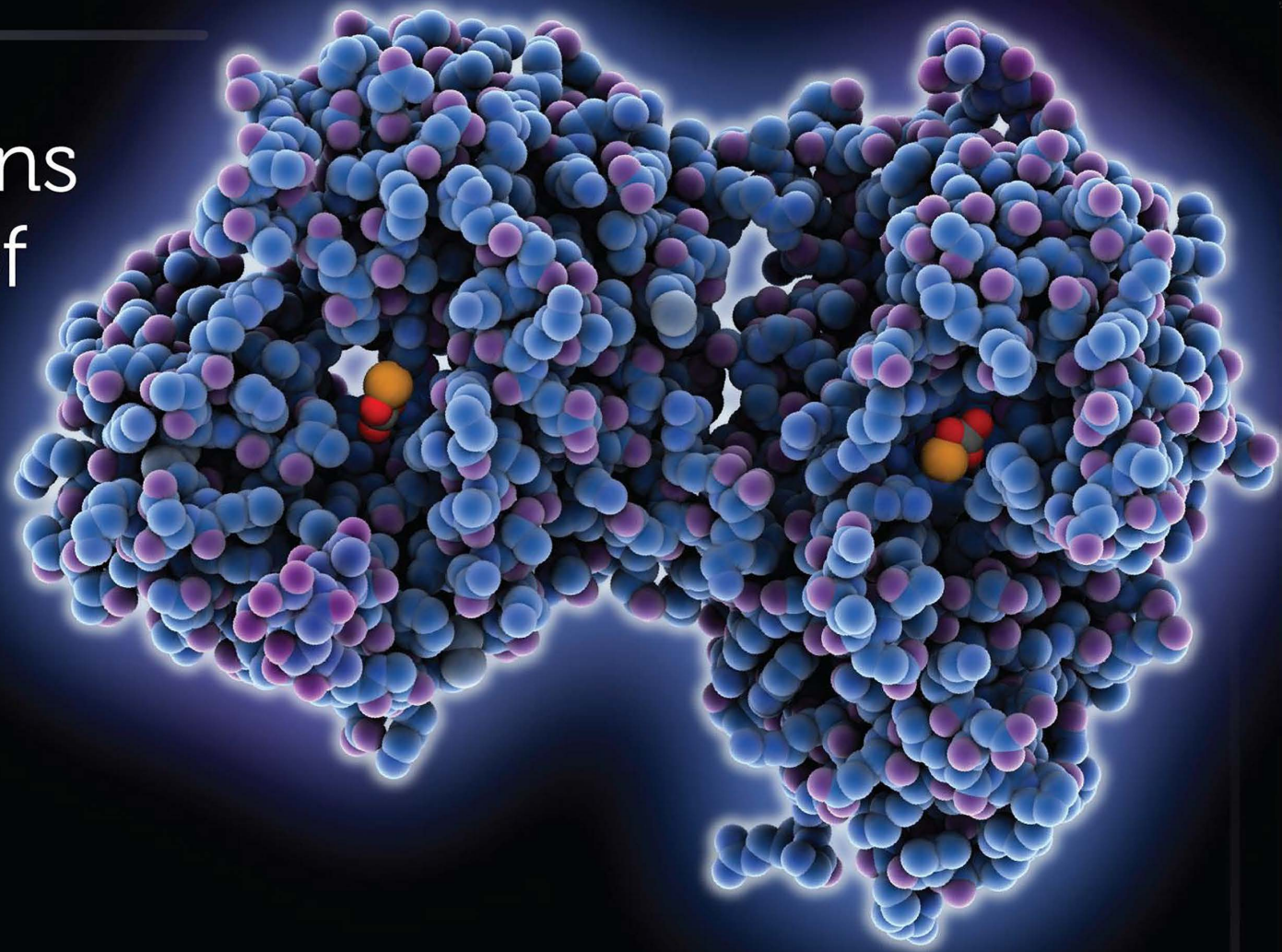
Neonate Feeding Regimens and the Expanding Role of **Lactoferrin**



Pediatric Nutrition
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Presented by
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Disclosures

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Speakers Bureau	AbbVie, Janssen, Mead Johnson Nutrition, Sodilac
Advisory Board	AbbVie, Janssen, MedImmune, Merck, Sanofi-Pasteur, Sodilac

Learning Objectives

- Evaluate clinical research that is expanding the understanding of the physiological and developmental properties of lactoferrin
- Develop evidence-based NICU feeding regimens with lactoferrin

Benefits of Human Milk

Fresh human milk prevents:

- Bronchopulmonary disease (BPD)/ chronic lung disease (CLD)[1]
- Retinopathy of prematurity (ROP)[2],[3]
- Necrotizing enterocolitis (NEC)[4],[5]
- Infections in neonates[4],[6]

1. Furman L, et al. *Arch Pediatr Adolesc Med.* 2003;157:66-71. 2. Hylander MA, et al. *J Perinatol.* 2001;21:356-362. 3. Manzoni P, et al. *Early Hum Dev.* 2014;90 Suppl 1:S60-5; 4. Lucas A, et al. *Lancet.* 1990;336:1519-1523. 5. Corpeleijn WE, et al. *JAMA Pediatr.* 2016;170:654-661. 6. Hylander MA, et al. *Pediatrics*.1998;102:E38.

Figure. The beneficial effects of fresh human milk are linearly associated with the intake volume.[1]

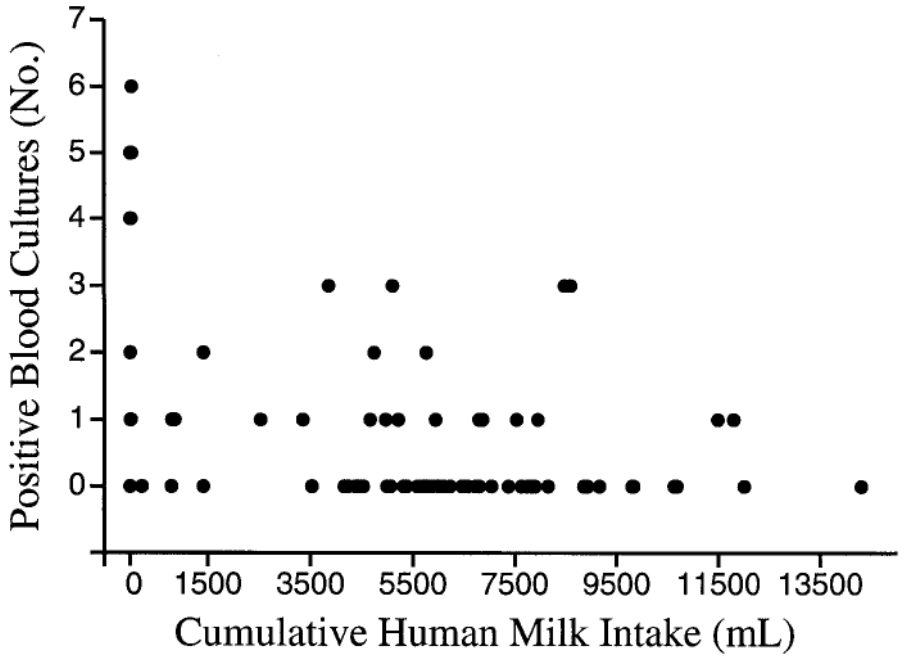


Table. Reduced logistic regression model for infection in relation to confounding variables[a],[2]

Variable	Odds Ratio	95% CI	*P* Value
Gestational age (wk)	0.80	(0.68–0.95)	.009
Apgar score at 5 mins	0.93	(0.77–1.14)	.494
Days without enteral feeding (NPO)	1.03	(0.99–1.07)	.153
Mechanical ventilator days	1.01	(0.99–1.03)	.184
Human milk fed	0.43	(0.23–0.81)	.010

a. The total for the regression model is 212 cases. The number of cases with imputed values on any single variable is 11 (5.0%).

Fresh human-milk feeding prevents infections in neonates.

CI, confidence interval; NPO, *nil per os* (nothing by mouth).

1. Schanler RJ, et al. *Pediatrics.* 1999;103:1150-1157. Reproduced with permission from Journal *Pediatrics*, Vol. 103, Pages 1150-1157, Copyright © 1999 by the AAP. 2. Hylander MA, et al. *Pediatrics.*1998;102:E38.

Major Bioactive Factors in Human Milk

Compound	Function
Cells	
Macrophages	Protection against infection, T-cell activation
Stem cells	Regeneration and repair
Immunoglobulins	
IgA/sIgA	Pathogen binding inhibition
IgG	Antimicrobial, activation of phagocytosis (IgG1, IgG2, IgG3); anti-inflammatory, response to allergens (IgG4)
IgM	Agglutination, complement activation
Cytokines	
IL-6	Stimulation of the acute phase response, B cell activation, proinflammatory
IL-7	Increased thymic size and output
IL-8	Recruitment of neutrophils, proinflammatory
IL-10	Repressing Th1-type inflammation, induction of antibody production, facilitation of tolerance
IFNγ	Proinflammatory, stimulates Th1 response
TGFβ	Anti-inflammatory, stimulation of T cell phenotype switch
TNFα	Stimulates inflammatory immune activation

Adapted from: Ballard O, Morrow AJ. *Pediatr Clin North Am.* 2013;60:49-74.

Major Bioactive Factors in Human Milk *(continued)*

Compound	Function
Chemokines	
G-CSF	Trophic factor in intestines
MIF	Macrophage Migratory Inhibitory Factor: prevents macrophage movement, increases antipathogen activity of macrophages
Cytokine Inhibitors	
TNFRI and II	Inhibition of TNFα, anti-inflammatory
Growth Factors	
EGF	Stimulation of cell proliferation and maturation
HB-EGF	Protective against damage from hypoxia and ischemia
VEGF	Promotion of angiogenesis and tissue repair
NGF	Promotion of neuron growth and maturation
IGF	Stimulation of growth and development, increased RBCs and hemoglobin
Erythropoietin	Erythropoiesis, intestinal development
Hormones	
Calcitonin	Development of enteric neurons
Somatostatin	Regulation of gastric epithelial growth

Adapted from: Ballard O, Morrow AJ. *Pediatr Clin North Am.* 2013;60:49-74.

