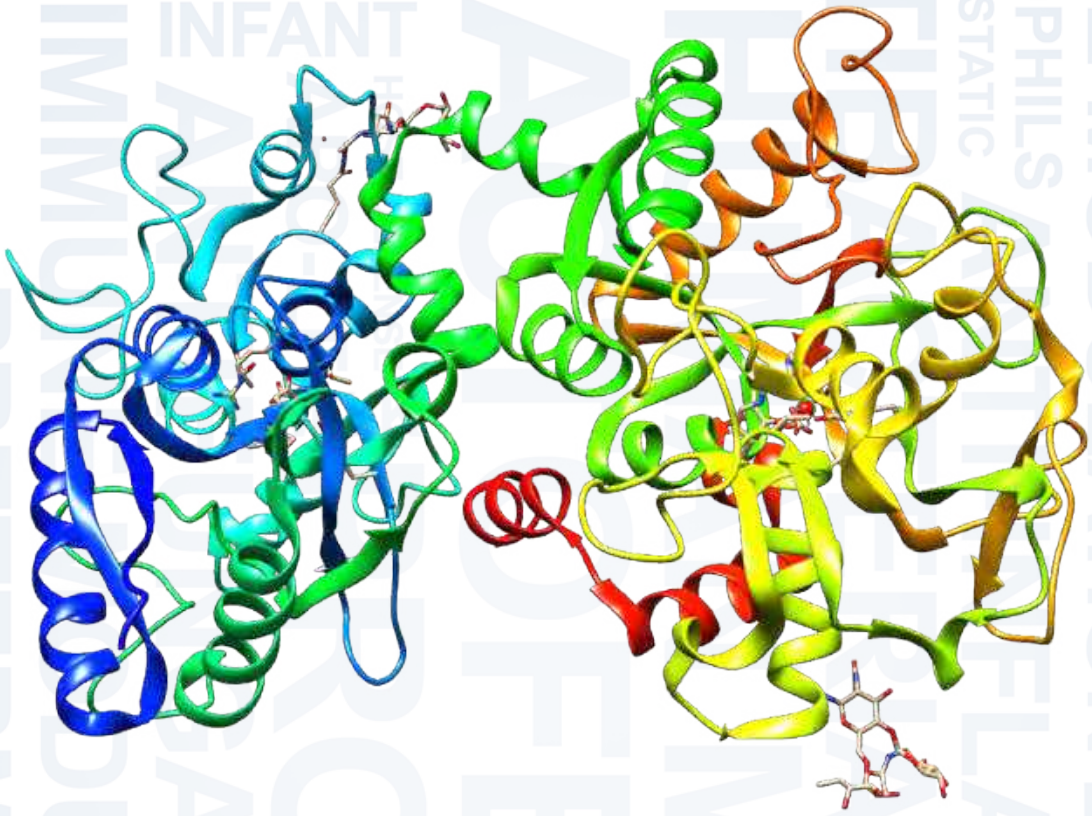
NEW INSIGHTS

INTO BIOACTIVE COMPONENTS OF HUMAN MILK: LACTOFERRIN



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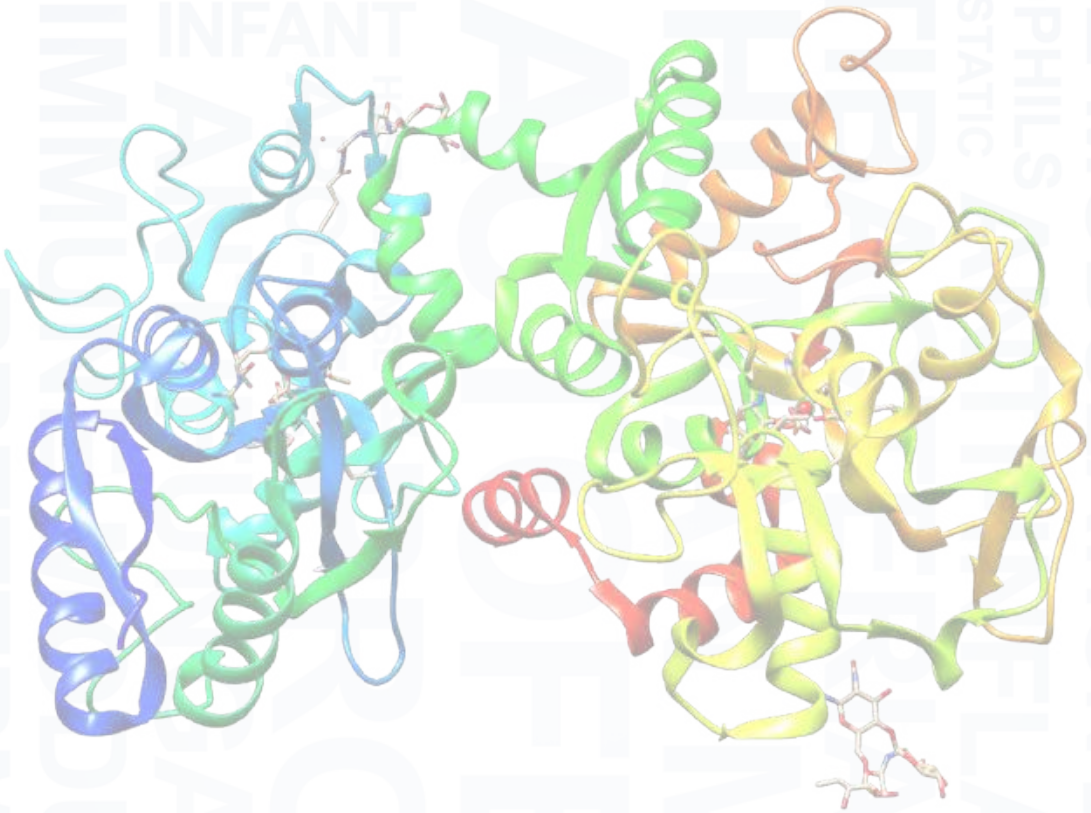

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Introduction

A. Nutrition and Early Development

Infancy and early childhood represent a critical period for nutrition, as proper dietary intake during this time is vital to supporting early growth and development, and promoting optimal health outcomes later in life.[1] Nutritional and environmental influences are especially important during the first two to three years of life, when growth is the most rapid. Normally, body weight triples in the first year of life, while length increases by 75% in the first two years.[2-4] This period of rapid physical growth is also critical for development of various body systems. For instance, tripling of brain size within the first two years is accompanied by a remarkable degree of functional maturation and rapid acquisition of cognitive milestones.[5,6] Likewise, the functions of the digestive tract and immune system—both central to healthy growth—also undergo rapid and significant maturation in early life. During this period, an infant establishes the intestinal structure and physiology that support nutrient absorption, barrier function, endocrine secretion, and antimicrobial defense.[7] An appropriate balance of nutrients—including proteins, carbohydrates, lipids, and micronutrients—is crucial to support the needs of the growing child. Furthermore, it has been recognized that proper nutrition also supports the growth of a healthy gut microbiome, which in turn can influence the growth, health, and possibly even mental functioning of the individual.[8] Therefore, optimal nutrition during early life must be targeted to the unique needs of infants and young children at each stage, and it is crucial for them to reach their full potential in growth, health, and cognitive development.[1]

