Emerging Developments in Human Milk Fortification:

Problem Solving for Clinical Practice

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Pediatric Nutrition
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Importance and Challenges of Optimal Nutrition for Preterm Infants

Challenges of Preterm Infant Nutrition

Developmental immaturity

Lack of provider confidence

Unknowns & limited standardization

Each of these challenges is compounded by limited NICU budgets and resources, competing demands of busy NICU providers, and potential lack of provider capacity or resources for establishing standardized nutrition practices.

Benefits of Optimal Feeding in Preterm Infants

Benefits of achieving recommended growth rates in preterm infants:

Potential reduced risk of late-onset sepsis, necrotizing enterocolitis, bronchopulmonary dysplasia, and retinopathy of prematurity[1],[2]

Improved short- and long-term body composition scores[3],[4]

Improved neurodevelopmental outcomes, which may persist into childhood and early adulthood[1]-[3]

Reduced rate of academic and behavioral school difficulties (ie, need for special educational accommodations, lower than average grades)[4]-[6]

[1]. Hellström A et al. *Acta Paediatr*. 2010;99(4):502-508. [2]. Isaacs EB et al. *J Pediatr*. 2009;155(2):229-234. [3]. Ramel S et al. *J Pediatr*. 2016;173:108-115. [4]. Kleinman RE, Greer FR, eds. *Pediatric Nutrition, 8th ed*. American Academy of Pediatrics; 2020. [5]. Guellec I et al. *J Pediatr*. 2016;175:93-99.e1. [6]. Sammallahti S et al. *J Pediatr*. 2014;165(6):1109-1115.e3.

2022 ESPGHAN Recommendations for Enteral Nutrient Intake for Preterm Infants

Nutrient	2022 ESPGHAN Guidelines[a] (per kg/day)
Fluid, mL	150–180 (135–200)
Energy, kcal	115–140 (–160)
Protein, g	3.5–4.0 (–4.5)
Carbohydrate, g	11–15 (–17)
Fat, g	4.8–8.1
Sodium, mg	69–115 (–184)
Potassium, mg	90–180
Chloride, mg	106–177 (–284)
Calcium, mg	120–200
Phosphorus, mg	68–115
Iron, mg	2–3 (–6)
Zinc, mg	2–3

a. Parentheses indicate ranges or upper intakes that may be needed for certain neonates.

- Data are limited regarding optimal intake for many macro- and micronutrients
- Recommendations are based on expert consensus
- Fluid and nutrient requirements vary with gestational age and birth weight
- Prior to increasing energy or protein beyond recommended intake for growth, optimize other nutrients and rule out alternate causes for suboptimal growth

Embleton ND et al. *J Pediatr Gastroenterol Nutr.* 2023;76(2):248-268.

2022 ESPGHAN Recommendations for Enteral Feeding in Preterm Infants

- **Start small-volume enteral feeds** (ie, minimal enteral feeding [MEF]) as early as possible and advance as clinically tolerated

- When advancing feeds in stable preterm infants, **increase volume by 18–30 mL/kg per day** (especially for those receiving breast milk)

- Establish a **standardized feeding protocol** for duration of MEFs, enteral feeding advancement, human milk fortification, and defining and managing gastric residuals, feeding intolerance, and full enteral feeds

Embleton ND et al. *J Pediatr Gastroenterol Nutr.* 2023;76(2):248-268.

2022 ESPGHAN Recommendations for Growth in Preterm Infants[1]

Defining Growth Abnormalities[2],[3]

- **Extrauterine growth restriction (EGR)**—growth restriction (<10th percentile of expected intrauterine growth) through 40 weeks' gestational age
- **Growth faltering (GF)**—difference in growth velocity through up to 1 year of life

Note: EGR has been defined in different ways in the literature. Current cutoffs for EGR, including percentile, are controversial and poorly predictive.[3]

- Growth can vary and is influenced by genetics, intrauterine environment, and morbidities[2]
- **Targets[1]:**
  - After initial weight loss of up to 7%–10% at days 3–4, regain birth weight by days 7–10
  - Then, growth along a target fetal growth centile and gradual transition to corresponding percentile on postnatal growth charts
  - Allow some catch-up growth in infants with GF but avoid very rapid catch-up growth

[1]. Embleton ND et al. *J Pediatr Gastroenterol Nutr.* 2023;76(2):248-268. [2]. Gounaris AK et al. *Nutrients*. 2023;15(14):3231. [3]. Bagga N et al. *Newborn (Clarksville).* 2023;2(3):198-202.

2021 ESPGHAN Position on Requirements for Critically Ill Neonates

- **No significant changes** to current guideline-recommended nutritional support
- Start or reduce nutritional support to the **lowest** amount necessary for basal metabolic and macronutrient needs during **early acute illness**
  - For most, parenteral nutrition (PN) will be necessary, but resume enteral nutrition as early as possible
- Assess critical illness phase **daily** to allow for nutrition adjustments
  - Full nutrient intake may not be reached until 5–10 days after acute illness onset
- Consider nutrition support in **upper range** of recommendations during **recovery phase**

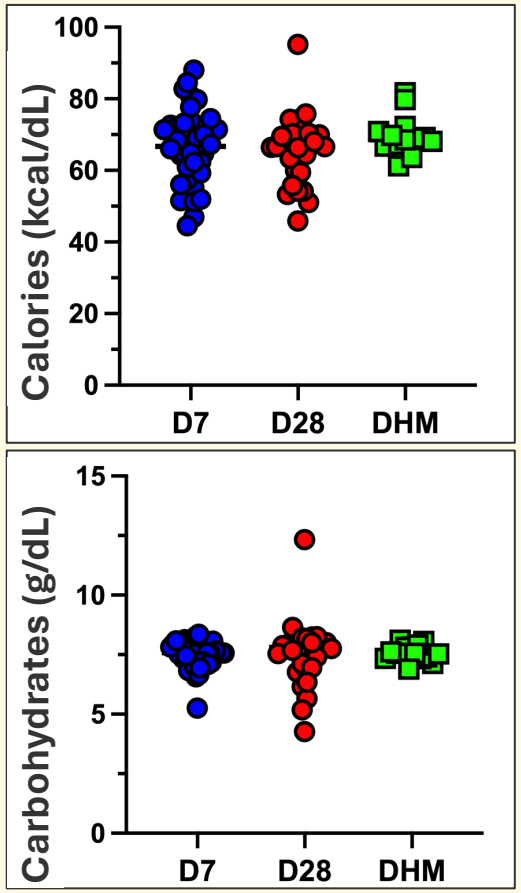
Moltu SJ et al. *J Pediatr Gastroenterol Nutr.* 2021;73(2):274-289.

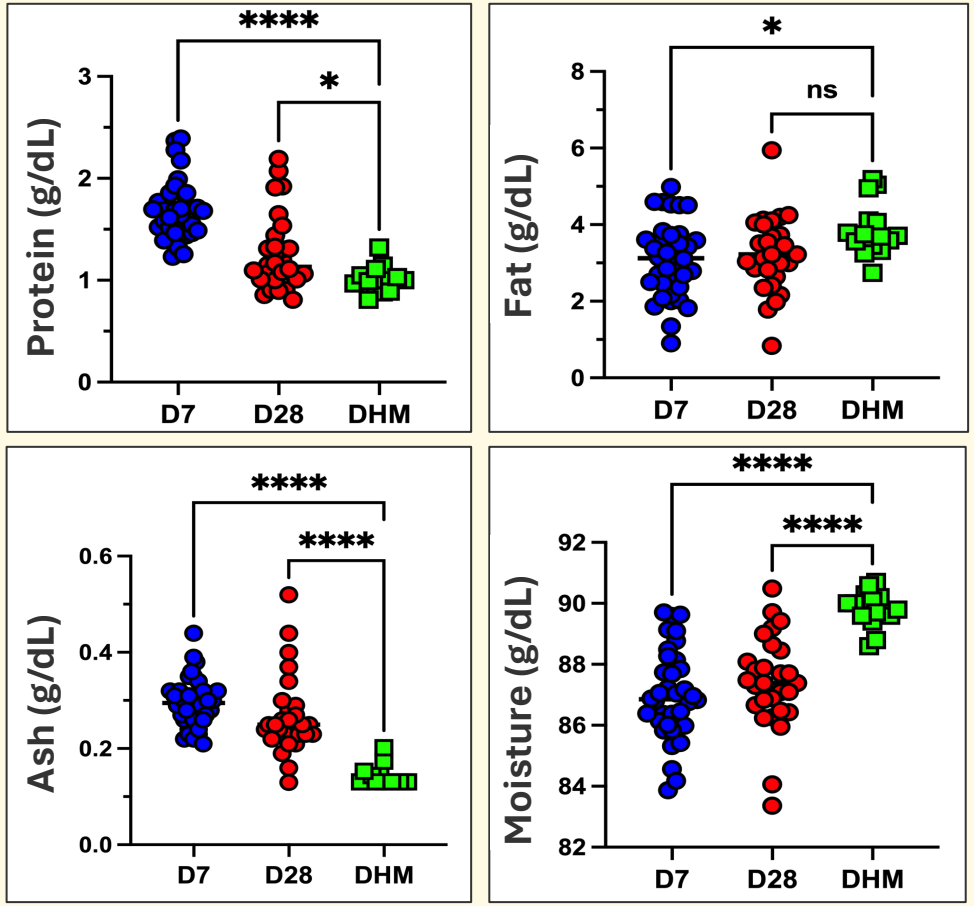
The Latest Evidence for the Role of Nutrition in Overcoming Developmental Immaturity

Donor Milk

Preterm Milk vs DM: Macronutrient Composition

No significant difference in **calorie** or **carbohydrate** content between preterm and donor milk