Major Bioactive Factors in Human Milk *(continued)*

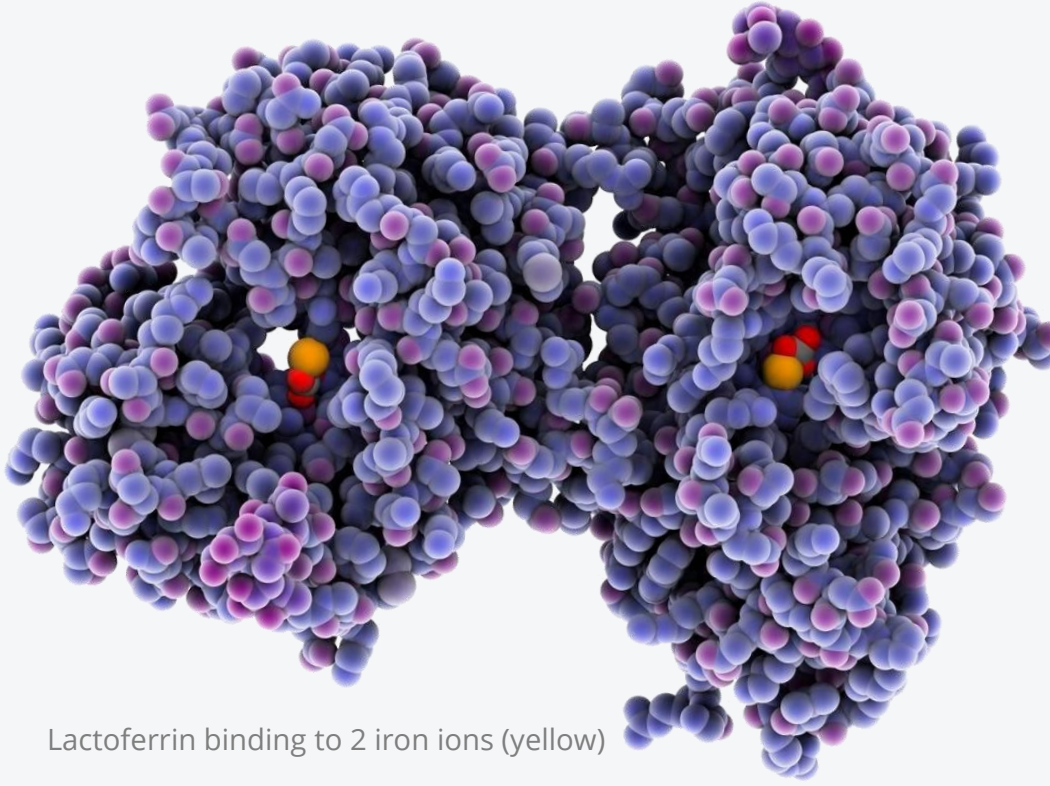
Compound	Function
Antimicrobial	
Lactoferrin	**Acute phase protein, chelates iron, antibacterial, antioxidant**
Lactadherin/MFG E8	Antiviral, prevents inflammation by enhancing phagocytosis of apoptotic cells
Metabolic and Hormones	
Adiponectin	Reduction of infant BMI and weight, anti-inflammatory
Leptin	Regulation of energy conversion and infant BMI, appetite regulation
Ghrelin	Regulation of energy conversion and infant BMI
Milk Fat Globule Membranes (MFGM)	Myelinization, immunitary
Oligosaccharides & Glycans	
HMOS	Prebiotic, stimulating beneficial colonization, reducing colonization with pathogens; reduced inflammation
Gangliosides	Brain development; anti-infectious
Glycosaminoglycans	Anti-infectious
Mucins	
MUC1	Block infection by viruses and bacteria
MUC4	Block infection by viruses and bacteria

Adapted from: Ballard O, Morrow AJ. *Pediatr Clin North Am.* 2013;60:49-74.

Lactoferrin Defined

Lactoferrin (LF) is a multifunctional, glycoprotein found in high concentration in mammalian milk with antimicrobial and immunomodulatory properties:

- Iron-binding characteristics
- Critical role protecting neonates and infants against infection



Lactoferrin binding to 2 iron ions (yellow)

LF, lactoferrin.

Jiang R, Lönnerdal B, et al. *J Pediatr Gastroenterol Nutr.* 2014;59:642-652. Telang S. *Nutrients.* 2018;10. pii: E1228.

Lactoferrin: A Multifunctional Milk Protein

Human Milk Whey Proteins[2]

- Lactoferrin is a biologically active protein with potentially important health benefits[1]
- Glycoprotein (80kDa), with iron-binding property
- Major whey protein in human milk[2]
- Also found in other mucosal secretions, such as tears and saliva,[3],[4] as well as in plasma, neutrophils, and epithelial cells
- Resistant to degradation in the newborn digestive tract[5]
- LF is historically low in infant formulas due to low levels in bovine milk[2]; newer technology allows LF to be concentrated for addition to infant formula
- Bovine and human lactoferrin share strong (77%) sequence homology[6] and the same antimicrobial peptide (n-Lactoferricin)
- Bovine LF is resistant to proteolytic digestion[7]

BSA, bovine serum albumin; IgG, Immunoglobulin G.

1. Kanwar JR, et al. *Molecules*. 2015;20:9703-9731. 2. de Wit JN. *J Dairy Sci.* 1998;81:597-608. 3. Reitamo S, et al. *Histochemistry*. 1980;66:285-291. 4. McClellan KA. *Surv Ophthalmol.* 1997;42:233-246. 5. Spik G, et al. *Acta Paediatr Scand.* 1982;71:979-985. 6. Manzoni P, et al. *JAMA*. 2009;302:1421-1428. 7. Davidson LA, et al. *Acta Paediatr Scand.* 1987;76:733-740.

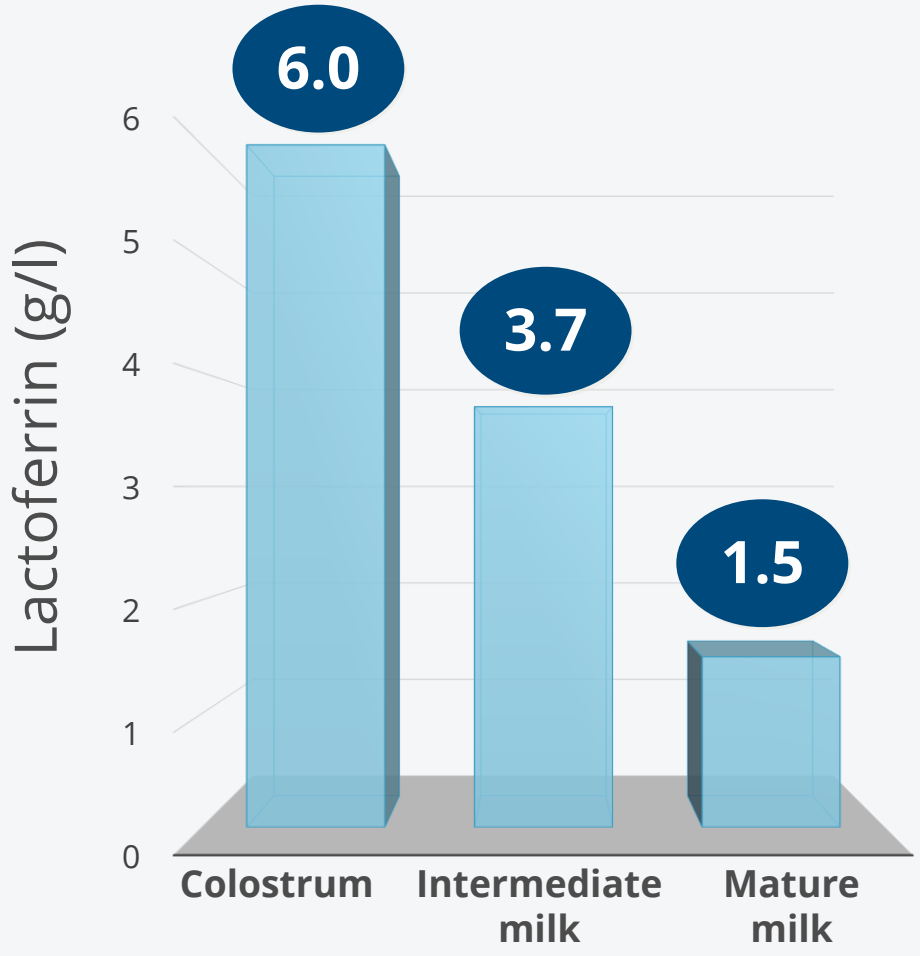
Lactoferrin Is Not Only a Milk Protein…

- Lactoferrin is part of the AMP network of inflammatory markers that can characterize the immune response during infections.
- As such, lactoferrin is released by neutrophils and mucosal secretions during an infection.
- Therefore, serum and plasma levels of lactoferrin can correlate with infection and sepsis.
- Berkestedt[1] et al 2010 show levels of several AMPs, including LF, are increased in sepsis and correlate with circulatory derangement. This probably reflects neutrophil activation as part of an innate immune response.

AMP, antimicrobial protein; LF, lactoferrin.

1. Berkestedt I, et al. *J Innate Immun.* 2010;2(5):478-482.

Lactoferrin Concentration Decreases in Mature Human Milk vs Colostrum



This decrease typically occurs in all mammals.

Milk	Concentrations of lactoferrin
Woman	2 (mature milk) – 6 (colostrum) mg/ml
Cow	0.2–0.5 mg/ml
Rat	<50 mcg/ml
Rabbit	<50 mcg/ml
Dog	<50 mcg/ml
Goat	0.2 mg/ml
Pig	0.2 mg/ml

Albenzio M, et al. *Am J Perinatol*. 2016;33(11):1085-9.