Since the ideal source for nutrition in infancy is healthy human milk,[9] a robust understanding of its composition and function is essential to develop the best nutritional strategies for all infants, whether breast-fed or formula-fed. The composition of human milk is complex and may vary across individuals, populations, environmental settings, and phases of lactation,[10-12] yet many fundamental aspects of human milk composition are shared across populations.[9,13] It is also important to recognize that nutrients in the diet perform three major distinct roles: they are sources of energy, they provide structural building blocks, and they act as functional or bioactive compounds. The latter can be defined as constituents that affect biological processes and thus have an impact on body function or condition and ultimately health, beyond basic nutrition.[10,14] Bioactive components in human milk represent a large and heterogeneous category that includes specific proteins, oligosaccharides, lipids and fatty acids, growth factors, hormones, and even intact cells; these are derived from a variety of sources and have diverse functions and potential health benefits.[8] Some nutrients may fulfill more than one role; for instance, lactose is both a carbohydrate energy source and a potential prebiotic, as some may reach the colon undigested and thereby influence the development of gut bacterial flora.[13,14] Another bioactive nutrient is the major human milk protein lactoferrin, a non-heme iron-binding glycoprotein that has been shown to be involved in several biological functions, including supporting gut health, immunity, and defense against multiple pathogens.

Infancy and early childhood represent a critical period for nutrition ...

B. Insights From Human Milk Research

Research into the composition and functionality of human milk over the past several decades has provided valuable insights into the nutritional needs of infants, as well as inspired innovations in the design of infant formula.

One example of such innovation is the addition to infant formula of prebiotic oligosaccharides, which have been demonstrated to support the gut immune system.[12,14] Another example is the addition of long-chain polyunsaturated fatty acids docosahexaenoic acid (DHA) and arachidonic acid (ARA), which are present in human milk but originally unsupplemented in infant formula. Following years of research into the safety and benefits of these fatty acids to brain, cognition, and visual development, DHA is now widely recognized to be necessary in the diet during the first 24 months of life[15,16] and is routinely supplemented in formula. Ongoing research continues to expand the understanding of human milk composition, and a number of national and international cohort studies have generated new biological insights into the function of various proteins, oligosaccharides, fatty acids, vitamins, and minerals.[11,12,17]

Advanced techniques including mass spectrometry, bioinformatics, and proteomics allow researchers to characterize both major and minor components present in human milk. In addition, research into functional proteins in human milk has provided significant insights into their varied biological roles in supporting infant growth, development, and health. Although the chief role of human milk proteins is to provide amino acids to rapidly growing infants for new protein synthesis,[18] many proteins such as immunoglobulins, κ-casein, lysozyme, haptocorrin, α-lactalbumin, lactoperoxidase, and lactoferrin are relatively resistant to proteolysis in the digestive tract, suggesting that they play other physiological roles. As will be discussed below, the structural characteristics of lactoferrin allow it to resist proteolytic digestion within the gut, preserving its bioactivity and allowing it to serve many of its beneficial functions, which include supporting gut health and exerting antipathogenic and immunomodulatory effects.

The purpose of this monograph is to provide an overview of the biological activities of lactoferrin. It will review its structure and physiology, highlight over 50 years of research into human lactoferrin and bovine lactoferrin (bLF), and examine the body of evidence supporting the potential benefits of lactoferrin in preclinical models as well as in the diet of infants and young children. The resulting insights can not only advance the understanding of infant health and development, but they can also provide a basis for further innovation and improvement in the design of infant formula.

Lactoferrin: An Overview

Lactoferrin is an 80 kDa, multifunctional, iron-binding glycoprotein naturally present in milk that has been shown to exert a number of beneficial physiological functions.[19] A member of the transferrin family, this protein was discovered in bovine milk in 1939 but was not well characterized until the early 1960s, when it could be isolated and extracted from bovine and human milk with sufficient purity.[20] Lactoferrin accounts for 15% to 20% of the total protein content in human milk,[21] making it the second most abundant whey protein.[22] At lower concentrations, lactoferrin is also present in other biological fluids such as saliva, tears, bile, pancreatic fluid, mucosal secretions, and secondary granules of neutrophils.[23] This widespread presence suggests the importance of lactoferrin in a variety of physiological functions, including host defense and immunity.[24] Although lactoferrin is present in bovine milk and some infant formulas, the concentrations are much lower than what is found in human milk. The bioactivities of human lactoferrin and bLF have been reported to be comparable.[25,26] Therefore, adding bLF to infant formulas has the potential to produce concentrations closer to those found in human milk, while also providing associated health benefits. The preclinical and clinical data outlined in the following sections support the functionality of bLF in pediatric nutrition.

A. General Structure and Properties of Lactoferrin

Lactoferrin Structure

The lactoferrin glycoprotein is a single polypeptide chain that is folded into two homologous lobes (N- and C-terminal lobes), and each lobe carries one metal-binding site[27] (Figure 1). The structures of human lactoferrin and bLF have been shown to be similar, given that they share a high degree of amino acid sequence homology.[23,28] Furthermore, human lactoferrin and bLF consist of 691 and 689 amino acids, respectively.

