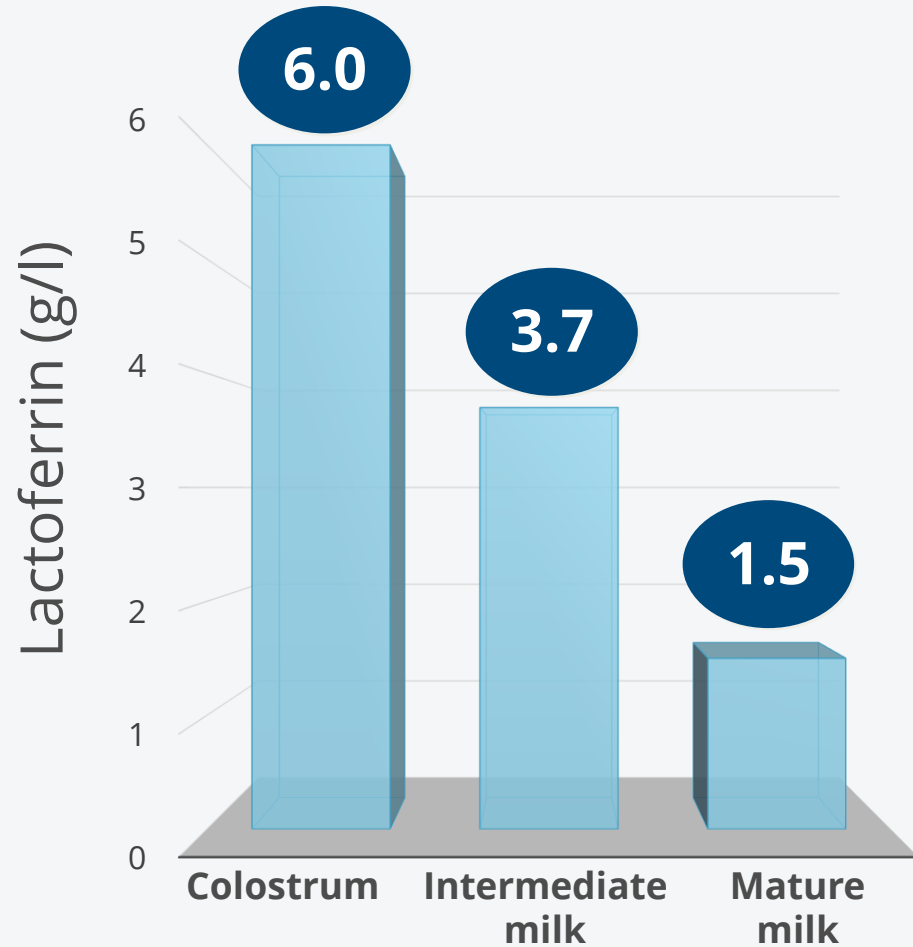


What is the ideal intake of lactoferrin to help reduce late-onset sepsis and necrotizing enterocolitis in preterm neonates?

Lactoferrin Concentration Decreases in Mature Human Milk vs Colostrum



This decrease typically occurs in all mammals.