Lactoferrin and Its Mechanisms for Anti-Infective Activity

Direct Mechanisms—antibiotic-like action

- Anti-LPS (vs Gram-negatives)
- Anti-LTA (vs Gram-positives)
- Anti-Candida cell-wall components

Indirect Mechanisms

- Iron-sequestring (→bacteriostasis)
- Functional modulation of intestinal proliferation and differentiation (→enhancement of gut barrier)
- Bifidogenic action on gut microflora

Immunomodulatory Actions in the Gut Lymphoid Tissues (GALT)

- IL-18 production, NK cell activity
- Maturation and differentiation of T-lymphocytes—Th1/Th2 balance
- $CD8^{+}/4^{-}$ DCs maturation
- Recruitment and activation of APCs

Antiflogistic Mechanisms

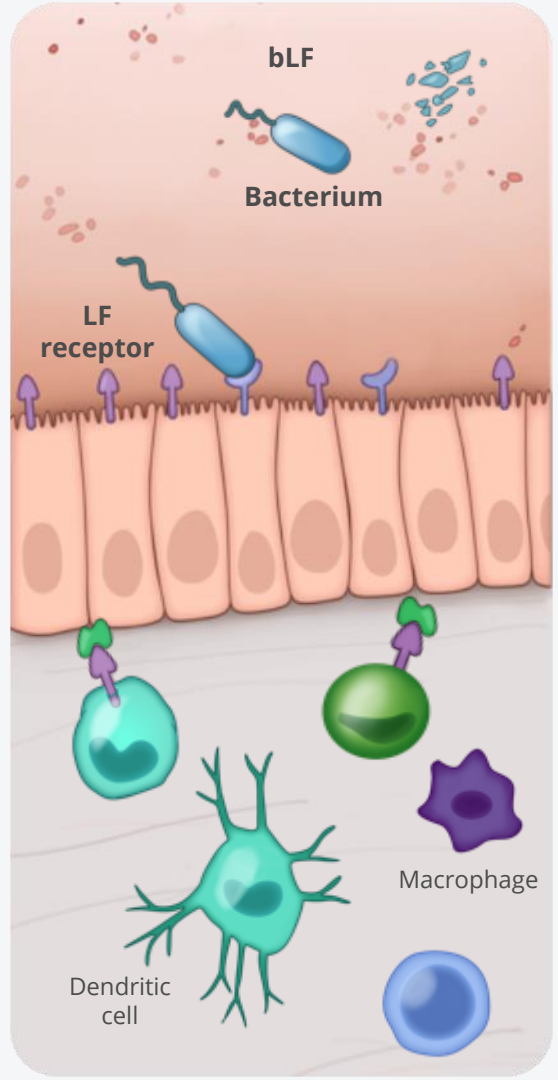
- Inhibition of formation of reactive oxygen species (ROS) by suppressing free-radical activity
- Decrease the levels of oxidative products when medicinal iron is present in a formula

APCs, antigen-presenting cells; NK cell, natural killer cells; Th1, T-helper 1; Th2, T-helper 2.

Fischer R, et al. *Biochem Cell Biol.* 2006;84:303-311. Kuhara T, et al. *J Interferon Cytokine Res.* 2006;26:489-499. de la Rosa G, et al. *J Immunol.* 2008;180:6868-6876. Ishii K, et al. *Hepatol Res.* 2003;25:226-233. Sherman MP, et al. *Neonatology.* 2011;100:409-411. van der Does AM, et al. *Biometals*. 2010;23:493-505. González-Chávez SA, et al. *Int J Antimicrob Agents.* 2009;33:301.e1-308. Lönnerdal B, et al. *J Pediatr.* 2010;156(2 Suppl):S26-S30. Lönnerdal B, et al. *J Pediatr Gastroenterol Nutr.* 2011;53:606-614. Legrand D, et al. *Biometals*. 2010;23:365-376. Raghuveer TS, et al. *Pediatr Res.* 2002;52:964-972. Sherman MP, et al. *Biometals*. 2004;17:285-289.

Functions of Lactoferrin in Gut Development and Immune Defense

- **Intestinal development**
  - Promotes cell proliferation and differentiation
  - Improves intestinal mucosal structure, increased villus height, and crypt proliferation[1],[2]
- **Antimicrobial effects**
  - Antibacterial, antiviral, and antiparasitic protein
  - Inhibits growth, adhesion, translocation, and virulence of pathogens[3],[4]
  - Sequesters iron
- **Immune modulation**
  - Stimulates cells involved in innate and acquired immunity[5]



1. Reznikov EA, et al. *J Nutr.* 2014;144:1401-1408. 2. Li Q, et al. *Mol Biol Rep.* 2014;41:2119-2128. 3. Ochoa TJ, *Biochimie.* 2009;91:30-34. 4. Teraguchi S, et al. *Appl Environ Microbiol.* 1995;61:4131-4134. 5. Shan T, et al. *J Anim Sci.* 2007;85:2140-2146.

Lactoferrin Is Bifidogenic

LF is able to promote growth of gut microbiota and establish/restore “healthy” microbiota

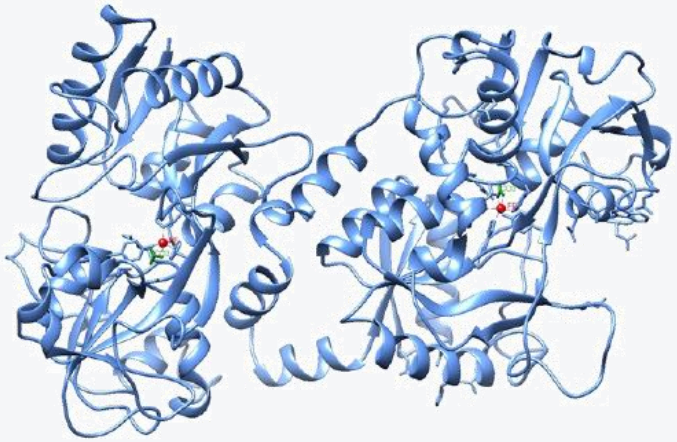
- Mastromarino[1] et al 2014 measured content of LF and microbiota of breast milk and feces of infants
- n=48 mother-infant pairs (34 full-term and 14 preterm) at birth and 30 DoL
- LF had positive influence on the microbiota → fecal count of *Bifidobacteria* and *Lactobacilli* was significantly associated with the concentration of fecal LF at 3 DoL (p= 0.01)

DoL, days of life.

1. Mastromarino P, et al. *Biometals.* 2014;27:1077-1086.

Bovine and Human Lactoferrin Similarities

- Bovine and human lactoferrin have 77% biochemical homology
- Bovine and human lactoferrin provide similar actions on the nascent gut:
  - Commercial bLF is biologically active, as well as purified bLF and hLF
  - Commercial bLF exerts several bioactivities if added to infant formula



bLF, bovine lactoferrin; hLF, human lactoferrin.

Lönnerdal B, et al. *J Pediatr Gastroenterol Nutr.* 2011 Dec;53(6):606-614. Jiang R, et al. *J Pediatr Gastroenterol Nutr.* 2014;59(5):642-652.

Lactoferrin Clinical Studies

Study (date)	Patients (n=)	Objective	Dosing	Started	Duration	Result
Manzoni P, et al. *JAMA*. 2009. Multicenter RCT, 10 months	n=472 VLBW (<1500g) infants bLF alone (n=153) bLF + LGG (n=151) or placebo (n=168)	Evaluate effectiveness of LF (alone or in combo w/LGG), compared with placebo, reduces incidence of LOS in VLBW neonates	**Group A1** – bLf 100 mg **Grp A2** – bLf 100 mg + LGG, 6 x 109 CFU/day; **Placebo** (2 ml of 5% glucose sol. to milk feeding, daily 4-to-6 weeks)	birth until day 30 of life (day 45 for neonates <1000 g at birth)		Compared with placebo, bLF supplementation alone or in combo w/LGG reduced incidence of a first episode of late-onset sepsis in VLBW neonates Supplemental lactoferrin reduced incidence of late-onset sepsis in VLBW infants.
Manzoni P, et al. *Pediatrics*. 2012.	n=472 neonates (<1500g) infants bLf alone (n=153) bLf + LGG (n=151) or placebo (n=168)	Secondary analysis of data from multicenter RCT where preterm VLBW neonates	**Group A1** – bLf 100 mg **Grp A2** – bLf 100 mg + LGG, 6 x 109 CFU/day; **Placebo** (2 ml of 5% glucose sol. to milk feeding)	4.0 ± 1.4 DoL	daily 4-to-6 weeks	Prophylactic oral administration of bLF reduces incidence of IFI in preterm VLBW neonates. No effect is seen on colonization. LF decreased infections but not colonization rates in the gut.
Ochoa TJ, et al. *J Peds*. 2013. (Peru) Study from Jan 2008–May 2011	n=277 lactoferrin n=278 placebo randomly assigned	Determine effect of bLF on prevention of diarrhea in children	Infants received 0.5 g twice/day bLF or placebo (diluted in 25 mL of water)	12 to ≤18 months old; 6 days/wk, twice daily	91,446 child/days of observation: 46,545 bLF 44,901 placebo	• No difference diarrhea: 5.4 vs 5.2 episodes/ child/year for lactoferrin and placebo, respectively (p= 0.375). • Although no decrease in diarrhea incidence, longitudinal prevalence and severity were decreased with lactoferrin.

bLF, bovine lactoferrin; DoL, days of life; IFI, invasive fungal infection; LGG, *Lactobacillus* GG; LOS, late-onset sepsis; VLBW, very low birth weight.

Manzoni P, et al. *JAMA*. 2009;302:1421-1428. Manzoni P, et al. *Pediatrics*. 2012;129(1)116-123. Ochoa TJ, et al. *J Pediatr*. 2013;162:349-356.

Study (date)	Patients (n=)	Objective	Dosing	Started	Duration	Result
Manzoni P, et al. *Early Hum Dev*. 2014.	n=743 VLBW neonates	Studies have shown reduction of NEC in animal models; enhanced by LGG. This study assessed whether bLF, alone or w/probiotic LGG, has a similar effect in human infants.	**bLF** (100 mg/day) alone (n=247) **or bLF w/LGG** (at 6×10(9) CFU/day; n=238) **Placebo** (control group; n=258)	birth until 30 DoL	assessed until discharge for development of NEC	Compared with placebo, bLF supplementation alone or in combo w/LGG reduced the incidence ≥ stage 2 NEC and of death-and/or ≥ stage 2 NEC in VLBW neonates. Decreased incidence of NEC. BLF might be a promising strategy to prevent NEC in NICU settings.
Akin IM, et al. *Am J Perinatol.* 2014.	n=50 VLBW or born <32 weeks • placebo (n=25) • 200 mg LF (n=25)	Does oral LF (200 mg/d) reduce nosocomial sepsis episodes and NEC in premature infants; Evaluate possible effects of LF on Treg levels.	200 mg LF daily throughout hospitalization		daily throughout hospitalization	Fewer sepsis episodes observed in LF-treated infants (4.4 vs 17.3/1,000 patient days, p= 0.007) with none developing NEC. LF prophylaxis reduced nosocomial sepsis episodes. Increase of Treg levels under LF prophylaxis was observed
Kaur G, et al. *J Trop Ped*. 2015. (India)	LBW infants (BW less than 2,000 g)	Evaluate efficacy of bLF to prevent first episode of LOS in LBW neonates.	bLF 100–200 mg/day, according to increasing BW 1000–2000g	<48 hrs of life	First 30 DoL	bLF supplementation in LBW neonates reduced incidence of first episode of LOS. **Note**: Ideal sample size would have been 114 per arm to have 80% power
NEOLACTO study (NCT01525316; Peru) Ochoa, et al. *J Peds.* 2015.	n=190 neonates; 80 (42.1%) had <1500 g BW	Determine the effect of bovine LF on the prevention of first episode of LOS in Peruvian infants	**bLF** (200mg/kg/day) **Placebo** (maltodextrin) given in 3-divided doses/day	500–2500 g at birth	4 wks since enrollment	Sepsis occurred less frequently in LF grp than in control grp. Although, primary outcome did not reach statistical significance, confidence interval is suggestive of an effect that justifies a larger trial.

bLF, bovine lactoferrin; DoL, days of life; LGG, *Lactobacillus* GG; LOS, late-onset sepsis; NEC, necrotizing enterocolitis; VLBW, very low birth weight.