Iron-Binding Protein

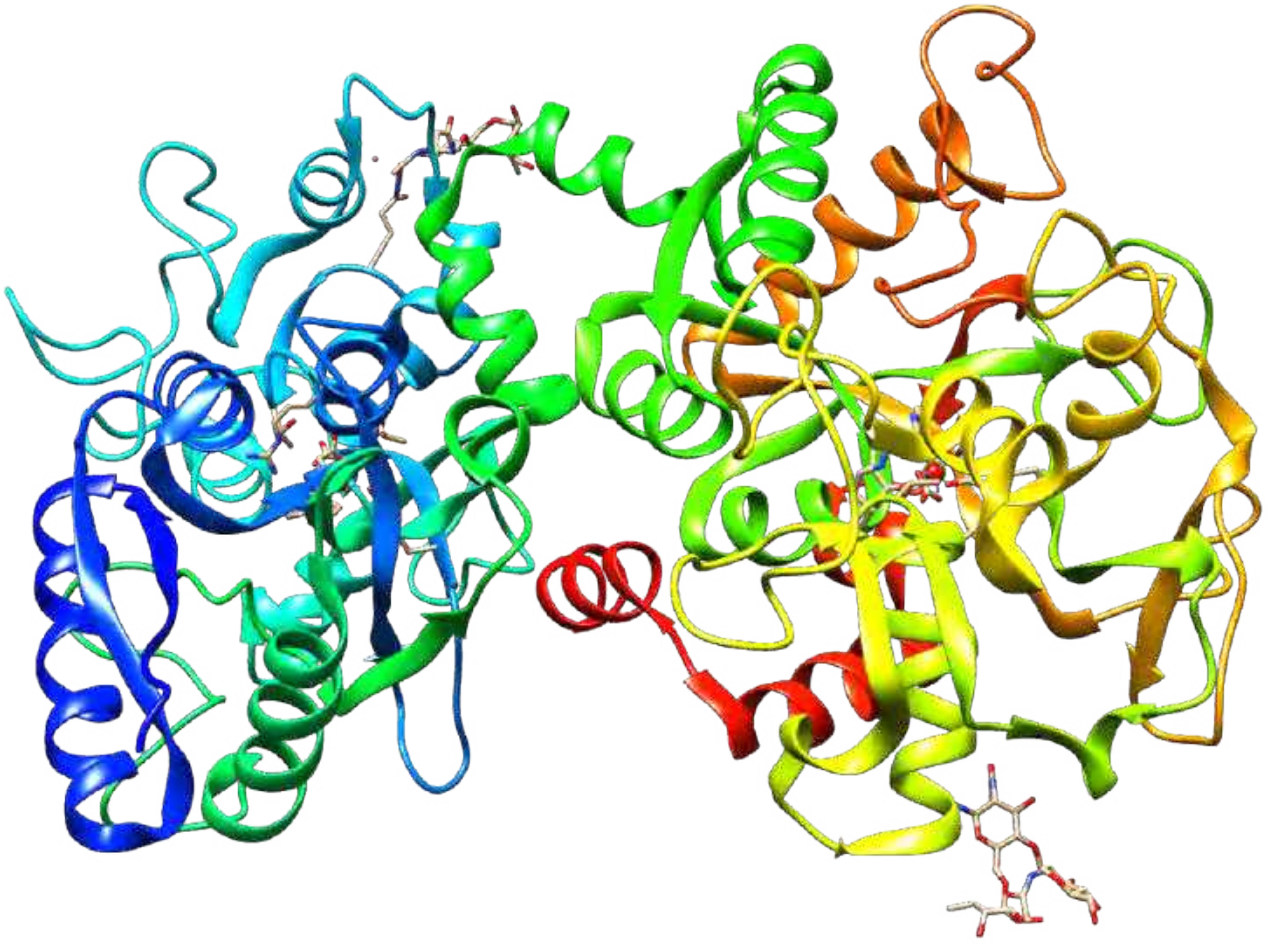
Lactoferrin has a high binding affinity for ferric iron (Fe^{3+}), enabling it to sequester iron from iron-requiring pathogens.[23] Lactoferrin can reversibly bind Fe^{3+}, which is associated with conformational changes in its three-dimensional structure.[29] Once bound to iron, lactoferrin has a closed conformation (holo-lactoferrin) and is more resistant to proteolysis than the open iron-free form (apo-lactoferrin).[30] The iron saturation of lactoferrin ranges from 8.7% to 13.1% in human milk.[31] The iron-lactoferrin complex is extremely strong at physiological pH.[32]

B. Digestion and Uptake of Lactoferrin

In addition to being a major component of human milk, lactoferrin has been reported to exert a number of physiological activities that support healthy growth and development. These functions include supporting intestinal health, exerting protective activities against pathogens, and modulating immune function.[25] The reason that lactoferrin can exert these beneficial activities is because a portion of it escapes proteolytic digestion,[30] allowing it to work locally in the gut as well as to become absorbed and work systemically.

> ... [lactoferrin] has been shown to exert a number of beneficial physiological functions[19] ... including host defense and immunity.[24]

Figure 1. Bovine Lactoferrin Structure



In infants who are exclusively breast-fed, a significant amount of intact lactoferrin can be found in their stools during the first 22 weeks of life.[33] Furthermore, in various preclinical studies, intact lactoferrin was detected in the peripheral blood within 10 minutes following oral ingestion and has been found in the liver, kidneys, gallbladder, spleen, brain, and cerebrospinal fluid.[34-39]

Additionally, during the digestive process, pepsin cleaves lactoferrin to release smaller bioactive peptides. One of the peptide fractions released during digestion—lactoferricin—has been shown to exert antimicrobial activities. Since it contains a cationic charge, lactoferricin can mediate iron-independent functions, exhibiting bactericidal, immune regulatory, and inflammatory activities.[40]

Both human lactoferrin and bLF have been shown to be absorbed in the gut via receptor-mediated mechanisms. The lactoferrin receptor was found responsible for the uptake of both apo- and holo-lactoferrin forms by enterocytes (Figure 2).[41] While the binding affinity of bLF is lower than that of human lactoferrin, it can still be taken up by the human intestinal lactoferrin receptor.[25]