Differences for Preterm vs Donor Milk

Higher protein and **ash** content

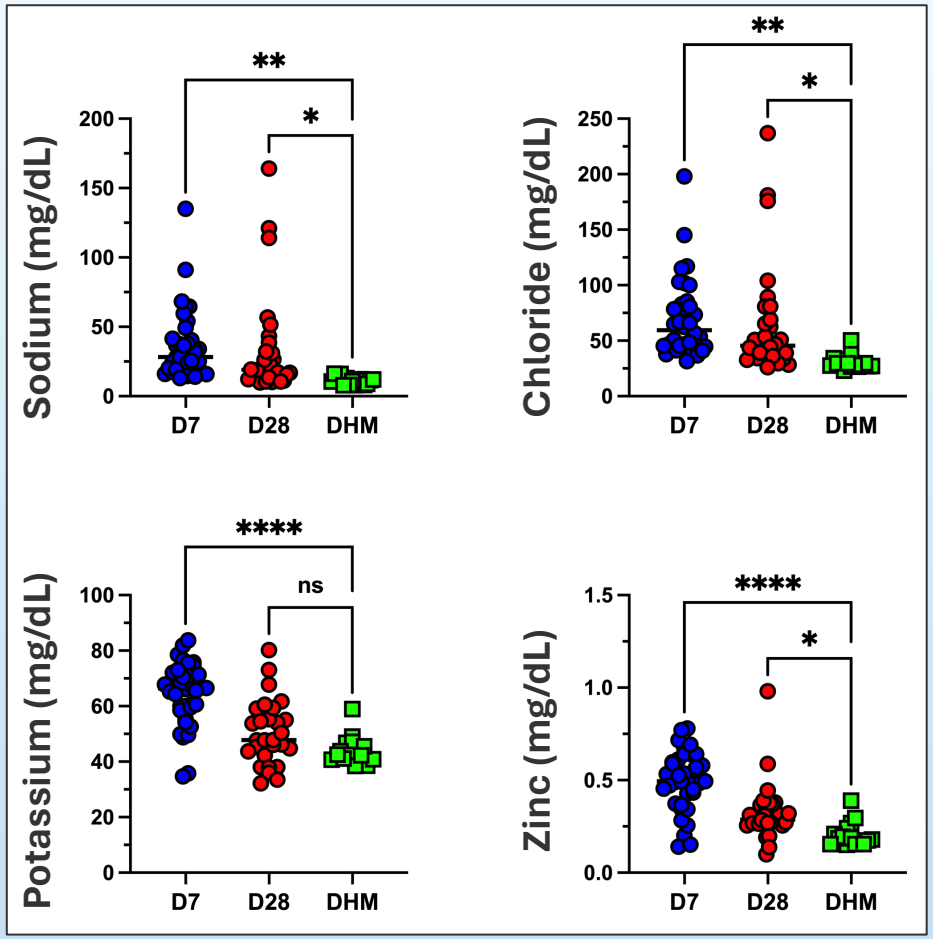
Lower moisture content

Lower fat content (day 7 only)

DM, donor milk.

*$P < .05$; **$P < .01$; ***$P < .001$; ****$P < .0001$

Preterm vs Donor Milk: Micronutrient Content



Differences for Preterm vs Donor Milk

Significantly higher levels of **sodium**, **chloride**, **potassium** (day 7 only), and **zinc**

*$P < .05$, **$P < .01$, ***$P < .001$, ****$P < .0001$.

Gates A et al. *J Nutr*. 2023;153(9):2622-2630.

Supplementation of MOM With DM vs Formula: MILK RCT Study Design

- **Objective:** Compare neurodevelopmental outcomes in preterm infants fed **DM + BMBF** with those in preterm infants fed **preterm infant formula**

- **Design:** Randomized 483 preterm infants from 14 NICHD Neonatal Research Network centers
  - Gestational age <29 weeks or birth weight <1000 g
  - Fed minimal maternal milk at time of enrollment
  - Other than randomization of base diet, followed site-specific feeding practices for initiation, fortification, and advancement; required fortified donor milk recipes to provide ~2.8–3.0 g/dL of protein

- **Primary outcome:** Bayley Scales of Infant Development (BSID) scores at 22–26 months' corrected age

NICHD, National Institute of Child Health and Human Development.

Supplementation of MOM With DM vs Formula: MILK RCT Neurodevelopmental Outcomes

	Donor milk	Preterm formula	Effect (95% CI)
Adjusted mean (SD) BSID score [n][a]			
Cognitive (primary)	80.7 (17.4) [206]	81.1 (16.7) [217]	-0.77 (-3.93 to 2.39)
Motor	80.3 (21.6) [202]	80.1 (19.9) [213]	-0.38 (-4.28 to -3.52)
Language	76.7 (19.6) [203]	75.8 (18.6) [212]	0.68 (-2.89 to 4.24)
Adjusted categorical BSID score, n (%)[a]			
Cognitive <85	95 (46)	106 (49)	0.96 (0.79–1.17)
Motor <85	90 (45)	102 (48)	0.96 (0.79–1.17)
Language <85	115 (57)	134 (63)	0.89 (0.77–1.04)
Moderate to severe NDI	87 (49)	96 (50)	0.99 (0.81–1.22)

NDI, neurodevelopmental impairment.
a. Deaths assigned lowest BSID-III score of 54

No significant difference in BSID-III scores at 22–26 months corrected age with DM vs formula in extremely preterm infants

Supplementation of MOM With DM vs Formula: MILK RCT Growth & Morbidity Outcomes

	Donor milk	Preterm formula	Adjusted between-group risk difference (95% CI)
Death before discharge, n (%)	24 (10)	18 (7.4)	NA
NEC, n (%)	10 (4.2)	22 (9.0)	-5% (-9% to -2%)
Late-onset sepsis, n (%)	47 (20)	37 (15)	5% (-1% to 11%)
Growth, mean (SD) change in *z* scores (Fenton) from randomization to end of study			
Weight	-0.43 (0.9)	-0.09 (0.9)	-0.35 (-0.50 to -0.20)
Length	-0.93 (1.12)	-0.77 (1.20)	-0.13 (-0.34 to 0.08)
Head circumference	0.39 (1.98)	0.44 (1.34)	-0.08 (-0.39 to 0.22)

NA, not available.

Although DM is protective against NEC, it may be associated with nutritional risk relative to preterm formula.

Outcomes With Preterm vs Term DM Supplementation

- Randomized trial comparing supplementation of MOM with term vs preterm DM for first 3 weeks of life in VLBW infants

- Compared with supplementation with term DM, preterm DM supplementation was associated with:
  - Greater protein intake
  - Higher *z* score for weight and head circumference at end of study period

Mean (SD) Protein Intake (g/kg/d)				
	Total (N = 120)	**MOM + Preterm DM (n = 43)**	**MOM + Term DM (n = 77)**	***P* value**
During hospitalization	3.03 (0.57)	3.20 (0.60)	2.93 (0.54)	.014
Donor milk period				
1st week	3.35 (1.05)	3.53 (1.10)	3.25 (1.01)	.023
2nd week	3.53 (1.13)	3.67 (1.05)	3.45 (1.17)	
3rd week	3.36 (0.99)	3.67 (0.96)	3.19 (0.97)	
At initiation of fortification	3.24 (0.84)	3.57 (0.82)	2.92 (0.85)	.006

MOM, mother's own milk; VLBW, very low birth weight

Considerations for Your Practice: Meeting Nutritional Needs and Minimizing Risk

- Target protein concentrations of 3.5–4.0 g/kg/d for both DM and MOM
- Use of DM requires mineral supplementation (eg, sodium, zinc)
- After optimizing volume, fortification, and mineral supplementation, monitor growth over the next 4–6 weeks
- If growth faltering persists, consider transitioning to preterm formula

Considerations for Your Practice: Discontinuing DM and Transitioning to Formula

- Limited evidence regarding the timing of DM discontinuation
- Considerations during decision making:
  - Availability of DM
  - Infant risk for NEC (eg, based on gestational age and postnatal age)

Average NEC Onset by Gestational Age[1]

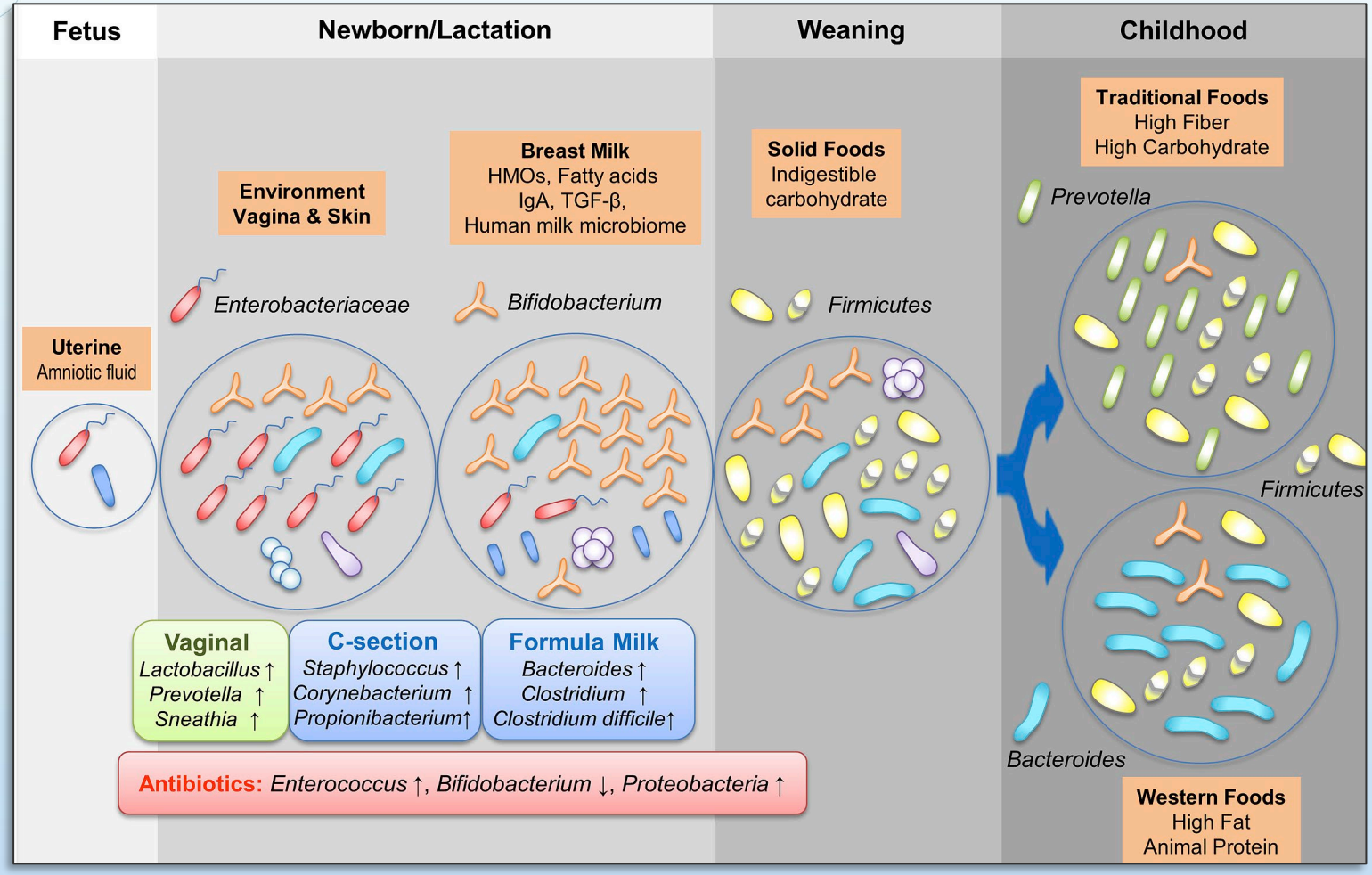
- For more **mature** preterm infants, the average age of NEC onset is **7 days**
- For preterm infants with **lower gestational age** or **smaller size**, the average age of NEC onset is **32 days**

[1]. Yee WH et al. *Pediatrics*. 2012;129(2):e298-e304.