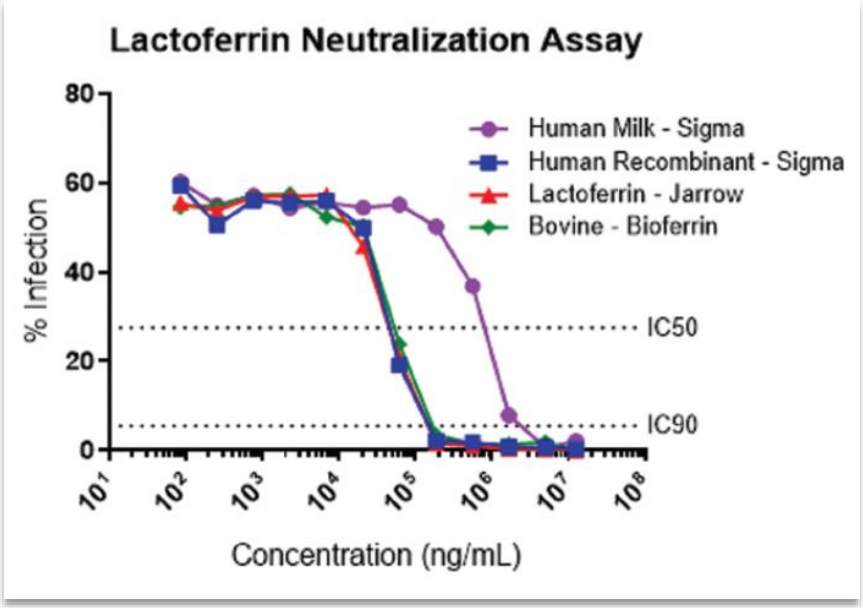
Manzoni P, et al. *Early Hum Dev.* 2014;90 Suppl 1:S60-65. Akin IM, et al. *Am J Perinatol.* 2014;31:1111-1120. Kaur G, et al. *J Trop Pediatr.* 2015;61:370-376. Ochoa TJ, et al. *Pediatr Infect Dis J.* 2015;34(6):571-576.

Study (date)	Patients (n=)	Objective	Dosing	Started	Duration	Result
Trial of lactoferrin for prevention of infections in very premature babies (**LACUNA**) trial (2016) Barrington et al. *J Perinatol*. 2016.	neonates >23 <30.6 wks GA First 48 hrs of age (n=79) Control group: milk w/o LF	Determine tolerability of bLF in VPI, and if intervention can be adequately masked	bLF 100 mg/day, 2 doses per day until 36 wks GA or discharge	<48 hours of life	36 wks GA or discharge	bLF is well tolerated, easy to administer, and its presence in prepared milk is not evident
Sherman et al, *J Pediatrics*. 2016.	TLf (n=60) or placebo (n=60)	Evaluate safety and efficacy of recombinant human LF (TLf) to reduce infection	TLf 150 mg/kg every 12 hours	Day 1–28 DoL	28 days	No clinical or lab toxicity; trend toward less infectious morbidity in infants treated with TLf
Cochrane Review 6 RCTs Effect of LF on Late-Onset Sepsis Pammi M, et al. *Cochrane Database Syst Rev*. 2017.	Six RCTs in 1071 preterm infants; Risk Ratio 0.59 (95% CI 0.40–0.87; *P*=0.008)	Three co-primary outcomes: • LOS (n=886) • NEC ≥ stage II (n=750) • Hospital mortality (n=1071)	Clarification regarding optimal dosing regimens, types of LF (human or bovine), and long-term outcomes is needed.			• Oral Lf prophylaxis with/wo probiotics decreases LOS and NEC ≥stage II in preterm infants without adverse effects • Current available evidence graded as "low-moderate quality"; *P* = 0.05 indicates significant heterogeneity between trials • Completed ongoing trials will provide data from more than 6000 preterm neonates, which may enhance the quality of the evidence

bLF, bovine lactoferrin; DoL, days of life; GA, gestational age; LOS, late-onset sepsis; NEC, necrotizing enterocolitis; TLf, Talactoferrin; VLBW, very low birth weight.

Barrington KJ, et al. *J Perinatol.* 2016;36:666-669. Sherman MP, et al. *J Pediatr.* 2016;175:68-73.e3. Pammi M, et al. *Cochrane Database Syst Rev.* 2017;6:CD007137.

Lactoferrin and Cytomegalovirus (CMV)



Neutralization potency of human and bovine lactoferrin against human cytomegalovirus. Neutralization of HCMV AD169 by human purified, human recombinant, and two bovine-purified lactoferrin preparations. The dotted lines represent the IC_{50} and IC_{90} (ng/mL).

HCMV, human cytomegalovirus.

- Lactoferrin (LF) neutralizes CMV in vitro
- Bovine LF has more potency than human
- LF concentrations in breast milk and saliva are likely too low for effective neutralization in vivo
- Breastfed, preterm neonates might need extra-LF to prevent CMV acquisition

Weimer KED, et al. *Am J Perinatol*. 2020. [published online ahead of print February 18, 2020] Used by permission of Thieme.

Benefits of Prophylaxis Oral Lactoferrin *(Cochrane Database Syst Rev)*

- 6 RCTs; n=1071 preterm infants
- 3 co-primary outcomes:

	n=	Risk Ratio	
Late-onset sepsis	886	0.59 (95% CI 0.40 to 0.87; *P*= 0.008)	NNT 17 Current available evidence graded as "low-moderate quality"
NEC ≥ stage II	750	0.40 (95% CI 0.18 to 0.86; *P*= 0.02)	NNT 25 Current available evidence graded as "low quality"
Hospital mortality	1071	0.65 (95% CI 0.37 to 1.11; *P*= 0.12)	

CI, confidence interval; NNT, Number Needed to Treat.

Meyer MP, et al. *J Neonatal Perinatal Med*. 2017;10:249-255. Pammi M, et al. *Cochrane Database Syst Rev.* 2017;6: CD007137.

Year 2018–2019–2020 — Two Mega Trials: ELFIN and LIFT Lactoferrin RCTs

Study	Population	Intervention Group	Control Group	Primary Outcome
Enteral Lactoferrin In Neonates (**ELFIN**); ISRCTN88261002	UK Neonates <32 wks GA First 72 h of age (n=2,200)	Bovine LF 150 mg/kg/day (max: 300 mg) until discharge	Milk with placebo	Culture-proven or clinically suspected LOS from trial entry until discharge
Lactoferrin Infant Feeding Trial (**LIFT**) to prevent sepsis and death in preterm infants; ACTRN12611000247976	AUSTRALIA, INDIA, CANADA, ITALY Neonates with BW <1,500 g GA 22–28 wks First 7 DoL (n=1,100)	Bovine LF 200 mg/kg/day until 34 weeks GA corrected or discharge	Breast milk or formula without bLF	Incidence of sepsis or brain injury or CLD or NEC or severe ROP

CLD, chronic lung disease; DoL, days of life; **ELFIN, Enteral Lactoferrin in Neonates**; GA, gestational age; **LIFT, Lactoferrin Infant Feeding Trial**; LOS, late-onset sepsis; NEC, necrotizing enterocolitis; ROP, retinopathy; RCT, randomized controlled trial.

ELFIN Trial (*The Lancet,* Jan 2019)

Primary and Secondary Outcomes	Lactoferrin group (n=1098)	Control group (n=1101)	Unadjusted risk ratio or median difference (95% CI or 99% CI)*†	Adjusted risk ratio or median difference (95% CI or 99% CI)*†‡	*p* value§
Microbiologically confirmed or clinically suspected late-onset infection	316/1093 (29%)	334/1089 (31%)	0.94 (0.83 to 1.07)	0.95 (0.86 to 1.04)	0.233
Microbiologically confirmed late-onset infection	190/1093 (17%)	180/1089 (17%)	1.05 (0.82 to 1.34)	1.05 (0.87 to 1.26)	0.490
All-cause mortality	71/1076 (7%)	68/1076 (6%)	1.04 (0.69 to 1.59)	1.05 (0.66 to 1.68)	0.782
NEC (Bell stage II or III)	63/1085 (6%)	56/1084 (5%)	1.12 (0.71 to 1.77)	1.13 (0.68 to 1.89)	0.538
Severe ROP treated medically or surgically	64/1080 (6%)	72/1080 (7%)	0.89 (0.58 to 1.35)	0.89 (0.62 to 1.28)	0.420
BPD at 36 weeks' postmenstrual age	358/1023 (35%)	355/1027 (35%)	1.01 (0.87 to 1.18)	1.01 (0.90 to 1.13)	0.867
Died before 36 weeks' postmenstrual age	64	60	..	..	..
Infection, NEC, ROP, BPD, or mortality	525/1092 (48%)	521/1094 (48%)	1.01 (0.90 to 1.13)	1.01 (0.94 to 1.08)	0.743
Total number of days of administration of antimicrobials from commencement of investigational medicinal product until 34 weeks' postmenstrual age, median (IQR)	2 (0 to 8)	3 (0 to 8)	0 (0 to 0)	0 (–1 to 1)	0.625
Length of hospital stay (days) to discharge, median (IQR)	59 (40 to 85)	58 (40 to 84)	1 (–2 to 4)	1 (–1 to 3)	0.446
Days in level 1 (intensive) care, median (IQR)	8 (4 to 16)	8 (4 to 16)	0 (–1 to 1)	0 (–1 to 1)	0.963
Days in level 2 (high dependency) care, median (IQR)	10 (3 to 30)	9 (3 to 29)	0 (–1 to 1)	1 (–1 to 3)	0.420
Days in level 3 (special) care, median (IQR)	29 (21 to 39)	30 (22 to 39)	–1 (–2 to 1)	–1 (–3 to 1)	0.216

Unless otherwise stated, data are n/N (%); when N is not equal to the total number of infants in the group it means that data are missing for some of the infants. NEC, necrotizing enterocolitis; BPD, bronchopulmonary dysplasia; ROP, retinopathy of prematurity. *Risk ratios for binary outcomes and median differences for continuous outcomes. †95% CI for microbiologically confirmed or clinically suspected late-onset invasive infection, 99% CI for all other outcomes. ‡Adjusted for minimization factors (i.e., collaborating hospital, sex, gestational age at birth, and single or multiple birth). §p value for testing whether adjusted risk ratio is equal to 1 or adjusted median difference is equal to 0).