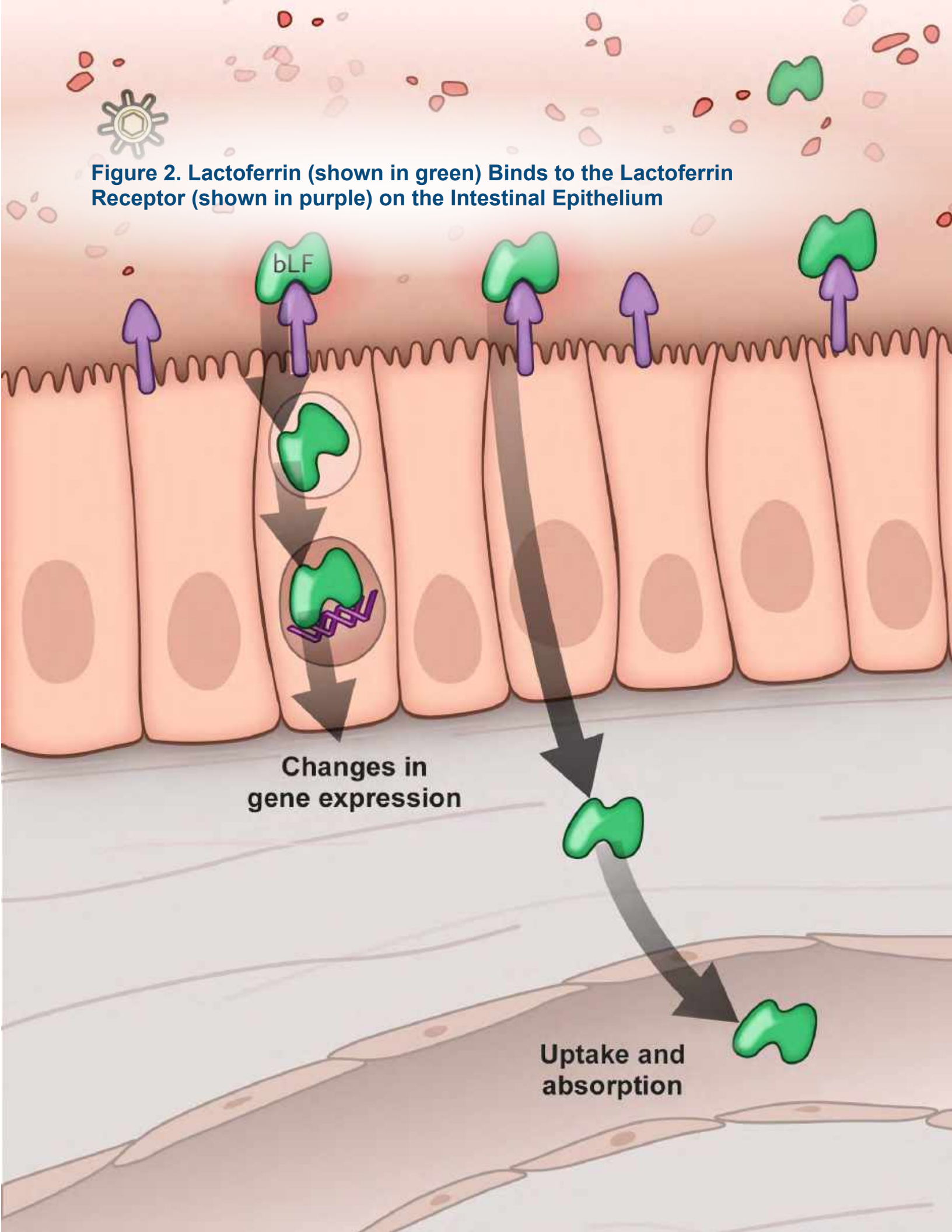
Since lactoferrin receptors are present in the small intestine, a role for lactoferrin in iron absorption has long been suggested. Although a number of clinical trials have assessed the role of lactoferrin in iron status in infants, the results remain inconsistent. King et al.[42] found that healthy term infants who were fed lactoferrin-fortified formula through the first year exhibited significantly higher hematocrit levels at nine months of age, but not at 12 months of age, compared to controls. In another study,[43] infants who received lactoferrin-fortified formula until three months of age had higher serum Hb (hemoglobin) levels and lower prevalence of anemia at four to six months compared to control infants, although the lactoferrin level (38 mg/100 g milk) in the experimental formula was relatively low. Conversely, other clinical studies do not support a role for lactoferrin in facilitating iron absorption.[44-46] For example, infants three to 10 months old fed breast milk with native lactoferrin demonstrated significantly reduced iron absorption compared to infants fed the same breast milk with the lactoferrin removed.[46] In summary, the potential role of lactoferrin in facilitating iron absorption in infants remains unclear.

C. Levels of Lactoferrin in Human and Bovine Milk and Infant Formula

Lactoferrin levels in human milk have been reported to decrease throughout the lactation period. Based on a comprehensive global review by Rai et al.,[23] lactoferrin levels in human milk were highest in the first five days of lactation but declined by 50% in the second week of lactation. Furthermore, lactoferrin concentrations in mature human milk (≥28 days of lactation) have been reported at a range of means of 0.44-4.40 g/L. Similar results were described in other studies.[47,48] Although lactoferrin is an iron-binding protein, its concentrations in human milk are generally independent of maternal iron status and/or maternal iron supplementation.[28]

In contrast to human milk, bovine milk and thus milk-based infant formula contain much lower levels of lactoferrin. Specifically, the levels of lactoferrin in mature bovine milk were described within ranges of 0.02-0.21 g/L.[25,49,50] However, since bLF is structurally and functionally comparable to human lactoferrin, its supplementation into infant formulas may potentially provide functional benefits to the growing infant.

Figure 2. Lactoferrin (shown in green) Binds to the Lactoferrin Receptor (shown in purple) on the Intestinal Epithelium



Scientific Evidence for Health Benefits of Lactoferrin

A. Data From Preclinical Studies

1. Intestinal Cell Growth and Differentiation and Barrier Function

There is increasing evidence demonstrating that lactoferrin supports intestinal health through various mechanisms, including stimulating proliferation and differentiation of epithelial cells,[51-54] modulating intestinal morphology,[55,56] supporting gut barrier integrity (Figure 2),[57] and decreasing translocation of bacteria.[58] In piglets, lactoferrin supplementation at both 1.1 g/L and 0.6 g/L concentrations resulted in significantly larger jejunal crypts compared to the control group, indicating greater cell proliferation.[54]

In addition to supporting intestinal cell growth, an intact intestinal epithelial barrier is critical for normal physiological functions and decreasing bacterial translocation. Research shows that healthy enterocytes provide a barrier against transcellular permeability, while the tight junctions act as a paracellular seal and functional barrier against translocation of microbes and substances from the lumen.[59] In a rodent model, oral administration of bLF significantly increased tight junction protein expression and reduced intestinal permeability, suggesting an improvement in intestinal barrier integrity.[60] These data suggest a role for lactoferrin in supporting gastrointestinal health.