The Latest Evidence for the Role of Nutrition in Overcoming Developmental Immaturity

The Preterm Microbiome

Microbiome Establishment and Development in Infancy

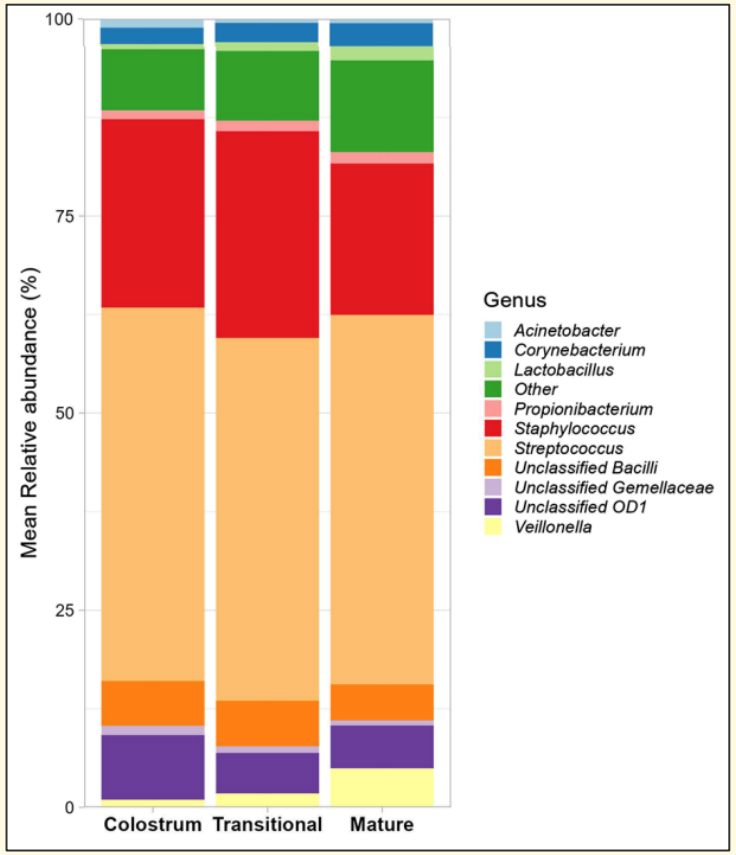


The microbiome is dynamic and increases in diversity through early childhood. This process is influenced by a variety of factors, including feeding practices.

The Breast Milk Microbiome by Lactation Stage and Gestational Age

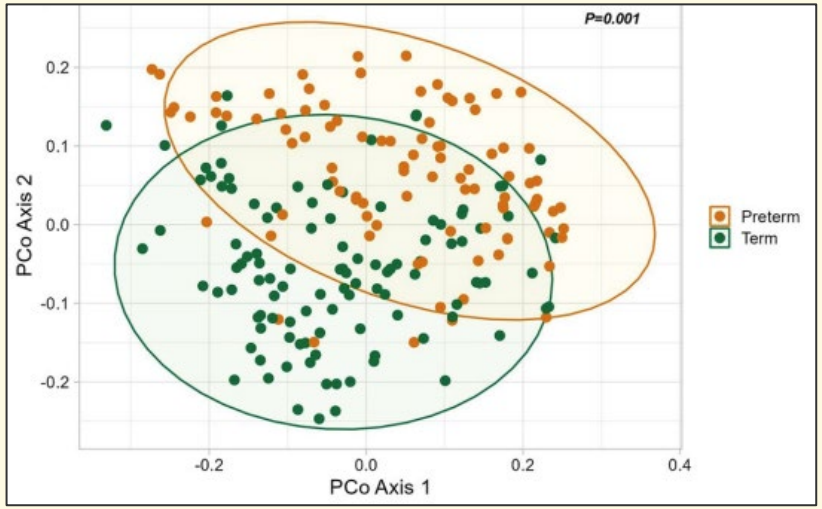
Relative abundance of top genera at 3 lactation stages

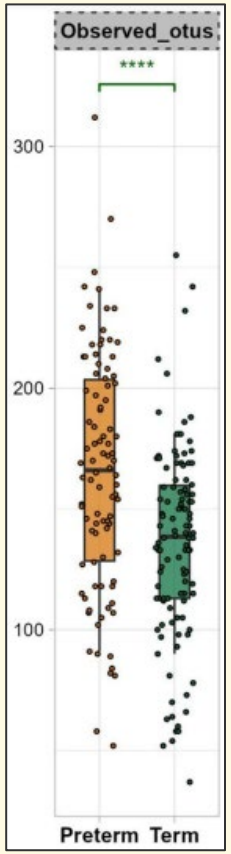
Lactobacillus and *Veillonella* become enriched in later stages of lactation



Bacterial diversity in preterm and term samples

Increased species richness in preterm breastmilk with distinct bacterial composition

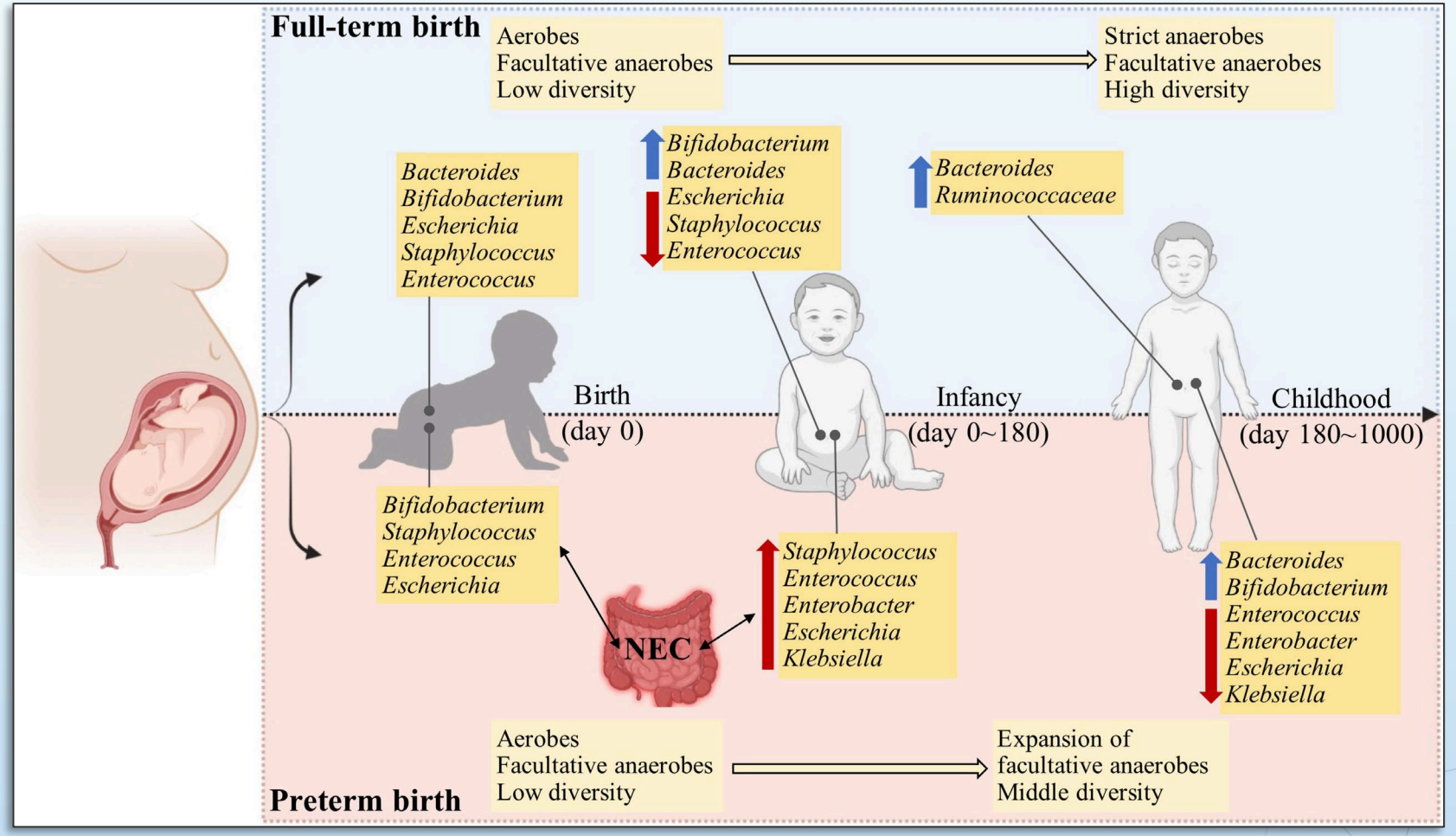




Preterm Infants Are at Risk for Dysbiosis & Less Bacterial Diversity

Microbiome of the Preterm Infant

- Higher seeding of skin- and hospital-associated microbes
- Less seeding of maternally derived microbes
- Delayed gut maturation and longer exposure to facultative anaerobes