" ...enteral lactoferrin supplementation (150 mg/kg/d until 34-weeks postmenstrual age) does not reduce the risk of late-onset infection, other morbidity, or mortality in very preterm infants... "

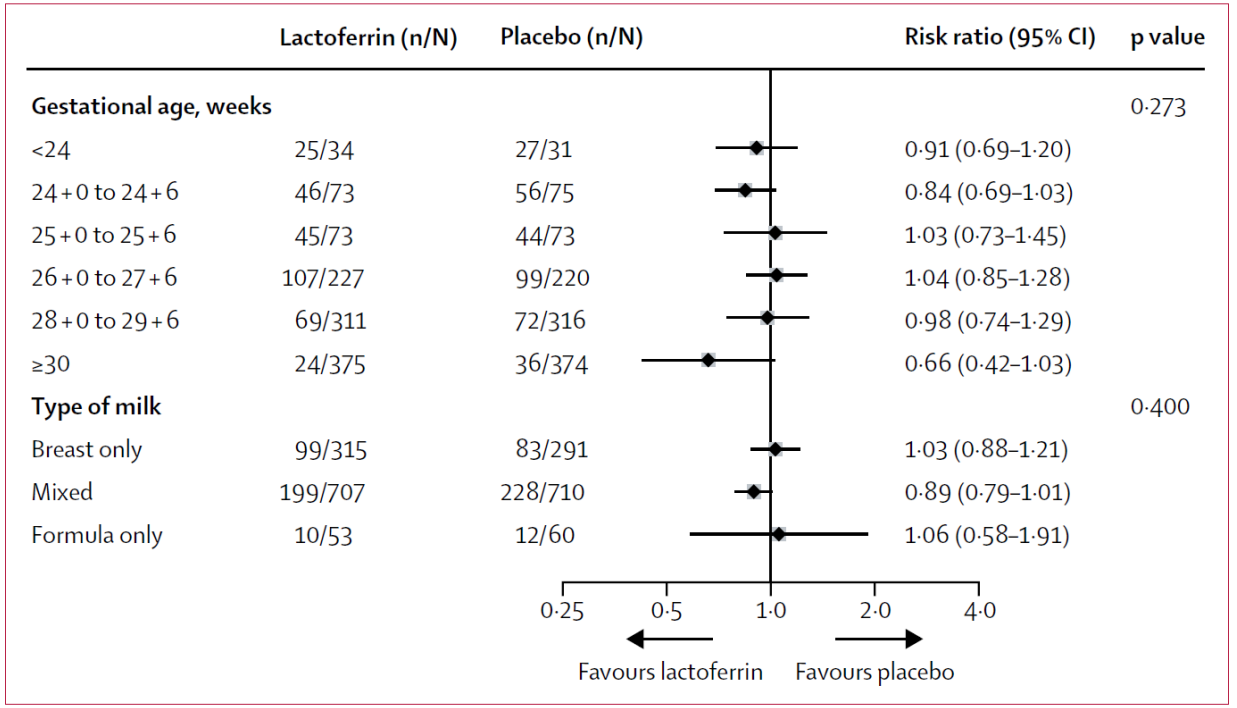
ELFIN trial investigators group. *Lancet.* 2019 Feb 2;393:423-433. Griffiths J, et al. *Health Technol Assess.* 2018;22:1-60.

ELFIN Trial (*The Lancet*, Jan 2019)

	Lactoferrin group (n=1098)	Control group (n=1101)
Microbiologically confirmed late-onset invasive infection from trial entry until hospital discharge	190/1093 (17·4%)	180/1089 (16·5%)
At least one Gram-positive organism confirmed	153/1093 (14·0%)	147/1089 (13·5%)
At least one CoNS group organism	122/1093 (11·2%)	111/1089 (10·2%)
At least one Gram-negative organism confirmed	46/1093 (4·2%)	39/1089 (3·6%)
At least one fungal organism confirmed	3/1093 (0·3%)	2/1089 (0·2%)
At least one other organism confirmed	3/1093 (0·3%)	2/1089 (0·2%)
Any record of probiotics being given		
Yes	99/354 (28·0%)	97/329 (29·5%)
No	208/728 (28·6%)	227/749 (30·3%)

Data for late-onset infection by classification of microorganism are n/N (%); when N is not equal to the total number of infants in the group it means that data are missing for some of the infants. Data for exposure to probiotics are number of infants with microbiologically confirmed or clinically suspected late-onset infection/number of infants who were given (or not) probiotics (%). CoNS=coagulase-negative staphylococcus.

Microbiologically confirmed late-onset infection by classification of microorganism (appendix) and microbiologically confirmed or clinically suspected late-onset infection from trial entry until hospital discharge by exposure to probiotics



Subgroup analyses for confirmed or suspected late-onset invasive infection p values are for statistical interactions. n=cases of late-inset invasive infection. N=group size.

LF dosing → 150 mg/kg per day; maximum 300 mg/day

Once daily until 34-weeks postmenstrual age

Trial participants were allocated to receive either bovine lactoferrin or sucrose

ELFIN trial investigators group. *Lancet*. 2019;393:423-433. © 2019 The Author(s). Griffiths J, et al. *Health Technol Assess.* 2018;22:1-60.

ELFIN Trial (*The Lancet*, Jan 2019)

Main take-home messages derived from 2,200 neonates enrolled

- "…does not support routine use of enteral bLF supplementation to prevent late-onset infection of other morbidity or mortality in very preterm infants"

- Highlights of trial data, clarifying conclusions
  - Significance of specific populations
  - Quantity of LF received with human milk (donor or mother's)

Please note only 113 of 2,182 patients (5%) were exposed to formula milk!

bLF, bovine lactoferrin; ELFIN, Enteral Lactoferrin in Neonates.

ELFIN trial investigators group. *Lancet*. 2019 Feb 2;393:423-433. Griffiths J, et al. *Health Technol Assess.* 2018;22:1-60.

Lactoferrin Infant Feeding Trial

First presentation of headline results: Torino, Italy—ICCN, May 2018

published results as of May 12, 2020,
The Lancet Child & Adolescent Health[1]

ICCN, International Conference on Clinical Neonatology.

1. Tarnow-Mordi WO, et al. *Lancet Child Adolesc Health*. 2020. pii: S2352-4642(20)30093-6. [published online ahead of print May 12, 2020]

NHMRC Lactoferrin Infant Feeding Trial

LIFT is a blind RCT to evaluate whether supplementing feeds in VLBW infants with once-daily study supplement of lactoferrin vs no lactoferrin (control) reduces:

- **Primary composite outcome**
  - Death, late-onset sepsis, brain injury, NEC, or ROP
- **Secondary outcome,** including
  - Death, late-onset sepsis, brain injury, NEC, chronic lung disease, blood transfusions

NHMRC, National Health and Medical Research Council; NEC, necrotizing enterocolitis; ROP, retinopathy; VLBW, very low birth weight.

NHMRC LIFT Trial *(continued)*

Intervention:

Bovine lactoferrin in breastmilk or formula milk to a daily dose of 200 mg/kg (control group received no bLF added to breast milk or formula milk) until 34 weeks corrected gestational age or for 2 weeks, whichever was longer, or until discharge home, if earlier.

Power and Sample Size:

n=1500, yields 85% power, with 2-sided 5% significance to detect difference in primary outcome, from 26% in controls to 19.5% in the bLF group.

Predefined subgroups:

(i) birth-weight <1000 g and 1000–1499 g;

(ii) randomized ≤72 hrs and >72 hrs from birth;

(iii) those who received or did not receive probiotics; and

(iv) ≤28-weeks and >28-weeks gestation

bLF, bovine lactoferrin; NHMRC, National Health and Medical Research Council.

NHMRC LIFT Trial *(continued)*

Table. Compliance With Study Treatment

Characteristic N (%)	Lactoferrin n=770	Control n=771
Days of study treatment median (IQR)	**29** (16 to 40)	**29** (17 to 40)
≥7 days of study treatment	719 **(93.4%)**	734 **(95.2%)**
≥14 days of study treatment	638 **(82.9%)**	665 **(86.3%)**
Study treatment completed	603 (**78.3%**)	634 (**82.2%**)
Study treatment incomplete	167 (21.7%)	137 (17.8%)

LIFT, Lactoferrin Infant Feeding Trial; NHMRC, National Health and Medical Research Council.

Martin A, et al. *BMJ Open.* 2018;8:e023044.

NHMRC LIFT Trial *(continued)*

Table. Descriptive Characteristics: Nutrition

Characteristic N (%)	Lactoferrin n=770	Control n=771
Any mother's milk	733 (**95.2%**)	725 (**94.0%**)
Any donor breast milk	54 (7.0%)	53 (6.9%)
Any formula milk	64 (8.3%)	63 (8.2%)
Received probiotics at any time	660 (**85.7%**)	658 (**85.3%**)

LIFT, Lactoferrin Infant Feeding Trial; NHMRC, National Health and Medical Research Council.

NHMRC LIFT Trial *(continued)*

Table. Primary Composite Outcome

Primary Outcome	Lactoferrin n=770	Control n=771	Relative Risk (95% CI)	*P*
Death or major morbidity	**162 (21.0%)**	**170 (22.0%)**	**0.95 (0.79 to 1.14)**	**0.60**

CI, confidence interval; LIFT, Lactoferrin Infant Feeding Trial; NHMRC, National Health and Medical Research Council.