2. Antimicrobial Activity

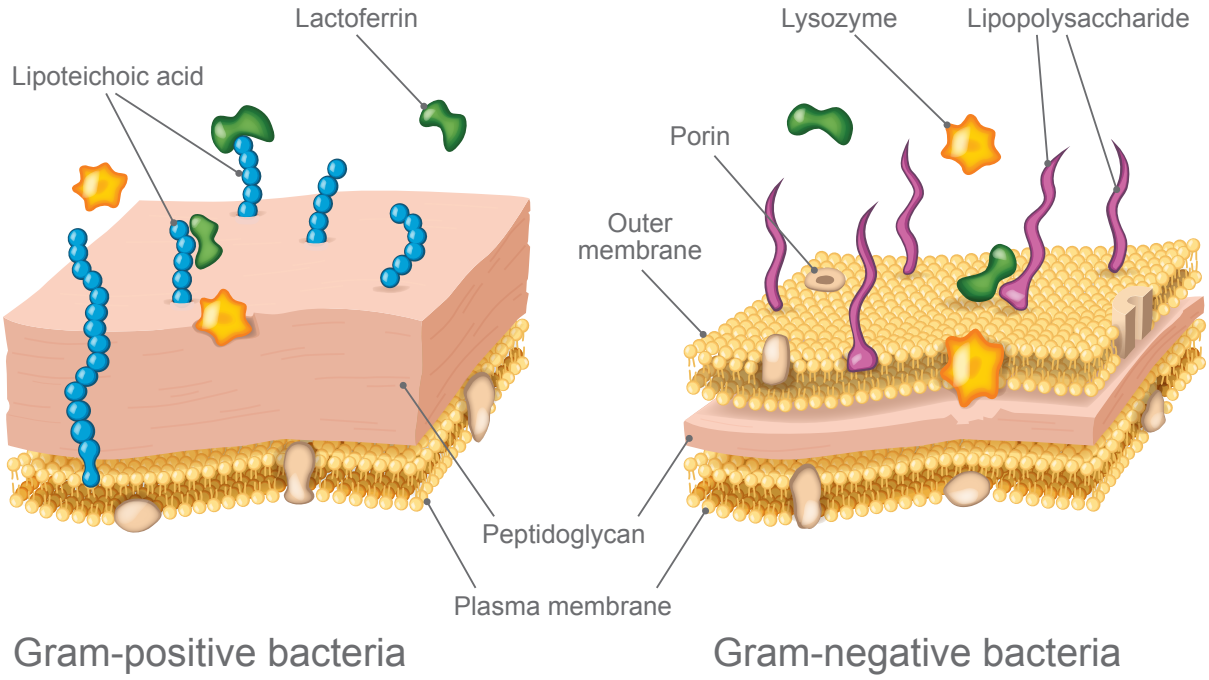
Lactoferrin can also potentially impact gut health through its direct antimicrobial effects. These antimicrobial functions have been well established and include activities against a broad spectrum of pathogens relevant to the pediatric population, including gram-negative and -positive bacteria as well as various viruses, parasites, and fungi.[23] Several direct and indirect mechanisms of action have been identified, and these include iron sequestration; binding to bacterial lipopolysaccharides (LPS), causing a release of LPS from the cell wall, an increase in membrane permeability, and damage to the bacteria; and decreasing bacterial aggregation, reduction of bacterial attachment to host cells, microbial cell lysis, and degradation of virulence factors.[23]

Antibacterial Activity

The antibacterial effects of lactoferrin have been among the most reported antimicrobial activities of this protein, and they occur via a wide range of mechanisms (see Figure 3 and Table 1). For example, lactoferrin can exert antibacterial effects by depriving iron from iron-requiring pathogenic bacteria. *In vitro*, lactoferrin from human milk has demonstrated a bacteriostatic effect against *Escherichia coli* by sequestering iron.[61] Furthermore, *in vitro*, bLF had an iron-dependent bacteriostatic effect on *Salmonella typhimurium*, and both bovine and human lactoferrin inhibited the adherence to and invasion by *Salmonella* to cultured cells.[26] Lactoferrin was also reported to damage the external membrane of gram-negative bacteria by forming a strong complex with LPS.[23] Furthermore, given that lactoferrin is highly positively charged, it can inhibit interactions between LPS and other positively charged cations.[32]

Human lactoferrin has been shown to inhibit the adhesion of enteropathogenic *E. coli* (EPEC)—a pathogen closely linked to infant diarrhea—to HeLa cells.[62] *In vitro*, recombinant human lactoferrin was capable of inhibiting EPEC-induced actin polymerization.[63] Additionally, in adherence assays with HEp-2 (human epithelial type 2) cells, bLF blocked EPEC attachment to the cells and decreased EPEC-mediated actin polymerization by 88%.[64]

Figure 3. Antibacterial Effects of Lactoferrin



Lactoferrin can damage the external membrane of gram-negative bacteria through interaction with LPS, causing LPS release from the cell wall and consequently an increase in cell membrane permeability and damage to the bacteria. In gram-positive bacteria, lactoferrin binds to anionic molecules on the bacterial surface, such as lipoteichoic acid. This reduces the negative surface charge, facilitating contact with lysozyme, which subsequently degrades the peptidoglycan cell wall.[23,32]

Lactoferrin has also been reported to decrease pathology and mortality in preclinical challenge models. In EHEC (enterohemorrhagic *E. coli*)-infected mice receiving bLF, the mortality rate was 50% compared to the 90% mortality rate in the control mice.[65] Furthermore, while bacteremia was reported in the control mice, the mice supplemented with bLF did not exhibit signs of bacteremia. Similarly, lactoferrin was shown to exert protective effects against *Salmonella ser. typhimurium* in orally challenged mice by reducing its adherence to the host cells.[66,67] In a study on mice infected with *Listeria monocytogenes*, the oral administration of human lactoferrin for seven days decreased the number of *L. monocytogenes* cells in the liver and the size and frequency of necrotic foci in liver samples.[68] In addition, the mRNA levels of inflammatory cytokines, such as IL-1, TNF-α, and IFN-γ, were reduced in the liver of mice receiving lactoferrin, indicating reduced inflammation, which is consistent with less *Listeria* infection in lactoferrin-supplemented mice.

In transgenic mice carrying a functional human lactoferrin gene and expressing lactoferrin at high levels in milk, infection with *Staphylococcus aureus* was significantly better cleared than in congenic littermates.[69] In a recent study, the concentration of human lactoferrin in preterm

human milk showed negative correlations with the colony-forming units of *E. coli* and *S. aureus* after incubation with breast milk *in vitro*.[47] Furthermore, the addition of human milk-derived lactoferrin to low-birth-weight infant formula exerted >50% bacteriostatic effect against all bacterial species *in vitro*. Although only a select few species were highlighted in the text, see Table 1 for additional bacteria against which lactoferrin has had reported effects.[63,68-77]

Antiviral Activity

Lactoferrin has demonstrated antiviral capacity against a range of RNA and DNA viruses that infect both humans and animals.[32] Based on *in vitro* data, the antiviral effects of lactoferrin have been described to include direct binding to the virus, reduced entry of the virus into host cells due to receptor blockade, or inhibition of viral replication within the cell.[78,79] The antiviral activities of lactoferrin have been reported for multiple viruses relevant to the pediatric population, including rotavirus. For example, an *in vitro* study showed that human lactoferrin exhibits activities against respiratory syncytial virus and rotavirus, even at concentrations 10-fold lower than those found in human milk.[80] In addition, *in vitro*, lactoferrin reduced attachment and replication of norovirus and attenuated viral infectivity.[81]