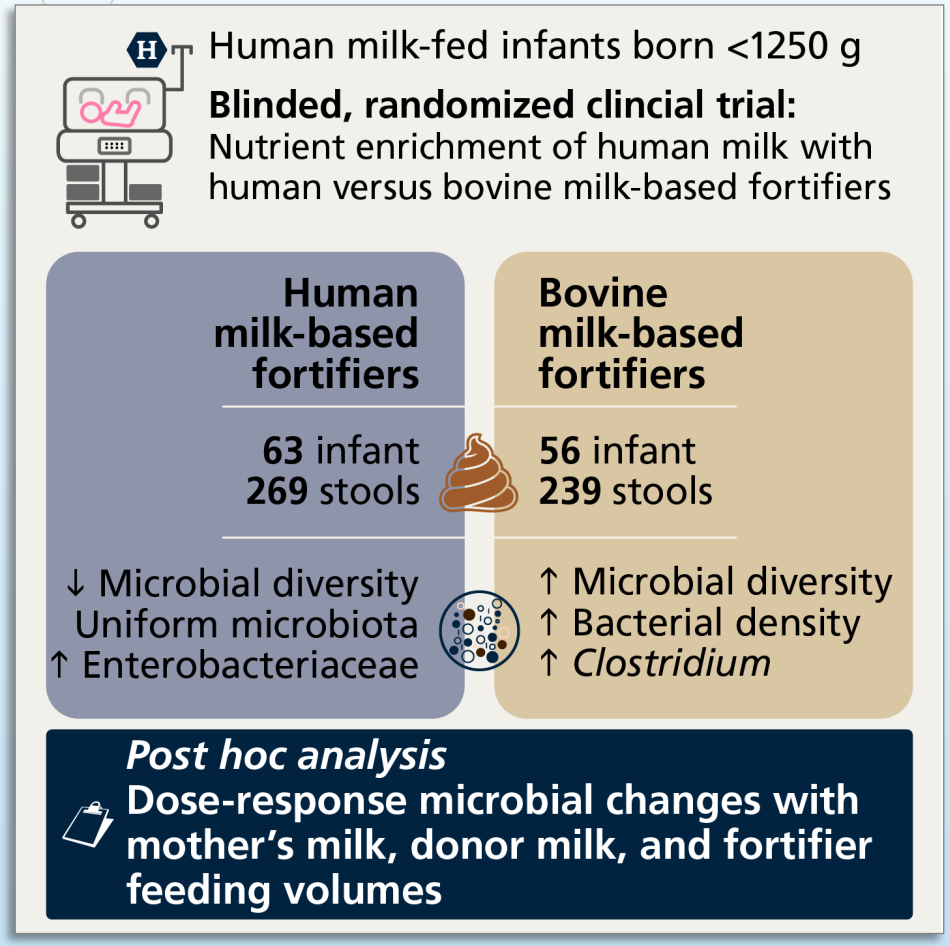
Effects of Dysbiosis in Preterm Infants

Data from observational studies indicate that dysbiosis may be associated with morbidity in preterm infants:

- Necrotizing enterocolitis[1],[2]
- Late-onset sepsis[2]
- Retinopathy of prematurity[2]
- Behavioral disorders (eg, attention-deficit hyperactivity disorder)[3]
- Neurodivergence (eg, autism spectrum disorder)[3],[4]

[1]. Pammi M et al. *Microbiome*. 2017;5(1):31. [2]. Westaway JAF et al. *Pediatr Res*. 2022;92(1):142-150. [3]. Bresesti I et al. *Cells*. 2022;11(3):379. [4]. Fujishiro S et al. *J Autism Dev Disord*. 2023;53(10):4012-4020.

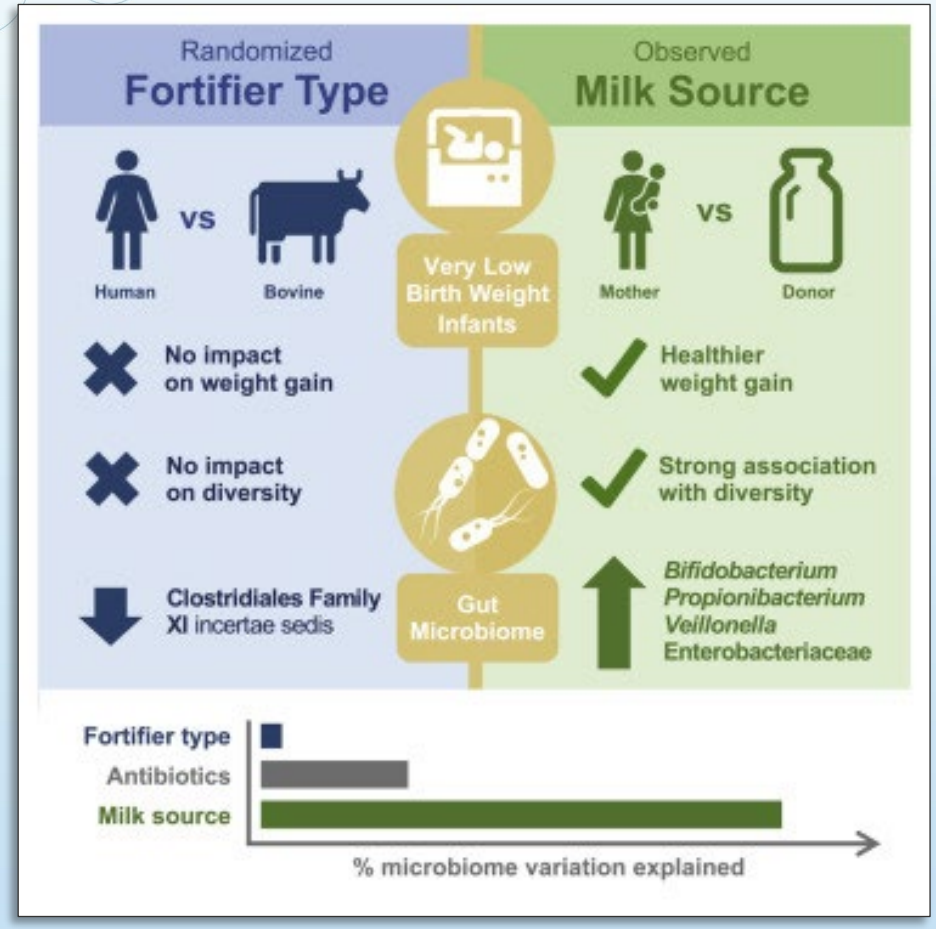
Fortifier Types and the Preterm Microbiome: a Post Hoc Analysis of the OptiMOM Trial



- Compared with BMBF, HMBF was associated with:
  - Less microbial diversity
  - Higher abundance of Proteobacteria and Enterobacteriaceae
  - Lower abundance of Firmicutes and *Clostridia*
  - More uniformity across samples
- Differences may be due to:
  - Less diversity of volatile compounds with HMBF, reducing viable substrates for microbes
  - Higher fat content with HMBF
  - Greater displacement with HMBF
  - Uniformity of HMBF due to pooling and pasteurization processes

Asbury MR et al. *Cell Host Microbe*. 2022;30(9):1328-1339.e5.

Fortifier Types and the Preterm Microbiome: RCT Comparing HMBF and BMBF



- Lower abundance of unclassified Clostridiales family species with HMBF
  - No other differences in microbiome diversity observed
- Compared with DM, MOM was associated with:
  - Greater abundance of *Bifidobacterium*, *Propionibacterium*, and *Veillonella*
  - Greater abundance of Enterobacteriaceae

Kumbhare SV et al. *Cell Rep Med.* 2022;3(9):100712.

RCT Comparing an Exclusive Human Milk Diet vs Bovine-Derived Formula and BMBF

- Randomized 126 preterm infants (<30 weeks gestation) to 2 groups through 34 weeks postmenstrual age:
  - DM for covering any shortfall in MOM and HMBF (intervention)
    OR
  - Bovine formula for covering any shortfall in MOM and BMBF (control)
- Compared with control, an exclusive human milk diet was associated with:
  - **No** difference in bacterial richness
  - **No** difference in bacterial diversity
  - **Reduced abundance** of *Lactobacillus*
- Findings suggest benefits of human milk-derived products are not due to microbiome-related mechanisms

Cohort Study: Prophylactic Probiotic Use in Preterm Infants and Rates of NEC in the NICU

Cohort Study of NICUs From 2012–2019

- Included 307,905 VLBW infants in 807 NICUs
- Defined probiotic adoption as treatment of ≥20% VLBW infants
- By 2019, 17% of NICUs were adopters (76.3% of infants at adopting NICUs received probiotics)

- **NEC declined by 18%** in NICUs adopting prophylactic probiotic use (relative to nonadopting NICUs) (*P* = .01)
- No significant change in the incidence of sepsis or in-hospital mortality

Agha L et al. *JAMA Health Forum*. 2023;4(5):e230960.

Effects of Probiotics vs Placebo on Clinical Outcomes in Preterm Infants: Meta-Analyses

Multistrain probiotics[a] reduced the mean number of days to reach full feeds by **3.3 days**[1]

Single-strain *B lactis* or *L reuteri* reduced hospital LOS by **7.9–13.0 days**[1]

Single-strain probiotics plus lactoferrin reduced the risk for sepsis by **67%**[2]

Multistrain probiotics[b] reduced the risk of severe NEC by **62%–65%**[1],[2]

Multistrain probiotics[c] reduced the risk of mortality by **31%–44%**[1],[2]

a. Combinations of ≥1 *Lactobacillus* spp, ≥1 *Bifidobacterium* spp, and *Saccharmoyces boulardii*
b. Combinations of ≥1 *Lactobacillus* spp and ≥1 *Bifidobacterium* spp, *Bifidobacterium animalis* subspecies *lactis*, *Lactobacillus reuteri*, or *Lactobacillus rhamnosus*[1] or combinations of 1 of any 16 probiotic strain (mostly containing ≥1 *Lactobacillus* spp and ≥1 *Bifidobacterium* spp)[2]
c. Combination of ≥1 *Lactobacillus* spp and ≥1 *Bifidobacterium* spp[1] or combinations of 1 of any 16 probiotic strain (mostly containing ≥1 *Lactobacillus* spp and ≥1 *Bifidobacterium* spp)[2]

[1]. Morgan RL et al. *Gastroenterology*. 2020;159(2):467-480. [2]. Wang Y et al. *JAMA Pediatr*. 2023;177(11):1158-1167.