NHMRC LIFT Trial *(continued)*

Table. RESULTS: Components of Primary Outcome

Primary Outcome	Lactoferrin n=770	Control n=771	Relative Risk (95% CI)	*P*
Death before discharge	32 (4.2%)	29 (3.8%)	1.12 (0.68 to 1.84)	0.66
Necrotizing enterocolitis	26 (3.4%)	25 (3.2%)	1.09 (0.63 to 1.9)	0.75
Late-onset sepsis	89 (11.6%)	108 (14.0%)	**0.83 (0.64 to 1.08)**	0.16
Brain injury	50 (6.5%)	47 (6.1%)	1.06 (0.72 to 1.54)	0.78
Treated retinopathy	29 (3.8%)	20 (2.6%)	1.43 (0.84 to 2.44)	0.19

Not significant; 17% reduction of sepsis in LF-treated infants.

LIFT Trial Summary Overview

- LF did not reduce the primary outcome, death, or major morbidity (Risk Ratio 0.95, 95% CI 0.79–1.14).
- Although a trend was observed, LF failed to reduce late-onset sepsis significantly (RR 0.83, 95% CI 0.64–1.08).
- LF did not affect other primary outcome components.
- The trial had 85% power to detect a 25% decrease or increase in primary outcome, but more moderate effects are not excluded.
- Treatment was well tolerated with good compliance, and there were no safety concerns with LF.

CI, confidence interval; LF, lactoferrin; RR, risk ratio.

LIFT Trial Summary Overview *(continued)*

- The LIFT results appear inconsistent with the previous Cochrane review of 6 RCTs.
- While some differences between LIFT and the other trials may reflect the play of chance, there was significant heterogeneity, both amongst earlier studies and between LIFT and earlier studies.
- This raises questions about whether exactly the same product was being tested, or the same population was tested.

CI, confidence interval; LF, lactoferrin; LIFT, Lactoferrin Infant Feeding Trial; RR, risk ratio.

Why Are There Inconsistencies Between the 3 Major RCTs in Terms of LF Efficacy?

	LIFT n=1541	ELFIN n=2199	Manzoni n=472	Ratio
Only mother's milk	86%	92%	24%	>3
Only formula milk	<8%	<5%	15%	~0.5
Any probiotic	86%	75%	32%	>2.5
Was bLF flash pasteurized?	Yes	Yes	No	

bLF, bovine lactoferrin; ELFIN, Enteral Lactoferrin in Neonates; LF, lactoferrin; LIFT, Lactoferrin Infant Feeding Trial.

Martin A, et al. *BMJ Open.* 2018;8:e023044; ELFIN trial investigators group. *Lancet.* 2019 Feb 2;393:423-433; Manzoni P, et al. *JAMA.* 2009;302:1421-1428.

Pending Issues Related to Supplementation Strategies With Lactoferrin

- LF levels vary in maternal milk during lactation
- Infant's GA and time of study sampling affect LF levels
- LF ranges in various breast milk types
  - Mother’s milk
  - Stored, refrigerated mother’s milk
  - Donor milk
- Clarification regarding optimal dosing regimens, types of lactoferrin (human or bovine), and long-term outcomes is needed.

GA, gestational age.

Albenzio M, et al. *Am J Perinatol*. 2016;33:1085-1089.

How Do We Correctly Interpret These Data? How Do We Reconcile Apparently Contrasting Findings?

- The evidence BEFORE 2020 was suggesting that lactoferrin supplementation to enteral feeds decreases late-onset in preterm infants without adverse effects.
- The recently completed large-sized trials have provided data from >6000 preterm neonates and should enhance the quality of the evidence.
- However, the populations studied in these 2 recent RCTs are likely different from those studied in the earlier trials!

Pammi M, et al. *Cochrane Database Syst Rev*. 2017;6: CD007137.

How Do We Correctly Interpret These Data? How Do We Reconcile Apparently Contrasting Findings?

- The key for a better understanding is NOT THE SUPPLEMENTATION OF LACTOFERRIN, but rather THE ACTUAL INTAKE OF LACTOFERRIN
- Evidence from 3 clinical studies:
  1. Trend[1] et al. (Australia)—2015
  2. Ochoa[2] et al. (Peru)—2020
  3. Manzoni[3] et al. (Italy & NZ)—2019
- Evidence from 2 Lab studies…

1. Trend S, et al. *PLOS One*. 2015;10: e0117038.
2. Ochoa TJ, et al. *Neonatology*. 2020:1-8.
3. Manzoni P, et al. *Am J Perinatol*. 2019;36:S120-S125.

Antimicrobial Protein and Peptide Concentrations and Activity in Human Breast Milk Consumed by Preterm Infants at Risk of Late-Onset Neonatal Sepsis[1]

This study assessed the levels and antimicrobial activity of antimicrobial proteins and peptides, including lactoferrin, in breast milk consumed by preterm (<32 wks) infants, and whether deficiencies of these factors were associated with late-onset neonatal sepsis

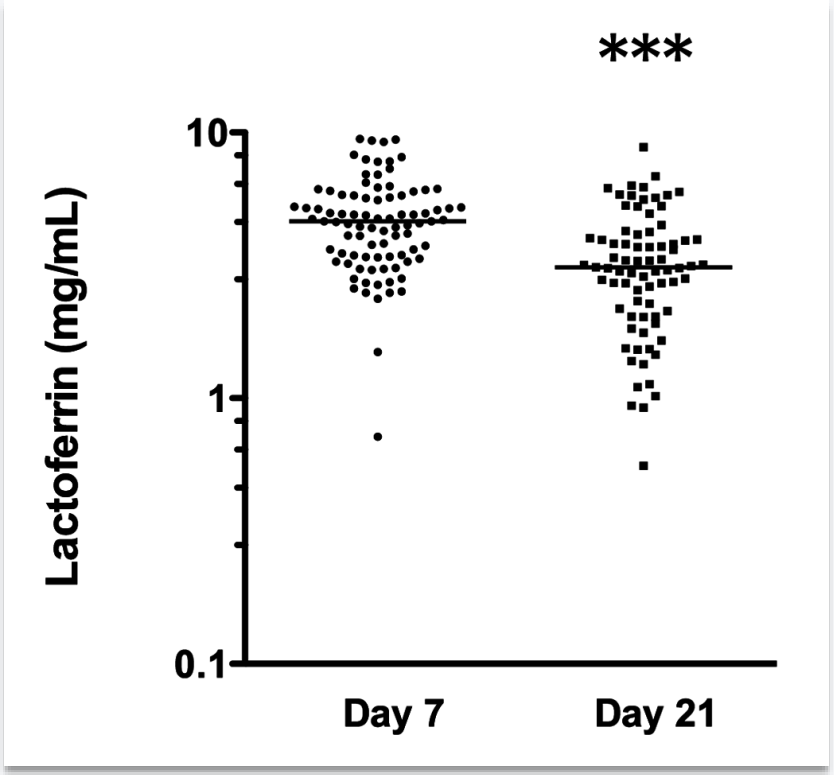
- Breast milk from mothers of preterm infants (32 wks GA) was collected on days 7 (n=88) and 21 (n=77) postpartum.
- Concentrations of lactoferrin, LL-37, beta-defensins 1 and 2, and alpha-defensin 5 were measured by ELISA.
- The antimicrobial activity of breast milk samples against *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Escherichia coli*, and *Streptococcus agalactiae* was compared to the activity of infant formula, alone or supplemented with physiological levels of AMPs.
- Samples of breast milk fed to infants with and without subsequent LOS were compared for levels of AMPs and inhibition of bacterial growth.

GA, gestational age; ELISA, enzyme-linked immunosorbent assay; LOS, late-onset sepsis; AMPs, antimicrobial proteins.

1. Trend S, et al. *PLOS One*. 2015;10(2):e0117038.

Results[1]

- Levels of most AMPs, including LF, and antibacterial activity in preterm breast milk were higher at day 7 than at day 21.
- The range of total daily LF consumed by infants ranged from 0–794 mg/kg on days 7 and 21 postpartum.
- **Lactoferrin was the only AMP that limited pathogen growth >50% when added to formula at a concentration equivalent to that present in breast milk.**
- Levels of AMPs were similar in the breast milk fed to infants with and without LOS, however, infants who developed LOS consumed significantly less breast milk and lower doses of milk AMPs than those who were free from LOS.



Levels of LF in breast milk are higher at 7 days than at 21 days (*P*<0.001).

AMPs, antimicrobial proteins; LF, lactoferrin; LOS, late-onset sepsis.

1. Trend S, et al. *PLOS One*. 2015;10(2):e0117038. Used under terms of a Creative Commons Attribution License. © 2015 Trend et al.

Antimicrobial Activity of Lactoferrin When Added to Infant Formula

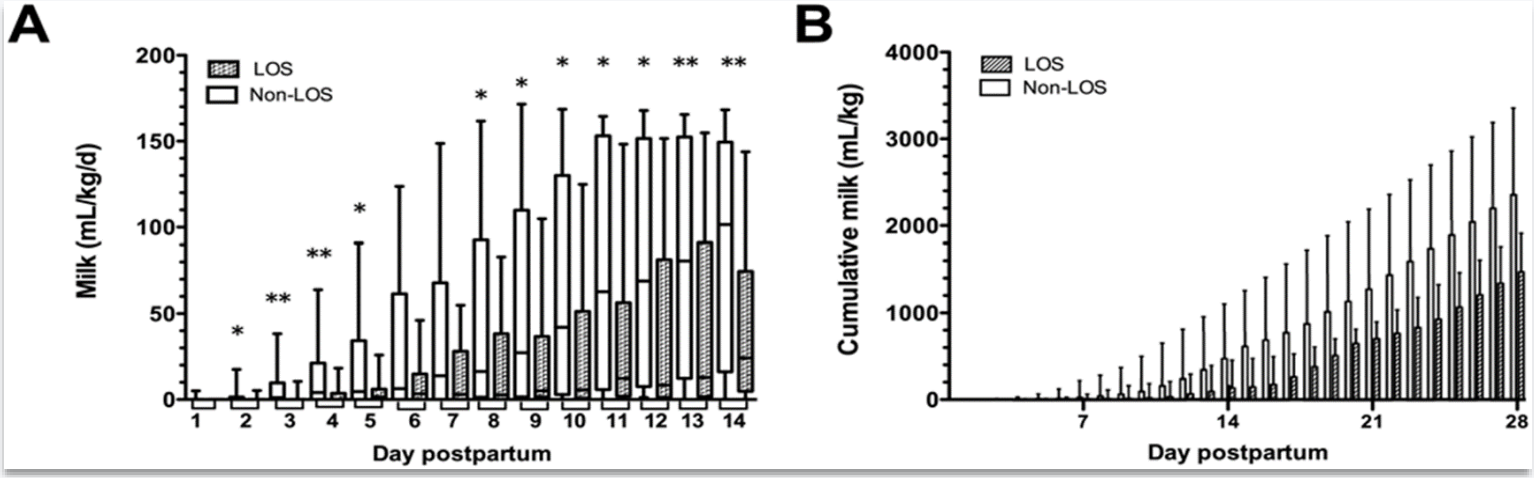
- In a secondary experiment, physiological milk levels of individual AMPs, including LF, were independently capable of inhibiting bacterial growth in LBWF.
- **The addition of LF to LBWF** at doses equivalent to the median concentration measured in preterm breast milk (3.8 mg/mL) had >50% bacteriostatic effect against all bacterial species, with **>97% inhibition of growth for *S. epidermidis, S. aureus,* and *E. coli,* and 67% for *S. agalactiae*** (Fig. 3, Trend[1] 2015).
- The **effect was dose-dependent**, with inhibition of all species >97% when 9.5 mg/mL LF (equivalent to the highest concentration detected in preterm breast milk) was used.
- No significant effect on growth inhibition was seen when the lowest concentration detected in preterm breast milk (0.5 mg/mL LF) was added to LBWF.
- The other AMPs tested did not show similar efficacy in inhibiting pathogens.