Antifungal Activity

There are emerging data that lactoferrin may also exert antifungal activity. Recent research has focused on *Candida* species, fungi which can be either commensal or opportunistic human pathogens. *Candida albicans* is the most commonly reported strain of fungi in infants.[82] Research conducted by Velliyagounder et al.[83] suggested that human lactoferrin anticandidal activity may be related to cell surface alterations of *C. albicans*. Furthermore, preclinical studies have suggested that although lactoferrin does not actively reduce *Candida* colonization, it can help support the gut barrier of the host[57] and inhibit gut microbial translocation.[58]

Lactoferrin knockout mice (LFKO-/-) have been reported to be more susceptible than wild-type mice to oral candidiasis; however, the addition of human lactoferrin to the drinking water of LFKO-/- mice reduced *C. albicans* colonization in the oral cavity compared to LFKO-/- mice not receiving lactoferrin.[83]

Table 1. Bacteria Against Which Lactoferrin Has a Reported Effect (adapted from Table 1 in Garcia-Montoya et al.)[26]

TARGET		MODE OF ACTION
Gram-Positive	*Klebsiella pneumoniae* *Listeria monocytogenes* *Staphylococcus aureus*	Iron-independent interaction with cell surface[70] Altering bacteria virulence[68,71] Iron sequestering[72,73]
Gram-Negative	*Haemophilus influenzae* EPEC (enteropathogenic *E. coli*) EAEC (enteroaggregative *E. coli*) DAEC (diffusely adherent *E. coli*) *Shigella flexneri* *Vibrio cholerae* *Salmonella enteritidis*	Altering bacteria virulence[70] Proteolytic activity of invasive mechanism[63] Inhibiting adherence of diffusely adherent[74] Inhibiting aggregative proliferation[74] Disrupting bacterial type III secretion system[75] Iron-independent mechanism of inhibition[76] Interfering with polysaccharide cell content[77]

Table 2. Clinical Studies Reporting Gut Health Effects of Lactoferrin

CLINICAL STUDY	POPULATION	MAIN OUTCOMES
Manzoni et al. 2014[96]	Preterm very low-birth-weight neonates	Lactoferrin administration reduced necrotizing enterocolitis (NEC) incidence. The incidence of death and/or NEC were lower in the lactoferrin groups compared to the control group.
Chen et al. 2016[98]	Healthy term infants	Diarrhea-related illness was significantly lower in the lactoferrin group compared to the control group.
Ochoa et al. 2008[99]	Young children 12-36 months old	Lactoferrin administration in young children for nine months significantly lowered colonization rates with *Giardia* species compared to the control group.
Ochoa et al. 2013[93]	Young children 12-18 months old	Lactoferrin administration for six months decreased the longitudinal prevalence and severity of diarrhea in young children compared to the control group.
Egashira et al. 2007[100]	Children younger than 5 years old	Children younger than five years old who were fed lactoferrin for 12 weeks exhibited similar incidences of rotaviral gastroenteritis but significantly lower frequency and duration of vomiting and diarrhea compared to the control group.

3. Immunomodulatory Effects

Another way lactoferrin can influence gut health is by acting as a modulator of both the innate and adaptive immune responses.[84] This modulation results in changes in the expression of cytokines that influence the balance between pro- and anti-inflammatory immune responses.[84] *In vitro* studies and studies in animal models have demonstrated a role of lactoferrin in modulating the expression of various anti-inflammatory (eg, IL-4, IL-10) and pro-inflammatory cytokines (eg, TNF-α, IL-2, IL-6, IL-12) and chemokines (eg, IL-8).[1,84,85] These changes may mediate the impact of lactoferrin on cellular development and functions of the immune system.[1,84,85] In a study with colostrum-deprived neonatal piglets receiving a formula enriched with bLF, increased cytokine production was observed compared to the control piglets.[86] Furthermore, in an intestinally challenged rat model, lactoferrin supplementation attenuated gut damage by significantly decreasing inflammatory cytokines.[87] Thus, the immunomodulatory effects and mechanisms of lactoferrin remain an area of active research interest.

4. Emerging Research on Brain Development and Functions

There is emerging preclinical evidence for a potential role of lactoferrin in brain development and cognitive functions. Preclinical studies in animal models have reported that lactoferrin can cross the blood-brain barrier by receptor-mediated endocytosis,

accumulate in the brain, and exert effects on neurodevelopment, cognitive performance, learning, and memory.[7,88,89] Feeding bLF to piglets supported cognitive function and learning, upregulating several signaling pathways associated with neurodevelopment and cognition.[88] In addition, some preclinical data suggest a potential modulatory role for lactoferrin on the gut microbiota.[48] Preclinical research studies have examined the bidirectional communication between gut microbiota and the central nervous system, which is referred to as the gut-brain axis.[90,91] This axis is believed to modulate digestive processes, immune functions, perception, and emotional response to visceral stimuli.[92] Although these preclinical findings suggest a potential role of lactoferrin in brain development and cognitive functions, future clinical studies are needed to corroborate such findings.

B. Data From Clinical Trials

1. Safety and Tolerance

A number of clinical studies have demonstrated that lactoferrin supplementation is safe, well tolerated, and supports normal growth.[23,42,93,94] For example, in healthy term infants fed bLF-fortified formula (850 mg/L) during the first year of life, similar growth was demonstrated compared to infants receiving the control formula.[43] In a more recent study, infants fed formula supplemented with bLF at either 0.6 g/L or 1.0 g/L during the first year of life exhibited normal growth compared to infants fed a control formula.[94] In 1986 in Japan, bLF was added to infant formula in low concentrations and has since demonstrated a history of safe use and healthy growth and tolerance at these concentrations in infants. It has also been granted GRAS (Generally Recognized as Safe) status by the FDA (U.S. Food and Drug Administration)[95] and has been added to commercial infant formulas in the US and China.

Table 3. Clinical Studies Reporting Respiratory Health Effects of Lactoferrin

CLINICAL STUDY	POPULATION	MAIN OUTCOMES
King et al. 2007[43]	Healthy term infants	Feeding a lactoferrin-enhanced formula until one year of age resulted in fewer episodes of lower respiratory tract infections vs those fed a standard formula in healthy term infants.
Zucotti et al. 2009[101]	Young children with recurrent respiratory tract infections	Young children with recurrent respiratory tract infections who were fed lactoferrin combined with curcumin had improved immunity markers and reductions in the number of respiratory tract infections.
Ajello et al. 2002[102]	Young children colonized by invasive strains of GAS	Young children given bLF and erythromycin had a lower number of intracellular Group A *streptococcus* (GAS) in their tonsil specimens compared to the control group receiving erythromycin alone.
Stecksen-Blicks et al. 2015[103]	Healthy infants	11% to 15% of the infants had oral *Candida* colonization; lactoferrin in human milk was negatively associated with colonization at 6 months of age.