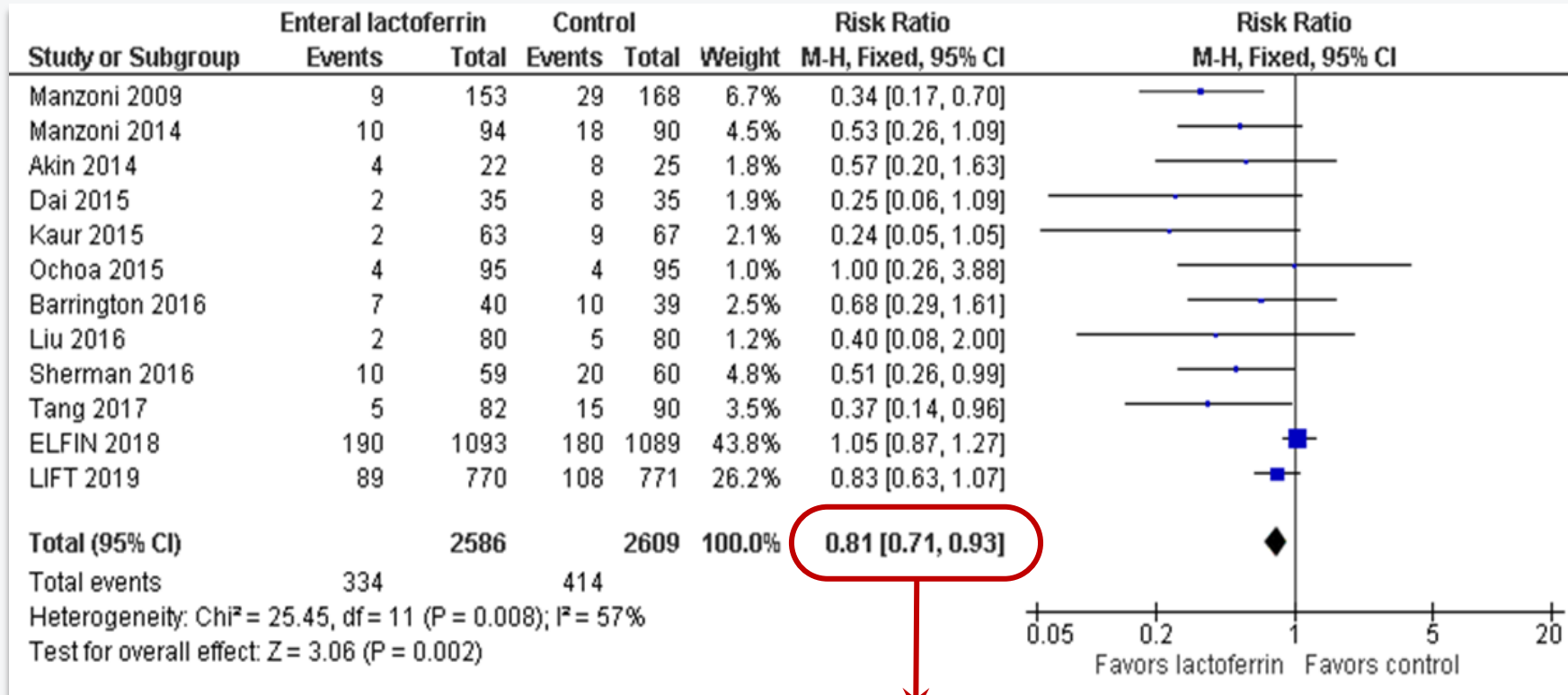
Milk Concentrations of lactoferrin

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



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



Lactoferrin provides a 19% reduction in late-onset sepsis



Inconsistencies Between the 3 Major RCTs of Lactoferrin Supplementation in Infants

	LIFT n=1541	ELFIN n=2199	Manzoni n=472	Ratio
Efficacy in prevention of sepsis	17% reduction	6% reduction	60% reduction	
Dose of lactoferrin	200 mg/kg day	150 mg/kg/day	100 mg/day fixed dose	
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;393:423-433; Manzoni P, et al. *JAMA*. 2009;302:1421-1428. Tarnow-Mordi W, et al. *Lancet Child Adolesc Health*. 2020



How to Give a Correct Interpretation of These Data? How to 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:
 - Trend et al (Australia) 2015
 - Ochoa et al (Peru) 2020
 - Manzoni et al (Italy-NZ) 2019
- Evidence from 2 lab studies:
 - Rollo et al (USA) 2014
 - Woodman et al (Australia) 2018