Probiotic Use in Preterm Infants

- Probiotics can influence the composition and diversity of the preterm gut microbiome[1],[2]
- Effects of probiotics are specific to probiotic strains and vary by single-strain and multistrain preparations[1],[2]
- Commonly used probiotic strains for preterm infants include ***Lactobacillus rhamnosus* GG (LGG)** and ***Bifidobacterium animalis* ssp. lactis (BB12)**

[1]. Beck LC et al. *Nat Microbiol.* 2022;7(10):1525-1535. [2]. Athalye-Jape G et al. *BMJ Open Gastroenterol*. 2022;9(1):e000811. [3]. Mercer EM, Arrieta MC. *Gut Microbes*. 2023;15(1):2201160.

Safety Concerns With Probiotic Use in Preterm Infants

- In October 2023, the US Food & Drug Administration (FDA) issued a warning to providers about the risks of certain probiotics in preterm infants
- Warning came following death of a preterm infant who developed *Bifidobacterium infantis* sepsis after receiving probiotics containing this strain of live bacteria
- Probiotics are regulated as dietary supplements (not as drugs) and must be marketed accordingly
  - There are currently no FDA-approved probiotics for preterm infants

US FDA. FDA Raises Concerns About Probiotic Products Sold for Use in Hospitalized Preterm Infants. October 26, 2023. Accessed April 1, 2024. https://www.fda.gov/news-events/press-announcements/fda-raises-concerns-about-probiotic-products-sold-use-hospitalized-preterm-infants.

ESPGHAN Position and Considerations About FDA Warning

- **Benefit-risk assessment:** ongoing process following an intervention's adoption in the market that includes consideration of RCT data and postmarketing surveillance data
- No studies support an increased risk of sepsis with probiotic use
- Extensive support for potential benefits of prophylactic probiotics (potential reduction in severe NEC)

Evidence for the Potential Benefits of Probiotics

>55,000	preterm infants studied
>60	RCTs conducted
30	High-quality nonrandomized studies conducted

van den Akker CHP et al. *J Pediatr Gastroenterol Nutr*. Published online April 4, 2024. doi:10.1002/jpn3.12204

Current Recommendations for Probiotic Use in Preterm Infants

- Probiotic use in preterm infants is **not routinely** recommended by the American Academy of Pediatrics (AAP)[1]
- ESPGHAN **conditionally recommends** the use of LGG to reduce severe NEC **only** if the following safety recommendations are met[2]:
  - Confirmation from local microbiologists of **ability to detect invasive probiotic infection** with standard cultures
  - Use of probiotic products **manufactured following CGMP** to ensure strain identity and lack of contamination
  - Use of products confirmed by the manufacturer to be **devoid of strains with antibiotic resistance genes**
  - Provision of information about probiotic use to parents regarding the potential risks and benefits

CGMP, Current Good Manufacturing Practice.

[1]. Poindexter B et al. *Pediatrics*. 2021;147(6):e2021051485. [2]. van den Akker CHP et al. *J Pediatr Gastroenterol Nutr*. 2020;70(5):664-680.

Considerations for Your Practice: Probiotic Use in the NICU

- Probiotics inherently carry a risk of sepsis that is largely unrelated to product quality
- Maintain hygienic workflows to reduce the risk of contamination of IV catheters with probiotic microbiota
- Explain risks and benefits of probiotics to parents using simple language
- Follow ESPGHAN guidelines for probiotic use

van den Akker CHP et al. *J Pediatr Gastroenterol Nutr*. Published online April 4, 2024. doi:10.1002/jpn3.12204

The Latest Evidence for Overcoming Provider Confidence Challenges

Enteral Nutrition & Fortification Practices

Dilemmas in Enteral Nutrition & Human Milk Fortification: Feeding Volume & Fortification Timing

Decision 1	Decision 2	Decision 3	Decision 4
Base milk?	**Feeding volume?**	**Fortifier type?**	**Duration of fortification?**
• Mother's own milk vs donor milk • Protein content differences • Effects of processing on bioactive components • Heterogeneity by gestational age and lactational stage	• Early (<100 mL/kg/d) vs late (≥100 mL/kg/d) fortification • Challenges meeting nutritional requirements	• BMBF vs HMBF • Liquid vs powder • Addition of supplements? • Extent of research support • Cost differences	• Discharge vs term postmenstrual age vs beyond • Patient characteristics and clinical needs

Hair AB et al. *J Perinatol.* 2023;43(1):103-107.

Early Fortification in Extremely Preterm Infants: a Randomized Controlled Trial

- Randomized 150 preterm infants (<28 weeks' gestation) to receive:
  - Early fortification:
    - » MOM or DM with HMBF beginning on feeding day 2 (intervention)

    OR
  - Routine fortification:
    - » MOM or DM with BMBF added as per routine clinical care (control)
- The primary efficacy outcome of mean fat-free mass (FFM) *z* score was **not** significantly different between intervention and control groups (-1.7 vs -1.6; *P* = .67)
- Compared with standard feeding, early fortification was associated with:
  - **Greater length gain** velocities (0.9 vs 0.8 cm/wk; *P* = .04)
  - **Less pronounced declines** in head circumference *z* score (-0.9 vs -1.3; *P* = .01)

Salas AA et al. *Pediatrics*. 2023;152(3):e2023061603.

Early and Exclusive Enteral Nutrition in Preterm Infants: a Randomized Controlled Trial

- Randomized 102 preterm infants (28–32 weeks' gestation) to receive enteral nutrition with MOM or DM in the following volumes within the first 36 hours of life:
  - 60–80 mL/kg/d (early enteral nutrition)
    OR
  - 20–30 mL/kg/d (standard nutrition)

- Early enteral nutrition was associated with:
  - **More full enteral** feeding days (+2 days; *P* = .004)
  - **Increased FFM** *z* scores at day 14 (+0.5; *P* = .02)
  - **Increased length** *z* scores at discharge (+0.6; *P* = .002)
  - **Reduced mean costs** of hospitalization (-$28,754; *P* = .04)

Razzaghy J et al. *Arch Dis Child Fetal Neonatal Ed*. Published online December 22, 2023. doi:10.1136/archdischild-2023-325969

ENACTPlus Trial: Early, Exclusive Enteral Nutrition With Early vs Delayed HMBF

- Randomized 80 preterm infants (29–33 weeks' gestation) receiving early, exclusive enteral nutrition with MOM or DM to either:
  - Early HMBF (between day 4–7)
  - Delayed BMBF (between day 10–14)
- Evaluated growth outcomes and compared between groups

	Early fortification (n = 38)	Delayed fortification (n = 36)	*P* value
Age and milk intake, median (IQR)			
Postnatal age at time of fortification	6 (6–9) days	12 (11–14) days	<.0001
Postnatal age at outcome assessment	22 (21–26) days	22 (21–24) days	.79
Maternal milk intake during first 14 days	54% (29%–81%)	48% (14%–77%)	.34
Growth at outcome assessment, mean ± SD			
FFM-for-age *z* score	-1.8 ± 0.9	-1.9 ± 0.9	.56
Weight, g	1876 ± 263	1780 ± 298	.15
Length, cm	41.8 ± 1.7	40.9 ± 1.9	.04
Head circumference, cm	30.0 ± 1.4	29.8 ± 1.7	.49
Mid-upper arm circumference, cm	7.9 ± 0.7	7.8 ± 0.8	.69

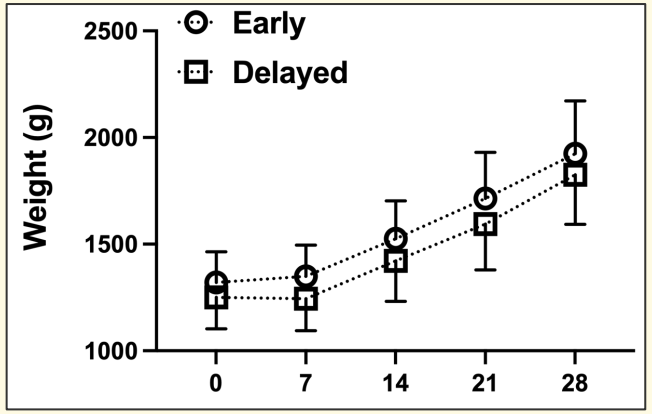
Salas A et al. Presented at: Pediatric Academic Societies Meeting. Abstract 0313. Toronto, CA: May 2-6, 2024.

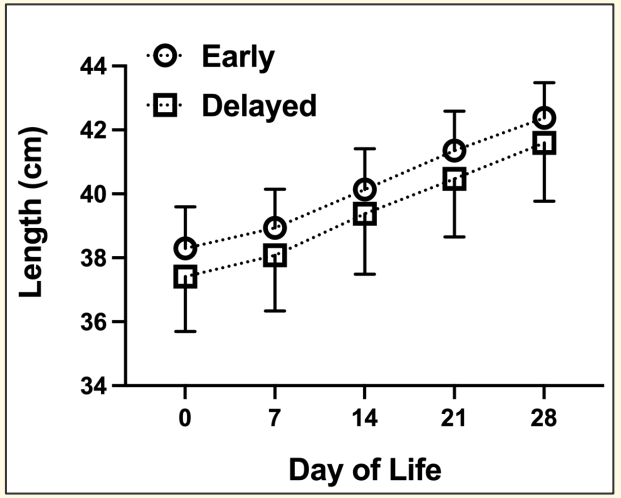
Randomized Controlled Trial: Growth With Early vs Delayed Fortification With BMBF

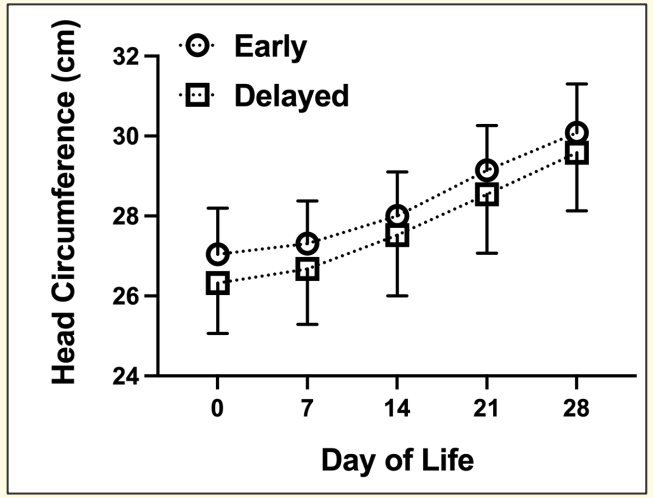
- Randomized 52 preterm infants to receive:
  - BMBF added on feeding day 1 (early) **OR**
  - BMBF added on feeding day 8 (delayed)
- Evaluated growth over the first 28 days of life and at 36 weeks

Growth by BMBF timing

Early BMBF is safe and may facilitate nutrient replacement and appropriate growth







Winter Z, Stansfield B. *J Perinatol*. Accepted April 2024.