LF, lactoferrin; LBWF, low birth weight formula.

1. Trend S, et al. *PLOS One*. 2015;10(2):e0117038.

Late-Onset Sepsis in Infants and Levels/Intakes of LF

- The concentration of LF in breast milk showed negative correlation with the colony forming units of *E. coli* and *S. aureus* after incubation with breast milk
- **The median doses of LF consumed by LOS cases were lower on day 7 (14 mg/kg LF in LOS cases and 52 mg/kg in controls, respectively; $p = 0.03$) and day 21 (131 mg/kg LF in LOS cases and 298 mg/kg LF in controls, respectively; $p = 0.04$).**



*p<0.05; **p<0.01. Consumption of breast milk by preterm infants in the case-control study.

In Light of ELFIN and LIFT Results… Let's Look Into *JAMA* 2009[1] Trial Results

- N=472; 361 (76%) infants were *not* exclusively human-milk fed
- Remaining 111 (24%) HM-fed infants had mean daily intake of 79 ml/kg/day (current evidence suggests >50 ml/kg/day is protective) (Schanler[2] 1999)
- **A post-hoc analysis shows the effect of bLF was not significant in the HM-only subgroup** (p=0.15 vs p<0.001)[3]
- **Why is this the case?**

bLF, bovine lactoferrin; DoL, days of life; HM, human milk; LF, lactoferrin.

1. Manzoni P, et al. *JAMA.* 2009;302:1421-1428.
2. Schanler RJ, et al. *Pediatrics.* 1999;103:1150-1157.
3. Manzoni P, et al. *Am J Perinatol*. 2019;36:S120-S125.

Why Was the Effect of bLF Not Significant in the Subgroup of Infants Fed HM?

Assuming a mean concentration of hLF comprised between 2–3.5 mg/ml, HM-fed infants in the LIFT study were likely exposed to 160–280 mg/kg/day during their stay in NICU, which is more than the bLF supplement (100 mg/kg/day) given to the experimental arms

Post-hoc analysis on the 2009 RCT patients

Is Lactoferrin More Effective in Reducing Late-Onset Sepsis in Preterm Neonates Fed Formula Than in Those Receiving Mother's Own Milk? Secondary Analyses of Two Multicenter Randomized Controlled Trials

Paolo Manzoni, MD[1,2,*] Maria Angela Militello, MD[1] Stefano Rizzollo, MD[1] Elena Tavella, MD[2] Alessandro Messina, MD[2] Marta Pieretto, MD[2] Elena Boano, MD[2] Martina Carlino, MD[2] Eleonora Tognato, MD[1] Roberta Spola, MD[1] Anna Perona, MD[1] Milena Maria Maule, MD[3] Ruben García Sánchez, MD[4] Mike Meyer, MD[5] Ilaria Stolfi, MD[6] Lorenza Pugni, MD[7] Hubert Messner, MD[8] Silvia Cattani, MD[9] Pasqua Maria Betta, MD[10] Luigi Memo, MD[11] Lidia Decembrino, MD[12] Lina Bollani, MD[12] Matteo Rinaldi, MD[13] Maria Fioretti, MD[14] Michele Quercia, MD[15] Chryssoula Tzialla, MD[12] Nicola Laforgia, MD[14] Fabio Mosca, MD[7,16] Rosario Magaldi, MD[13] Michael Mostert, MD[17] Daniele Farina, MD[1] William Tarnow-Mordi, MD[18] on behalf of the Italian Task Force for the Study Prevention of Neonatal Fungal Infections; the Italian Society of Neonatology

bLF, bovine lactoferrin; HM, human milk; hLF, human lactoferrin.

Manzoni P, et al. *Am J Perinatol*. 2019;36:S120-S125.

Is Mother's Own Milk Lactoferrin Intake Associated With Reduced Neonatal Sepsis, Necrotizing Enterocolitis, and Death?

OBJECTIVES → to determine the association of maternal LF intake and mother's own milk intake in the first 10 days of life on the prevention of late-onset sepsis (LOS), necrotizing enterocolitis (NEC), or death in the first 8 weeks of life in newborns with a birth weight <2,000 g.

METHODS → retrospective cohort study on 240 mother/infant pairs. Intakes of maternal milk, and content of LF in the maternal milk feeds, were measured.

RESULTS →

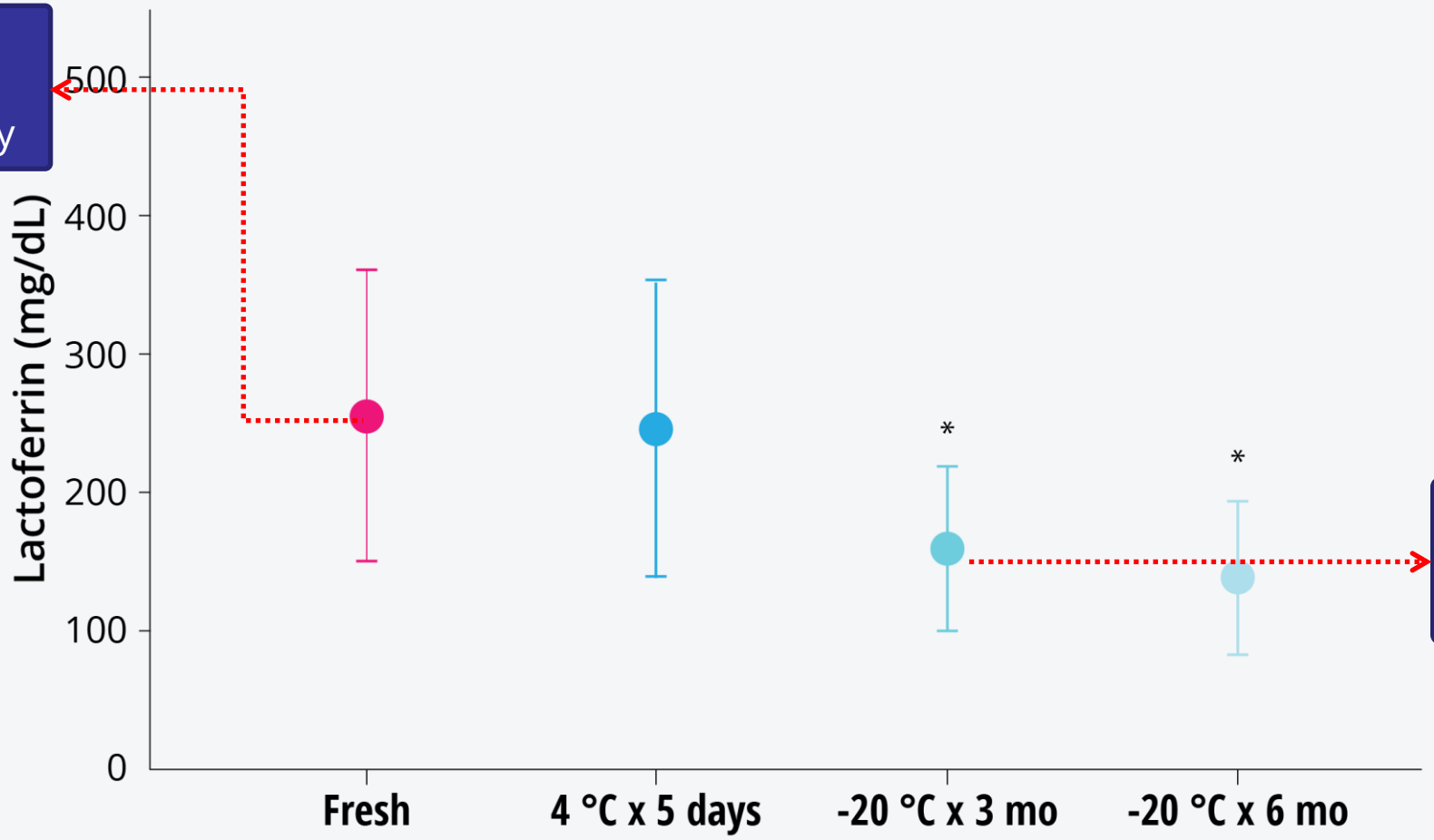
1. The average daily human LF intake over days 4–10 of life was 283 mg/kg/day
2. The adjusted hazard ratio (HR) of mother's own milk LF intake ≥100 mg/kg/day in days 4–10 for LOS, NEC, or death 0.752 (95% CI 0.301–1.877, $p = 0.541$)
3. The adjusted HR of mother's own milk cumulative intake (days 4–10) of 54–344 mL/kg (25–75 quartiles) for LOS, NEC, or death was 0.414 (95% CI 0.196–0.873, $p = 0.02$). **Infants who developed an event (LOS, NEC, or death) had significantly less median daily human LF intake than those that did not (89 vs 334 mg/kg/day, respectively, $p < 0.0001$).**

CONCLUSION → Consumption of higher amounts of mother's own milk in the first days of life is associated with less infection, NEC, and death.

