönnerdal:** Certainly, it’s not identical when it comes to the amino acid sequence, but it seems like several of these structural motives that I talked about are similar and some smaller peptides are identical. For example, the calcium-binding site in bovine \( \alpha \)-lactalbumin is identical or very similar to the one in human \( \alpha \)-lactalbumin. Then, there may be stretches in the molecule where you have very different amino acid peptides, but they may not be related at all to these bioactivities that I talked about; however, the antibacterial peptides being formed were identical.

**Dr. Anderson:** You haven’t commented on obesity. Is there a difference in gastric emptying rate between formula and breast milk? That could in part be a determinant
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of excess caloric intake. I am puzzled why high-protein formulas don’t associate with suppressed food intake rather than increased caloric intake and obesity.

Dr. Lönnerdal: You brought up several things here. With regard to implications for obesity, there are at least two scenarios. If you have hyperglycemia and hyperinsulinemia in infants because of high-protein formula, there is the possibility later on in life for diabetes and obesity. The other one is the recent studies by Jeff Gordon and his group with communication between the microbiota and the diet and the crosstalk with the intestinal mucosa, where they have shown a clear connection to energy metabolism. This is a very novel area, but it’s exciting because there are some human studies now, following the mouse studies, showing that at least it’s possible that it could affect things like obesity and energy metabolism. When it comes to gastric emptying, I think little has been done in human infants. We often use young rat pups as a model for digestion studies, and it’s very obvious if you do sequential killings that there is much more rapid clearing of the stomach when you feed them breast milk than if you give them infant formula. My expectation would be that it’s the same in the human infants, but I don’t think we have firm data on that. When it comes to overall food intake though, we know that formula intake is much higher than the milk intake of breastfed infants, so it doesn’t seem to have any suppressive effect; in contrast, it may be peptides of formula that stimulate the intake.

Dr. Netrebenko: Prof. Hernell mentioned in his lecture that breast milk composition varies greatly from one woman to another. Have you seen cases of low lactoferrin or other protective factors in the human, and does it result in worse immune function in infants?

Dr. Lönnerdal: I haven’t seen any direct studies on it. I actually have thought about how such studies could be done. Even if the concentration is at the lower range of what is considered normal, there would still be enough to perform the biological function. This is something that has to be borne in mind when discussing nutrient recommendations. As we have heard, there is a large variation in the composition of breast milk from one woman to another, and there is a lot of tracking. Thus, if a mother has a low concentration of a nutrient at the beginning of lactation, this will remain so throughout lactation. The infants seem to be doing quite well, and then recommendations are set at the medium level, not at the lower level, even though the lower level intakes seem to be adequate. This would be interesting to study for both individual proteins, but also when it comes to how much protein breast milk should provide and therefore in its extension how much protein there should be in infant formula.

Dr. Jongpiputvanich: To mimic the protein composition of breast milk, why don’t we make infant formula that has higher α-lactalbumin and has no β-lactoglobulin as in human milk?

Dr. Lönnerdal: There are formulas now that have increased levels of α-lactalbumin, so steps have been taken in that direction. We will have to ask Dr. Haschke, but there have also been attempts to remove β-lactoglobulin, but then the formula becomes too costly. Therefore, you may be able to make a very nice formula without β-lactoglobulin, but if the mother cannot afford it, you haven’t gained that much. In addition, I haven’t seen any evidence that it would eliminate or reduce the risk of allergy if you just take out β-lactoglobulin. If an infant develops an allergy against one cow’s milk protein, it often develops an allergy against other components too, so just removing β-lactoglobulin may not be enough.

Dr. Sankaranarayanan: Can you comment on the role of bovine colostrum?

Dr. Lönnerdal: Bovine colostrum is a very interesting source of bioactive components, although I think the supply is relatively limited. Just like human colostrum is
higher in many components, bovine colostrum is high in immunoglobulins, lactoferrin, osteopontin, and many other things.

*Dr. Thorsdottir:* I read somewhere that amino acid cysteine was a stimulator for insulin-like growth factor, and you said that α-lactalbumin increases the amount of cysteine. Do you know anything about this, maybe this is wrong?

*Dr. Lönnerdal:* I was not aware of this. Maybe Dr. Martin who is going to speak tomorrow will have a chance to address that.

*Dr. Mouane:* I have a question about the milk fat globule membrane. I was expecting better neurological development or vision, and there is an anti-diarrhea effect, so what is the mechanism?

*Dr. Lönnerdal:* It’s a fairly complex mixture with several components. I wouldn’t be surprised if there was an effect on neurodevelopment, but no such studies have been done. When it comes to defense against infection, there may be other components.

*Dr. Melnik:* Concerning the insulinotropic effect of α-lactalbumin, it is known that hydrolyzed α-lactalbumin is a source of highly insulinotropic amino acids. α-Lactalbu-min is especially enriched in tryptophan, an amino acid precursor of serotonin synthesis which stimulates pituitary growth hormone and prolactin release. This fits in experimental evidence observed in α-lactalbumin-enriched diets in humans and animals [1].

Both growth hormone and prolactin increase insulin secretion of pancreatic β-cells. In this regard, α-lactalbumin appears to be a most important signaling molecule of the mammalian lactation genome promoting insulinotropic effects of milk consumption.

**Reference**