Randomized Controlled Trial: Tolerability With Early vs Delayed Fortification With BMBF

	Early (n = 26)	Late (n = 26)
Days of life HMF added	1 ± 0.6	9 ± 2.0
HMF >24 kcal/ounce	8 (31)	11 (42)
Total parenteral nutrition (d)	12 ± 6	11 ± 3
Stool (# per day)	2.6 ± 0.8	2.7 ± 0.87
Emesis (mL)	1.9 ± 2.5	1.5 ± 2.3
No emesis	14 (54)	16 (62)
Feeding-related NPO occurrence	7 (27)	6 (23)

No major tolerability differences with early vs late fortification

Winter Z, Stansfield B. *J Perinatol*. Accepted April 2024.

Considerations for Your Practice: Timing of Feeding & Fortification

- “Aggressive” feeding advancements with either DM or MOM do not increase NEC risk and may reduce sepsis risk
- Evidence suggests that stable infants may experience growth benefits with early BMBF or HMBF fortification
- Uncertainty remains regarding whether both strategies can work together

The Latest Evidence for Overcoming the Unknowns

Fortification Types & Strategies: Fortifiers

Dilemmas in Enteral Nutrition & Human Milk Fortification: Decision Points for Clinicians

Decision 1	Decision 2	Decision 3	Decision 4
Base milk?	**Feeding volume?**	**Fortifier type?**	**Duration of fortification?**
• Mother's own milk vs donor milk • Protein content differences • Effects of processing on bioactive components • Heterogeneity by gestational age and lactational stage	• Early (<100 mL/kg/d) vs late (≥100 mL/kg/d) fortification • Challenges meeting nutritional requirements	• BMBF vs HMBF • Liquid vs powder • Addition of supplements? • Extent of research support • Cost differences	• Discharge vs term postmenstrual age vs beyond • Patient characteristics and clinical needs

Hair AB et al. *J Perinatol.* 2023;43(1):103-107.

Effects of BMBF vs HMBF on Feeding Intolerance and Neurodevelopment: a Randomized Controlled Trial

- Multicenter, triple-blind RCT
- Enrolled 232 VLBW (<1250 g) infants fed mother's milk supplemented with donor milk, as necessary
- Compared BMBF with HMBF

Signs of Feeding Intolerance During Intervention[1]

	HMBF (n = 64)	BMBF (n = 61)	Adjusted *P* value
Feeding interruption (primary)	27%	33%	.45
Parental nutrition restarted	5%	2%	.33
Feedings withheld for 24 h not due to clinical procedure/breastfeeding	11%	16%	.37
Gastric residuals	41%	41%	.97
Abdominal distension	80%	85%	.41

Neurodevelopmental Composite Scores at 18 Months[2]

Mean score	HMBF	BMBF	Adjusted *P* value
Cognitive	95	96	.67
Language	92	93	.85
Motor	96	98	.43

VLBW, very low birth weight.

[1]. O'Connor DL et. al. *Am J Clin Nutr*. 2018;108(1):108-116. [2]. Hopperton KE et al. *Curr Dev Nutr*. 2019;3(12):nzz129.

Effects of BMBF vs HMBF on Severe Morbidity and Mortality: a Randomized Controlled Trial

- Randomized 229 extremely preterm infants (22–28 weeks' gestation) fed MOM or DM to receive either HMBF or BMBF
- Evaluated NEC and sepsis by blinded review
- **No** differences in outcomes

Morbidity and Mortality From Inclusion to Discharge			
	HMBF (n = 115)	**BMBF (n = 113)**	***P* value**
Composite of NEC, culture-proven sepsis, and mortality (primary)	41 (35.7%)	39 (34.5%)	.86
NEC II-III	8 (7.0%)	9 (8.0%)	.77
Death	7 (6.1%)	13 (11.5%)	.15
Culture-proven sepsis	33 (28.7%)	28 (24.8%)	.50
Bronchopulmonary dysplasia	60/108 (55.6%)	66/102 (64.7%)	.18
Retinopathy of prematurity	50/113 (44.2%)	47/110 (42.7%)	.82

Exclusive Human vs Bovine-Based Diet in Very Preterm Infants: a Randomized Controlled Trial

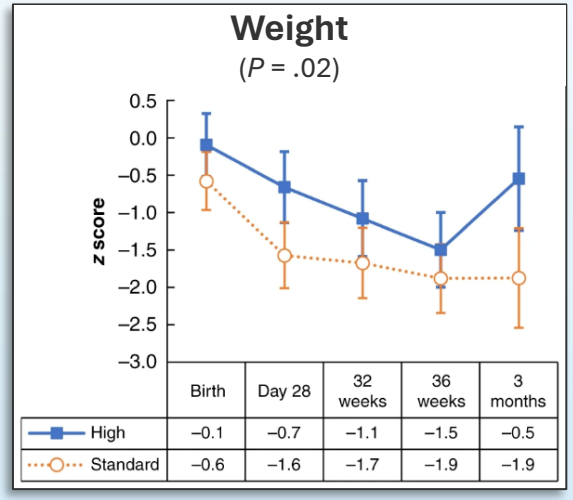
- Randomized 38 preterm infants (<30 weeks' gestation) receiving MOM to 2 groups to cover shortfalls in MOM:
  - Human milk-derived preterm formula plus HMBF
  - Cow's milk-derived preterm formula plus BMBF

- **No significant differences** between groups in the primary outcomes of adipose tissue mass or FFM at term

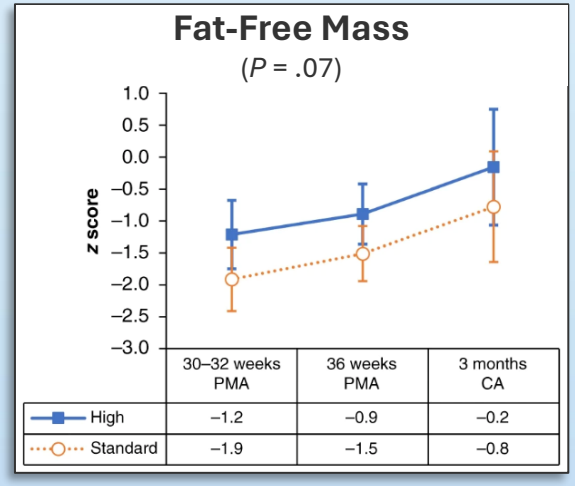
Uthaya S et al. *Early Hum Dev*. 2022;171:105619.

Addition of Protein Supplement to HMBF in Extremely Preterm Infants: a Randomized Controlled Trial

- Randomized 56 preterm infants (25–28 weeks' gestation) receiving MOM or DM to:
  - HMBF plus fixed protein supplementation (high) **OR**
  - HMBF without protein supplementation (standard)
- Intervention increased protein intake and protein:energy ratio through 36 weeks



	Birth	Day 28	32 weeks	36 weeks	3 months
High	−0.1	−0.7	−1.1	−1.5	−0.5
Standard	−0.6	−1.6	−1.7	−1.9	−1.9



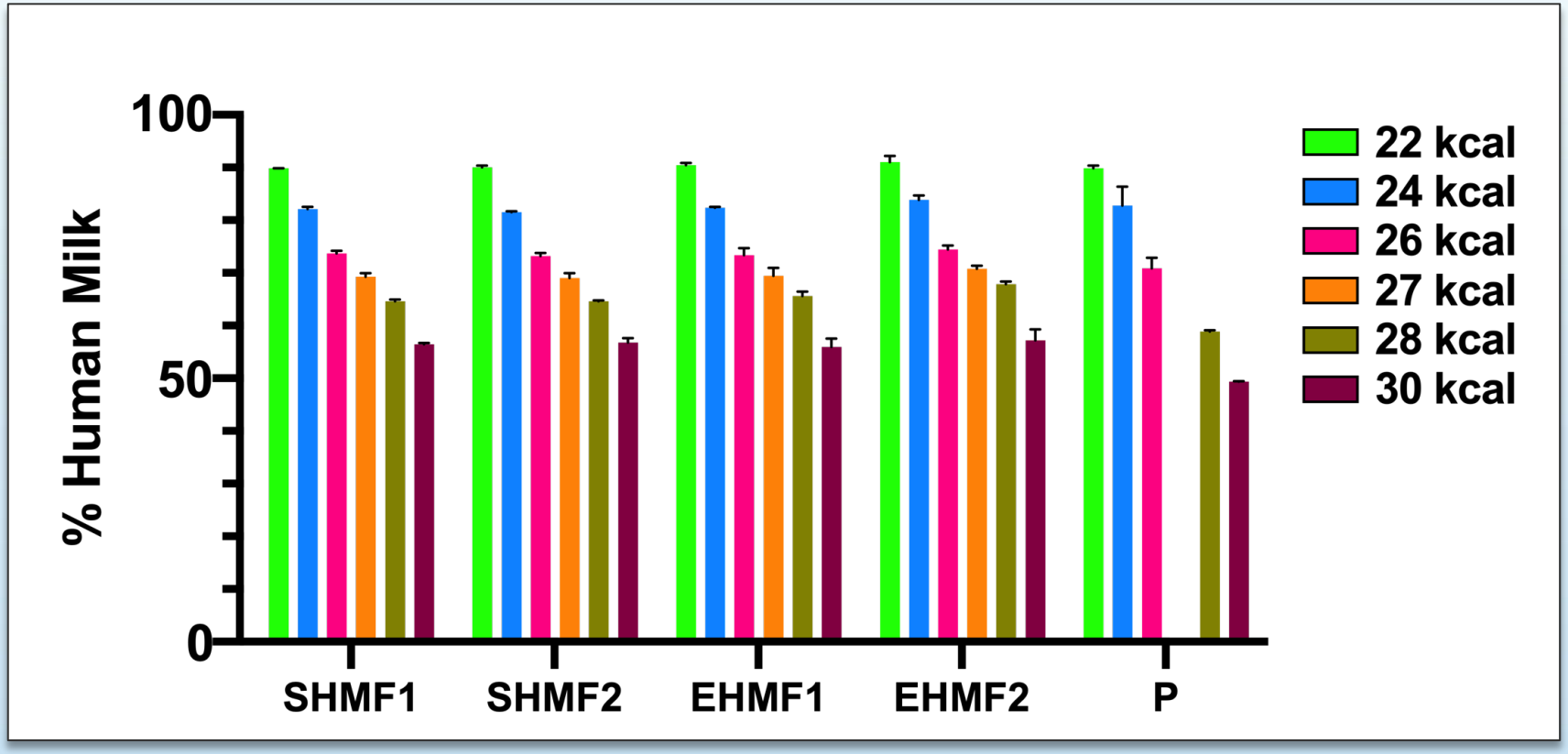
	30–32 weeks PMA	36 weeks PMA	3 months CA
High	−1.2	−0.9	−0.2
Standard	−1.9	−1.5	−0.8