Several clinical studies have reported beneficial effects of lactoferrin on gut health ...

2. Gastrointestinal Health

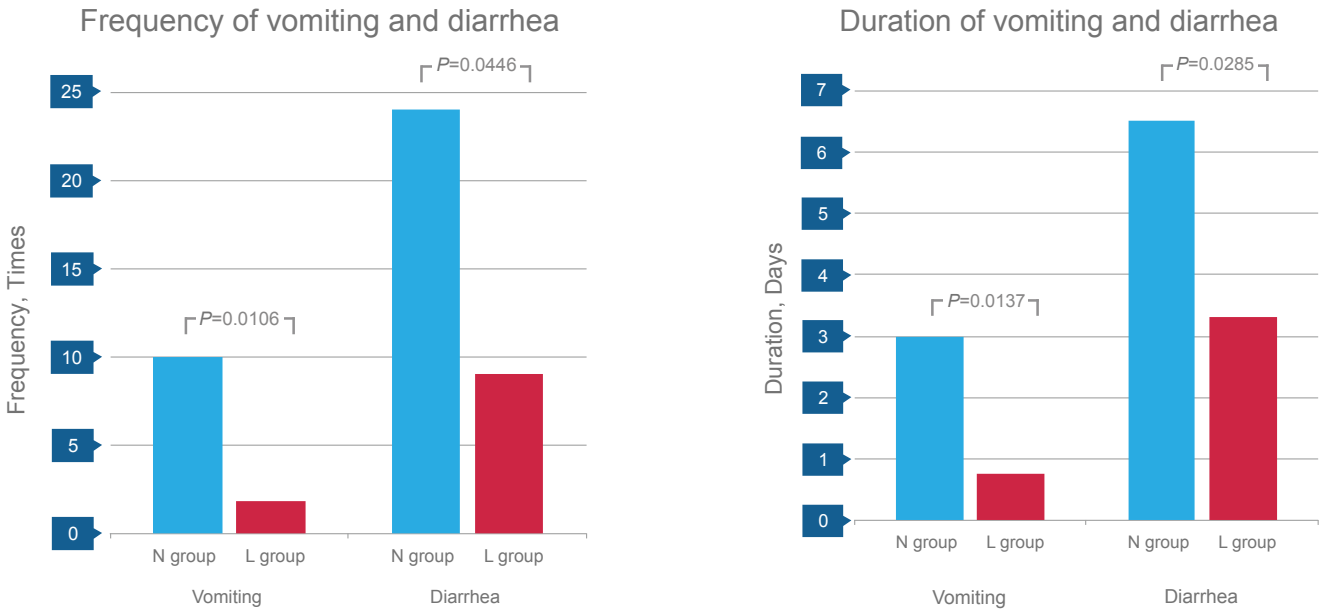
Several clinical studies have reported beneficial effects of lactoferrin on gut health, particularly in premature infants. In a prospective, double-blind, placebo-controlled, randomized trial, a reduced incidence of NEC and associated mortality were reported with lactoferrin supplementation in preterm very low-birth-weight neonates.[96] The infants were randomly assigned to receive orally administered bLF (100 mg/day), bLF with *Lactobacillus rhamnosus* GG (LGG) (bLF + LGG), or placebo from birth until day 30 of life (day 45 for neonates <1000 g at birth). Both bLF and bLF + LGG groups had significantly lower incidence of NEC (5/247 [2.0%] and 0/238 [0%], respectively) than in controls (14/258 [5.4%]). The mortality rate was also significantly reduced with bLF and bLF + LGG supplementation. Manzoni et al.[96] concluded that lactoferrin may be a promising strategy to reduce the risk of NEC in NICU (neonatal intensive care unit) settings. In a previous study by the same authors,[97] although there was no statistical difference in the diagnosis of NEC stage ≥2, overall NEC incidence was significantly reduced with oral lactoferrin supplementation.

Promising results of lactoferrin supplementation on gastrointestinal health are also seen in healthy term infants and in young children. Chen et al.[98] conducted a study in infants previously breast-fed for four to six months who were randomized to receive formula with or without bLF or who continued exclusive breast-feeding. The incidence of diarrhea-related illnesses were lower in the breast-fed and bLF-supplemented groups.[98] Ochoa et al.[99] conducted a small community-based trial in young children in Peru that compared the effects of oral supplementation with bLF (2 x 0.5 g/day) to a placebo group for nine months on incidence of diarrhea. The prevalence of colonization with *Giardia* species was lower in the bLF group.[99] There was also a trend toward less colonization with other pathogens, as well as a trend toward shorter duration of diarrheal episodes with lactoferrin intake. In a larger study with a similar protocol, bLF administration decreased the longitudinal prevalence and severity of diarrhea compared to the control group.[93] In another study, when bLF was supplemented (100 mg/day for 12 weeks) to children younger than five years of age as either a dietary supplement or in yogurt that also contained lactulose and bifidobacterium, the incidence of rotaviral gastroenteritis was similar compared to children who did not receive supplementation. However, the frequency and duration of vomiting and diarrhea were significantly reduced in the bLF groups compared to the control group (Figure 4).[100] While it is impossible to conclude that lactoferrin was the only bioactive component mediating the reduction of diarrhea in this particular study, these data are consistent with the preclinical data showing the activity of lactoferrin against rotavirus, as well as the other clinical data on lactoferrin and diarrhea.[98] A summary of these clinical studies is provided in Table 2.

3. Respiratory Health

Clinical studies have demonstrated a role of lactoferrin in supporting respiratory health. In a pilot study by King et al.,[42] healthy term infants fed a lactoferrin-enriched formula (850 mg/L) until one year of age demonstrated significantly fewer episodes of lower respiratory tract infections (0.15 episode per child year) compared to those fed a control formula (0.5 episode per child year). In another

Figure 4. Lactoferrin and Rotavirus Symptoms in Children[100]



N group=no lactoferrin, L group=lactoferrin 100 mg/day

small study of young children with recurrent respiratory tract infections, supplementation of lactoferrin (2.7 g/day) and curcumin (0.3 g/day) together for four weeks reduced the number of respiratory tract infections in 80% of the cases during a one-year follow-up.[101]

The beneficial effects of lactoferrin on respiratory health may in part be attributed to its antibacterial and antifungal activities, which have also been reported in clinical studies. In a small clinical trial of young children colonized by invasive strains of *Group A streptococcus* (GAS), children given bLF (100 mg three times daily) and erythromycin had a lower number of intracellular GAS in their tonsil specimens compared to those given erythromycin alone.[102] In another clinical study, lactoferrin levels in breast milk were negatively associated with oral *Candida* colonization in healthy infants at six months of age.[103] A summary of these clinical studies is provided in Table 3.