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

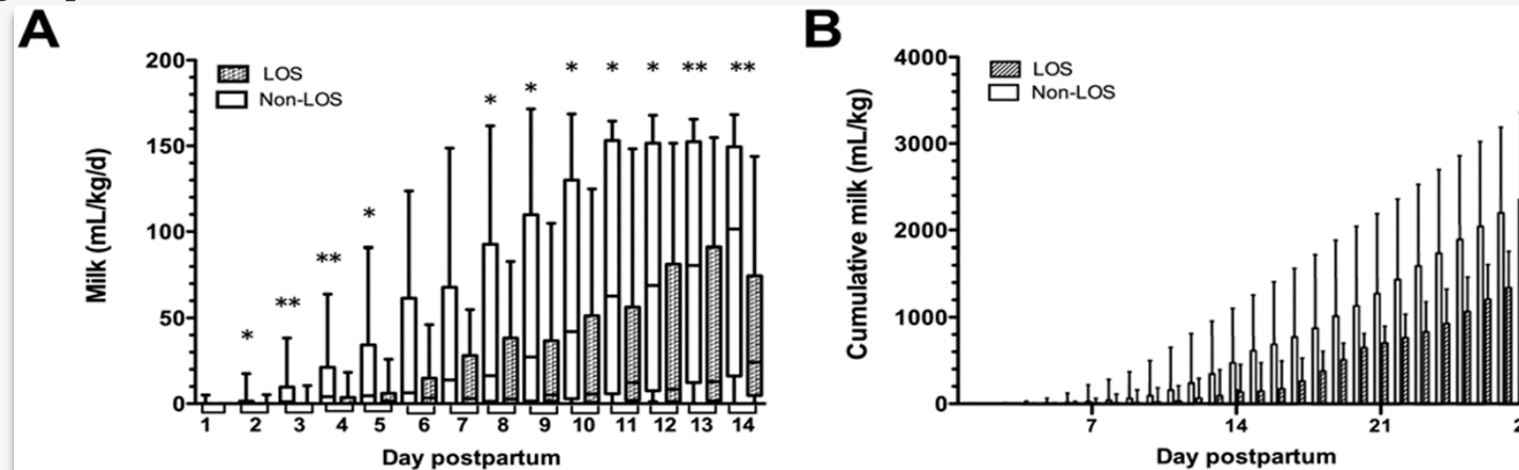
- Breast milk from mothers of preterm infants (32 wks GA) was collected on days 7 (n = 88) and 21 (n = 77) postpartum.
- Concentrations of **lactoferrin** were measured by ELISA
- Levels of LF and antibacterial activity in preterm breast milk were higher at day 7 than at day 21
- The range of total daily LF consumed by infants ranged from 0–794 mg/kg on days 7 and 21 postpartum
- **Lactoferrin limited pathogen growth >50% when added to formula at a concentration equivalent to that present in breast milk.**
- Levels of LF 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.

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



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 1999^[2])
- **A post-hoc analysis shows the effect of bLF was not significant in the HM-only subgroup ($p=0.15$ vs $p<0.001$)**
(Manzoni et al. *Am J Perinatol.* 2019)
- **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.



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

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Michele Quercia, MD¹⁵ Chryssoula Tzialla, MD¹² Nicola Laforgia, MD¹⁴ Fabio Mosca, MD^{7,16}
Rosario Magaldi, MD¹³ Michael Mostert, MD¹⁷ Daniele Farina, MD¹ William Tarnow-Mordi, MD¹⁸ 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.



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.



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]	220	100
Trend S, et al. 2015 ^[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.



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

Day of Life	Human milk intakes (ml/feed) and no. of feeds	Concentration of LF in colostrum and early human milk (mg/ml) ^[a]	Presumed weight in grams	Estimates of mean daily amounts of human lactoferrin ingested with feeds (mg/kg)
1	0.5 x 6	7	1,000	21
2	0.5 x 8	7	950	26.6
3	1 x 8	6.5	900	46.8
4	1 x 12	6.5	850	66.3
5	2 x 12	6	850	122.4
6	2 x 12	6	850	122.4
7	3 x 12	5.5	870	172.6
8	3 x 12	5.5	870	172.6
9	4 x 12	5.5	870	229.6
10	4 x 12	5	890	213.6
11	5 x 12	5	890	267
12	5 x 12	5	910	273
13	6 x 12	4.5	910	294.8
12	6 x 12	4.5	930	301.3
13	7 x 12	4.5	930	351.4
14	7 x 12	4	950	319.2
15	8 x 12	4	950	364.8
16	8 x 12	4	950	364.8

a. Patterns of mean daily human lactoferrin amounts for a 1000g birth weight preterm infant in the first 2 weeks of life.



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

 **Bovine Lactoferrin supplementation is advisable if intakes are below these figures!**

DoL, days of life.