Salas AA et al. *Pediatr Res.* 2022;91(5):1231-1237.

Considerations for Your Practice: BMBF and HMBF

- No differences in short-term morbidity and mortality with BMBF vs HMBF
- No differences in body composition and neurodevelopmental outcomes with BMBF vs HMBF
- Protein supplementation of HMBF may improve protein intake and protein:energy ratio

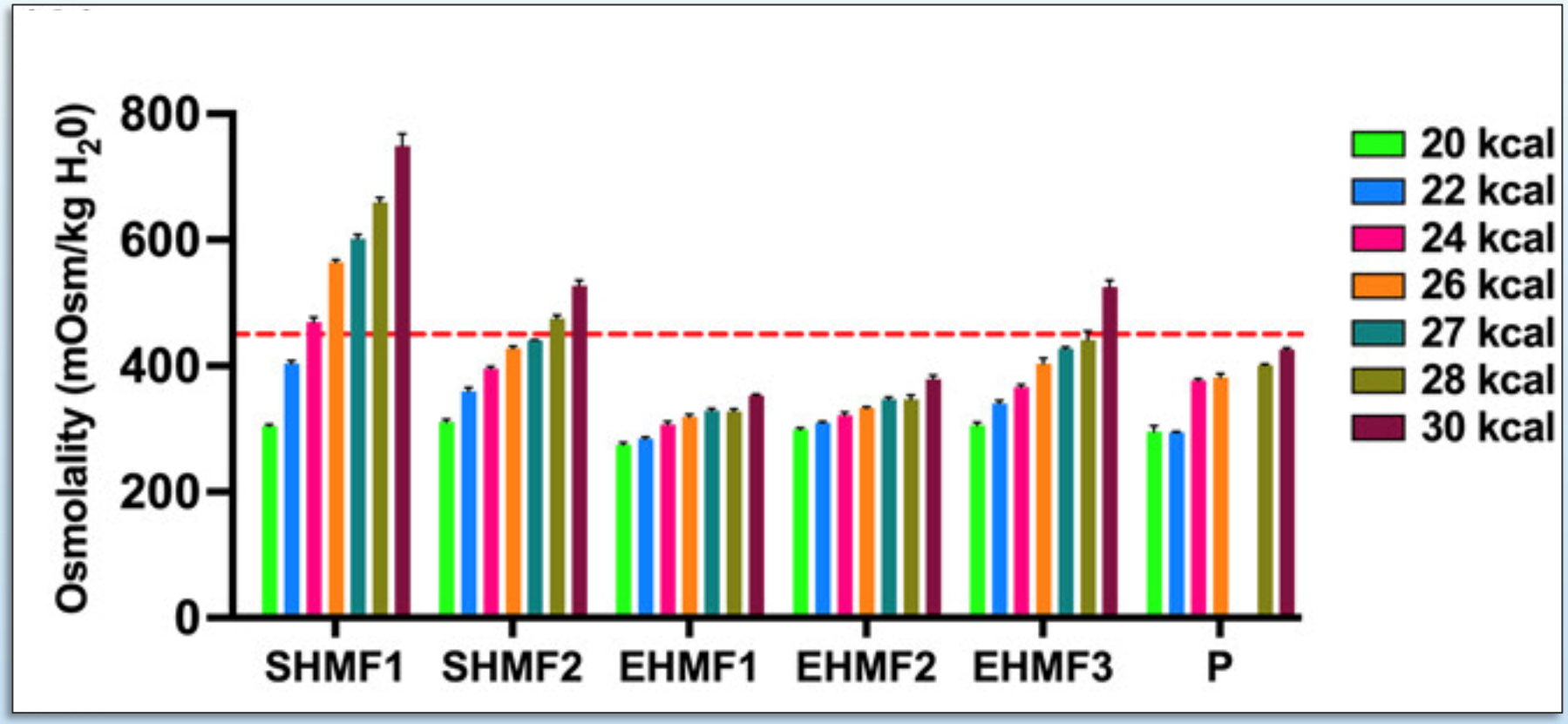
The Balancing Act of Fortifying Human Milk: Displacement by Fortifier Type & Target Caloric Density



SHMF1, Similac® Human Milk Fortifier Extensively Hydrolyzed Liquid; SHMF2 , Similac® Human Milk Fortifier Concentrated Liquid; EHMF1 , Enfamil® Liquid Human Milk Fortifier Standard Protein; EHMF2 ,Enfamil® Liquid Human Milk Fortifier High Protein; P, Prolact CR; Prolact +4, +6, +8, +10 H^2MF.

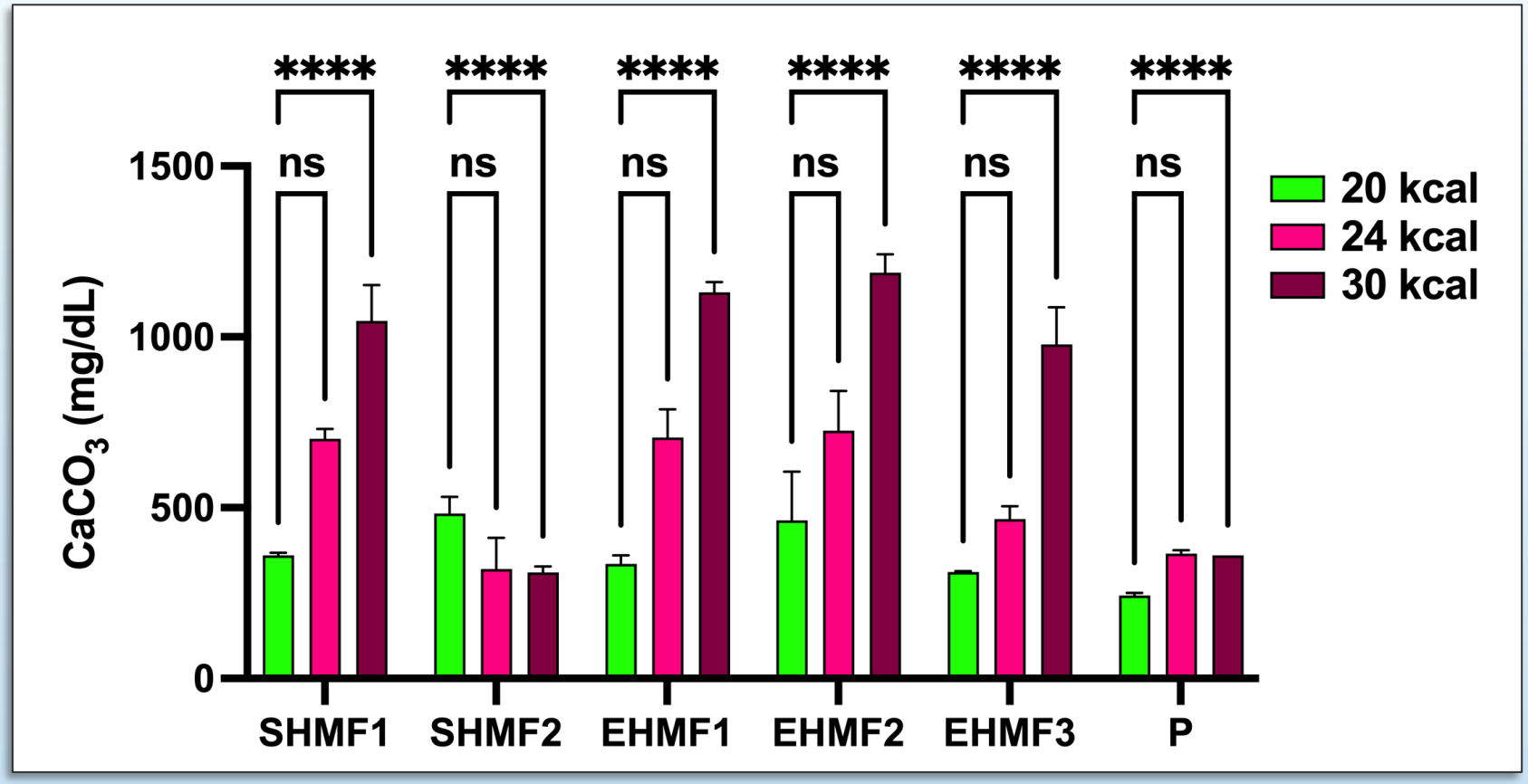
Bingham R et al. *JPEN J Parenter Enteral Nutr*. 2023;47(8):1062-1066.