4. Premature Infant Health

In a study by Trend et al.,[47] the median amounts of total daily lactoferrin from human milk consumed by preterm infants on day 7 and day 21 were significantly lower in cases with late-onset neonatal sepsis vs infants without sepsis. This result may be due to lactoferrin intake, or may simply be due to differences in human milk consumption, because there were no significant differences in the lactoferrin levels in the human milk samples from the two groups. Nevertheless, these data are consistent with other clinical studies that showed beneficial effects of lactoferrin on sepsis in preterm infants.[50] In a prospective double-blind, placebo-controlled, randomized trial, preterm very low-birth-weight neonates fed 0.1 g of bLF (with and without LGG) experienced a lower incidence of bacterial or fungal late-onset sepsis and a lower frequency of late-onset sepsis mortality compared to infants given the placebo.[97] In another study by the same researchers, oral administration of bLF reduced the incidence of invasive fungal infection

Table 4. Clinical Studies Reporting Lactoferrin Effects in Preterm Infants

CLINICAL STUDY	POPULATION	MAIN OUTCOMES
Trend et al. 2015[47]	Preterm infants	Higher lactoferrin intake was associated with lower cases of late-onset neonatal sepsis in preterm infants.
Manzoni et al. 2009[97]	Preterm very low-birth-weight neonates	The consumption of lactoferrin with or without LGG from birth until day 30 of life reduced the frequency of and the mortality attributed to late-onset sepsis in preterm very low-birth-weight neonates.
Manzoni et al. 2012[104]	Preterm very low-birth-weight neonates	Administration of bLF with or without LGG reduced incidence of invasive fungal infections compared to the control group.
Akin et al. 2014[105]	Very low-birth-weight infants	Fewer sepsis episodes were observed in the bLF-supplemented infants compared to the placebo group.

in preterm very low-birth-weight neonates.[104] Infants received bLF, bLF with LGG (bLF + LGG), or placebo. While the incidence of fungal colonization was comparable among the three groups (16.6-18.5%), the intensity of colonization (>3 different sites concomitantly) was significantly lower in the bLf and bLf + LGG groups compared to the control group.[104] In very low-birth-weight infants receiving bLF (200 mg/day), significantly fewer episodes of sepsis were reported compared to the control group.[105] A summary of these clinical studies is provided in Table 4.

Summary and Conclusions

Optimal nutrition during early life targets the unique needs of infants and young children at each stage, and it is crucial for adequate growth and healthy gut and immune development. A number of studies have identified lactoferrin—a major human milk protein—as being involved in several important biological functions. However, because bovine milk contains lower concentrations of lactoferrin compared to human milk, unsupplemented infant formula also provides lower levels of lactoferrin. In regards to structure and bioactivity, bLF has been shown to be similar to human lactoferrin; this allows bLF to be supplemented in infant formula to provide a lactoferrin concentration closer to human milk. A variety of evidence suggests that such bLF supplementation may also provide functional benefits. Preclinical studies have demonstrated effects of lactoferrin on intestinal cell proliferation and differentiation, intestinal epithelial function, immune cell composition, and antimicrobial activities, with emerging preclinical evidence for a potential role in cognitive development. Similarly, pediatric clinical trials have reported beneficial effects of lactoferrin on gut health and immunity, including protection against microbial infections and support of respiratory health in preterm infants as well as term infants and young children. In conclusion, available evidence shows that dietary supplementation of lactoferrin may help narrow the gap, both nutritionally and functionally, between infant formula and human milk.

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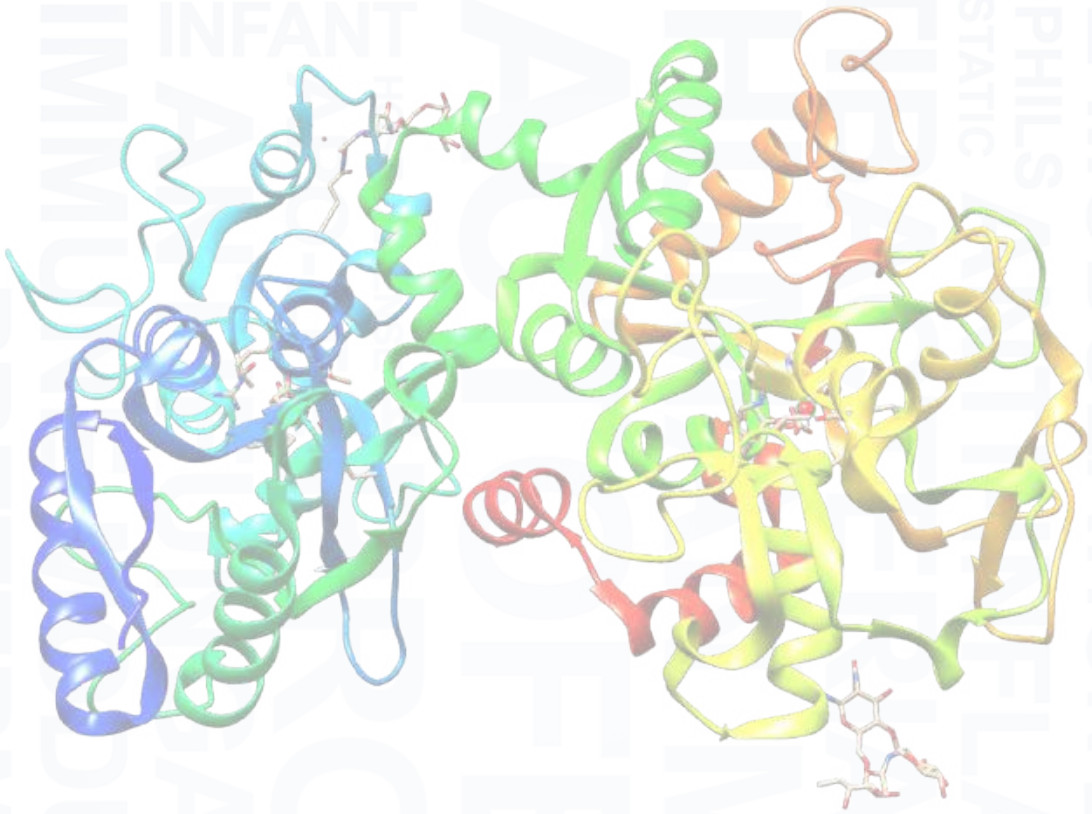
Notes



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