Ochoa TJ, et al. *Neonatology*. 2020;1-8.

Stability of Lactoferrin in Stored Human Milk

250 mg/dL=
300–375 mg/kg/day
@120–150 mL/kg/day



150 mg/dL=
180–225 mg/kg/day
@120–150 mL/kg/day

Lactoferrin concentrations (mg/dL, mean ± SD) at described storage conditions (± 2 days). *P <0.001 compared with freshly expressed human milk. mo, months.

Adapted from Rollo DE, et al. *J Perinatol.* 2014;34:284-286.

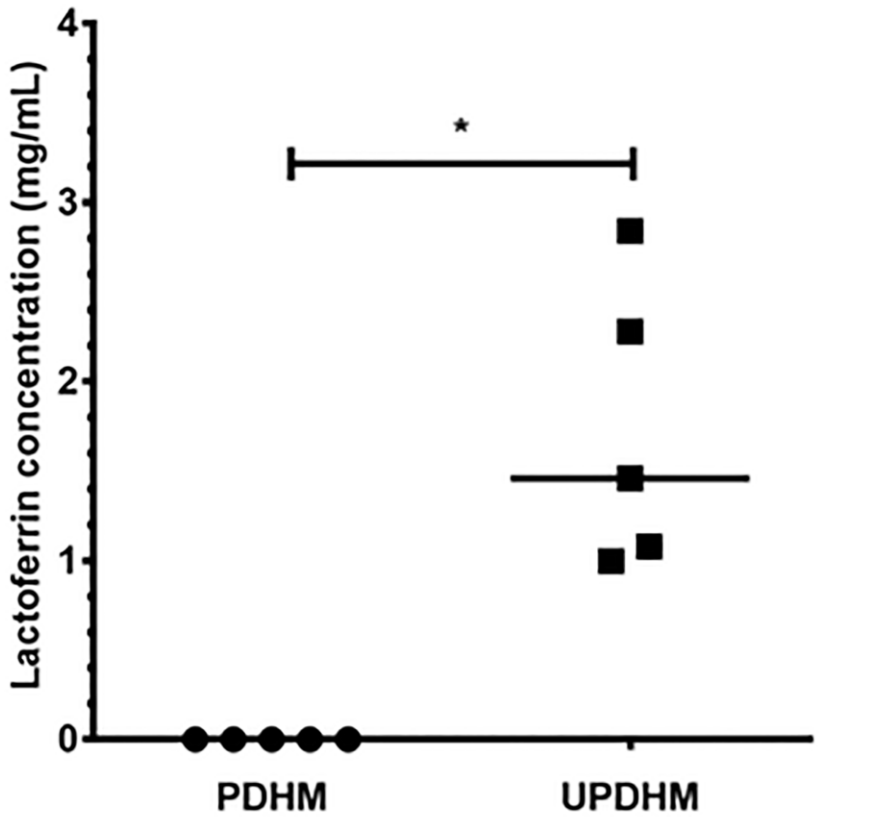
Detectable Lactoferrin Is Significantly Lower in Pasteurized vs Unpasteurized Donated Human Breast Milk Samples

PLOS ONE

RESEARCH ARTICLE

Effects of lactoferrin on neonatal pathogens and *Bifidobacterium breve* in human breast milk

Tabitha Woodman[1], Tobias Strunk[2,3], Sanjay Patole[2,3], Benjamin Hartmann[4], Karen Simmer[2,3], Andrew Currie[1,2]*



Detectable lactoferrin (Lf) in pasteurised (PDHM) and unpasteurised (UDHM) donated human breast milk samples. Data expressed as mg/mL of Lf. Where there were undetectable quantities of Lf, samples were given an arbitrary value of 7.8125×10^{-4} mg/mL Lf, which was the lowest point detected on the standard curve. Data show median (n = 5), $^{*}p < 0.05$ Wilcoxon matched-pairs signed rank test.

PDHM, pasteurized donor human milk; UPDHM, unpasteurized donor human milk.

Let Us Focus on the Figures…
LF Mean Daily Intakes and Neonatal Sepsis

Study	Infants WITHOUT sepsis	Infants WITH sepsis
Manzoni P, et al. 2019[1]	160-280	100
Trend S, et al. 2016[2]	298	131
Ochoa RJ, et al. 2020[3]	334	89

Lactoferrin intakes are expressed in mg/kg

1. Manzoni P, et al. *Am J Perinatal*. 2019;36:S120-S125.
2. Trend S, et al. *PLOS One*. 2015;10: e0117038.
3. Ochoa TJ, et al. *Neonatology*. 2020:1-8.

How Much Lactoferrin Do We Need? The Natural Model[a]

Table. Typical intake of hLF in a 1000g infant after starting trophic feeding with breast milk

Day	1–2	3–4	5–6	7–8	9–10	11–12	13–14	15–16
ml feed	0.5	1	2	3	4	5	6	8
No. of feeds	6–8	8–12	12	12	12	12	12	12
Mean daily volume of feeds	3–4	8–12	24	36	48	60	72	96
hLF concentration [mg/ml]	7	6.5	6	5.5	5	5	4.5	4
Presumed weight in grams[b]	1000	900	850	870	870	890	920	950
Mean daily hLF (mg/kg)	21–27	47–66	130	**172**	**209**	**267**	**298**	**365**

a. Patterns of mean daily human lactoferrin amounts for a 1000 g birth weight preterm infant in the first 2 weeks of life.
b. Assuming a typical weight loss of up to 15% in the first week.

hLF, human lactoferrin.

Dosing Summary: Ideal Ingestion of Lactoferrin

Studies suggest ideal scenario…

A newborn with exposure to fresh maternal milk since birth would ingest the following daily amounts of bioactive LF:

- At least 50 mg/kg at DoL 3
- At least 150 mg/kg at DoL 7
- Around 300 mg/kg at DoL 15 to 21

Please remember these figures!

DoL, days of life.

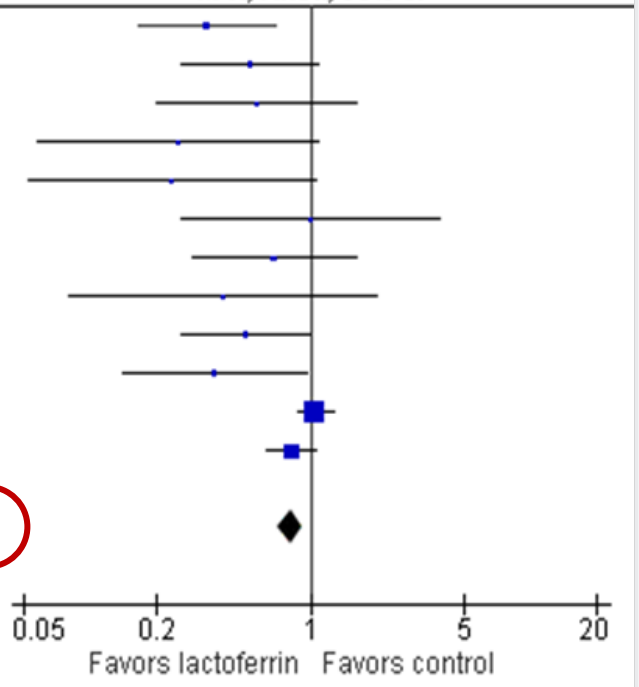
A Final Word: Prespecified Synthesis of Effects of Lactoferrin Supplementation in LIFT and 11 Other Trials (up to August 31, 2019), Assuming Fixed Effects in Each Trial.

Effect of lactoferrin supplementation vs control on late-onset sepsis

Study or Subgroup	Enteral lactoferrin Events	Total	Control Events	Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Manzoni 2009	9	153	29	168	6.7%	0.34 [0.17, 0.70]
Manzoni 2014	10	94	18	90	4.5%	0.53 [0.26, 1.09]
Akin 2014	4	22	8	25	1.8%	0.57 [0.20, 1.63]
Dai 2015	2	35	8	35	1.9%	0.25 [0.06, 1.09]
Kaur 2015	2	63	9	67	2.1%	0.24 [0.05, 1.05]
Ochoa 2015	4	95	4	95	1.0%	1.00 [0.26, 3.88]
Barrington 2016	7	40	10	39	2.5%	0.68 [0.29, 1.61]
Liu 2016	2	80	5	80	1.2%	0.40 [0.08, 2.00]
Sherman 2016	10	59	20	60	4.8%	0.51 [0.26, 0.99]
Tang 2017	5	82	15	90	3.5%	0.37 [0.14, 0.96]
ELFIN 2018	190	1093	180	1089	43.8%	1.05 [0.87, 1.27]
LIFT 2019	89	770	108	771	26.2%	0.83 [0.63, 1.07]
Total (95% CI)		**2586**		**2609**	**100.0%**	**0.81 [0.71, 0.93]**
Total events	334		414			

Heterogeneity: Chi² = 25.45, df = 11 (P = 0.008); I² = 57%

Test for overall effect: Z = 3.06 (P = 0.002)



Lactoferrin provides a 19% reduction in Late-Onset Sepsis

Tarnow-Mordi WO, et al. *Lancet Child Adolesc Health*. 2020. pii: S2352-4642(20)30093-6. [published online ahead of print May 12, 2020]

Speculation Rising From Current Data

- Lactoferrin (either human or bovine) supplementation appears to have clinically measurable benefits *only* when LF intakes from HM are below a certain threshold (see LIFT; Manzoni[1] et al. *JAMA.* 2009; Sherman[2] et al. *J Pediatr.* 2016.).
- Possible protective threshold levels of LF intake—according to the experimental data and to natural breastfeeding trends—could be comprised between 50–150 mg/kg at 7 DoL, and between 300–400 mg/kg at 21 DoL.
- When breastfeeding already provides these intakes, an external supplementation might not be needed, nor effective, nor confer additional advantages.
- However, in all situations where LF intake is not needed, (including processed HM and donor-banked HM), LF external supplememntation could be considered.

DoL, days of life; HM, human milk; LF, lactoferrin.

1. Manzoni P, et al. *JAMA*. 2009;302:1421-1428.
2. Sherman MP, et al. *J Pediatr.* 2016;175:68-73.e3.

Ongoing Issues With Supplemental Lactoferrin

- Quality control
- Correct intakes
- Optimal dosing regimens
- Types of LF: human or bovine, milk and formulas
- Interactions with probiotics
- Long-term outcomes

LF, lactoferrin.