The Balancing Act of Fortifying Human Milk: Osmolality by Fortifier Type & Target Caloric Density



SHMF1, Similac® Human Milk Fortifier Extensively Hydrolyzed Liquid; SHMF2 , Similac® Human Milk Fortifier Concentrated Liquid; EHMF1 , Enfamil® Liquid Human Milk Fortifier Standard Protein; EHMF2 ,Enfamil® Liquid Human Milk Fortifier High Protein; EHMF3, Enfamil® Human Milk Fortifier Powder, P, Prolact CR; Prolact +4, +6, +8, +10 H^2MF.

The Balancing Act of Fortifying Human Milk: Acid-Base Balance by Fortifier Type & Target Caloric Density



SHMF1, Similac® Human Milk Fortifier Extensively Hydrolyzed Liquid; SHMF2 , Similac® Human Milk Fortifier Concentrated Liquid; EHMF1 , Enfamil® Liquid Human Milk Fortifier Standard Protein; EHMF2 ,Enfamil® Liquid Human Milk Fortifier High Protein; EHMF3, Enfamil® Human Milk Fortifier Powder, P, Prolact CR; Prolact +4, +6, +8, +10 H^2MF.
****$P < .001$ for each caloric density compared with all other caloric densities for each brand.

Pineda D et al. *JPEN J Parenter Enteral Nutr*. 2024;48(1):57-63.

Considerations for Your Practice: Fortification Strategies

Start by considering what base milk is being fortified

- If MOM, displacement should be major driver for fortifier selection
- Acidosis and osmolality have less impact on fortifier selection

The Latest Evidence for Overcoming the Unknowns

Fortification Types & Strategies: Feeding Protocols

Effect of Individualizing Nutrition on Neurodevelopment: a Randomized Controlled Trial

- Randomized 114 preterm infants to receive fortification adjustments based on either[1]:
  - Individualized measurements of macronutrients in MOM (individualized nutrition)
  - Measurements of infant growth and serum nutrients (optimized nutrition)

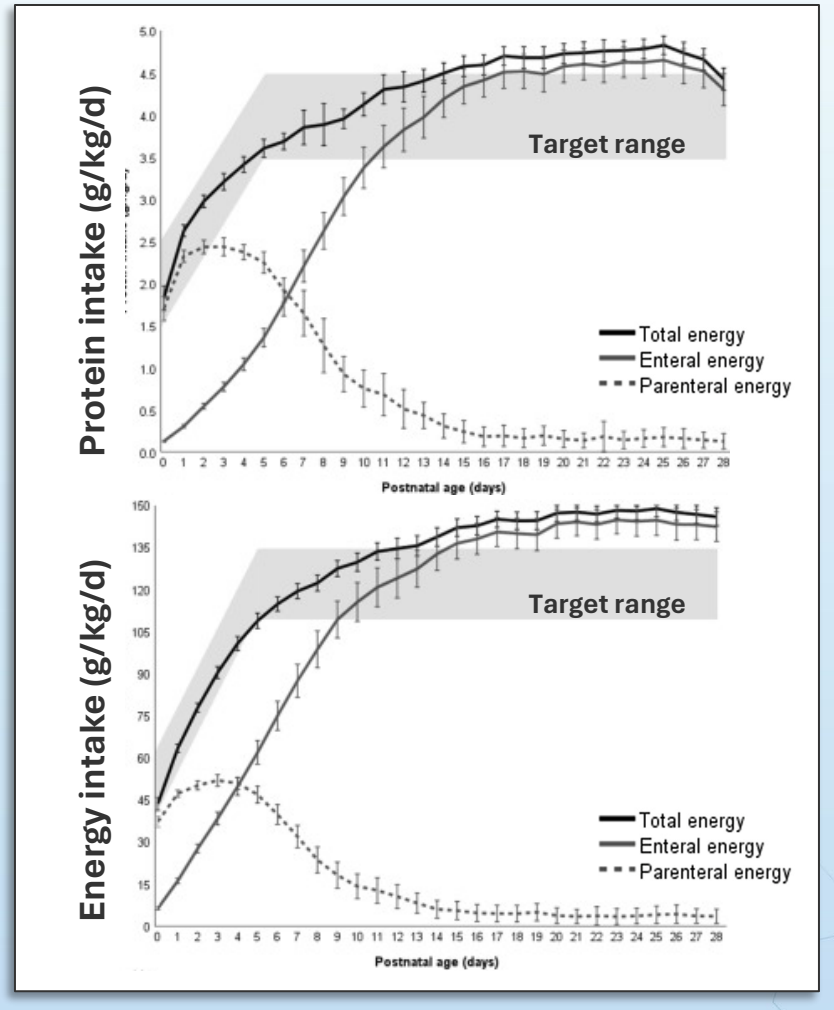
- Feeding adjustment strategy had **no** effect on Bayley scores at ages 18 to 38 months (n = 91/114 measurements)[1]

- At ages 33–48 months, the individualized nutrition group had **higher** rates of central obesity but similar renal function and blood pressure[2]

[1]. Reis JD et al. *J Perinatol*. 2023;43(1):81-85. [2]. Reis JD et al. *J Perinatol*. 2024;44(1):78-86.

Standardized Feeding Protocols to Improve Nutritional Adequacy & Preterm Infant Growth During Transition

- Secondary analysis of the **120 preterm infants** in the ImNuT RCT
- **Standardized feeding protocol:**
  - Combination of PN and human milk beginning at birth
  - Milk advanced in 12–18 mL/kg/d as tolerated
  - EN administered by GI tube
  - Fortifier added at 100–115 mL/kg/d
  - Fortification based on estimated milk composition
- Associated with **near-target nutrient intake** and **growth**



Rossholt et al. *Clin Nutr ESPEN*. 2023;53:251-259.

Postdischarge Fortification of MOM: Follow-up Study of Randomized Trial

- Compared long-term neurodevelopmental outcomes of preterm infants randomized to either fortified or unfortified MOM (n = 141) and infants fed preterm formula (n = 73)
  - Feeding strategy began shortly before discharge and continued to 4 months corrected age

- At 6 years' corrected age, **no** difference in IQ test results with vs without postdischarge fortification among infants fed MOM
  - However, infants fed MOM had significantly better verbal comprehension and motor development scores

Klamer A et al. *Nutrients*. 2022;14(13):2709.

Considerations for Your Practice: Feeding Protocols

- Advocate for standardized feeding protocols at your institutions
  - Feeding protocols adjusted based on infant growth and serum nutrient concentrations are ideal

- Balance potential benefits of postdischarge fortification with costs and burden to families

Considerations for Your Practice: Use of Supplements

- Sodium supplementation is rational after 10–14 days of life
- Zinc supplementation may also be important

In meta-analyses of randomized controlled trials evaluating zinc supplementation in preterm infants, zinc supplementation was associated with:

- Improved weight gain and linear growth[1]
- Better motor development scores [1]
- Moderately decreased mortality [2]

[1]. Alshaikh B et al. *J Perinatol*. 2022;42(4):430-439. [2]. Staub E et al. *Cochrane Database Syst Rev*. 2021;3(3):CD012797.

Emerging Solutions in Preterm Nutrition

Overview of Bioactives

Bioactives are defined as “essential and nonessential compounds (eg, vitamins or polyphenols) that occur in nature, are part of the food chain, and can be shown to have an effect on human health… beyond the basic nutritional value.”[1]

- Major bioactives in human milk include immune cells, antibodies, cytokines, hormones, glycoproteins (eg, lactoferrin), milk fat globule membrane (MFGM), and oligosaccharides (eg, human milk oligosaccharides [HMOs])[2]
- Bioactives in human milk are believed to play a role in immune modulation, protection against infection, metabolism, and neurodevelopment, among many other functions[3]

[1]. Biesalski HK et al. *Nutrition*. 2009;25(11-12):1202-1205. [2]. Ballard O, Morrow AL. *Pediatr Clin North Am*. 2013;60(1):49-74. [3]. Almeida CC et al. *Int J Food Sci*. 2021;2021:8850080.

Human Milk Oligosaccharides

- Nonnutritive carbohydrates that are unique to human breast milk [1]
  - Nondigestible
  - Remain virtually intact throughout the GI tract
- After lipids and lactose, HMOs are the third most abundant solid component in human breast milk[1]
- Synthesis is highly energy intensive[1]
- HMOs have prebiotic, antimicrobial, and immunomodulatory effects[2]

Major Solid Components in Human and Cow's Milk[2]

	Human milk	Cow's milk
Lactose (g/L)	70	48
Fat (g/L)	41	37
Oligosaccharides (g/L)	**5–15**	**0.05**
Protein (g/L)	8	32

GI, gastrointestinal.

[1]. Sánchez C, et al. *Nutrients*. 2021;13(3):1026. [2]. Bode L. *Glycobiology*. 2012;22(9):1147–1162.

Infant Formula Supplementation With HMOs and Term Infant Outcomes

- Common HMOs used to supplement infant formula include **2'fucosyllactose (2'FL)** and **lacto-N-neotetraose (LNnT)**
- Compared with healthy term infants fed cow's milk formula, those who receive cow's milk formula supplemented with HMOs had:
  - **Lower** rates of parent-reported **bronchitis** at 2 and 12 months[1]
  - **Lower** rates of **antipyretic** and **antibiotic use** through 4 months and 12 months, respectively[1]
  - **Softer stools** more similar to human milk-fed infants[2]

Most data for HMO-supplemented formula come from studies in term infants

[1]. Puccio G et al. *J Pediatr Gastroenterol Nutr*. 2017;64(4):624-631. [2]. Lasekan J et al. *Nutrients*. 2022;14(13):2625.

RCT Evaluating HMOs in Preterm Infants: Growth and Time to Full Enteral Feeds

- Randomized 86 preterm infants (27–33 weeks' gestation) to receive either:
  - Liquid HMO supplement with 2'-FL and LNnT
  - Isocaloric placebo
- Supplements were administered through discharge
- HMO was noninferior to control for time to full enteral feeding with a nonsignificant 2-day reduction
- **Length-for-age *z* scores** were **higher** with HMO supplementation at days 14 and 21

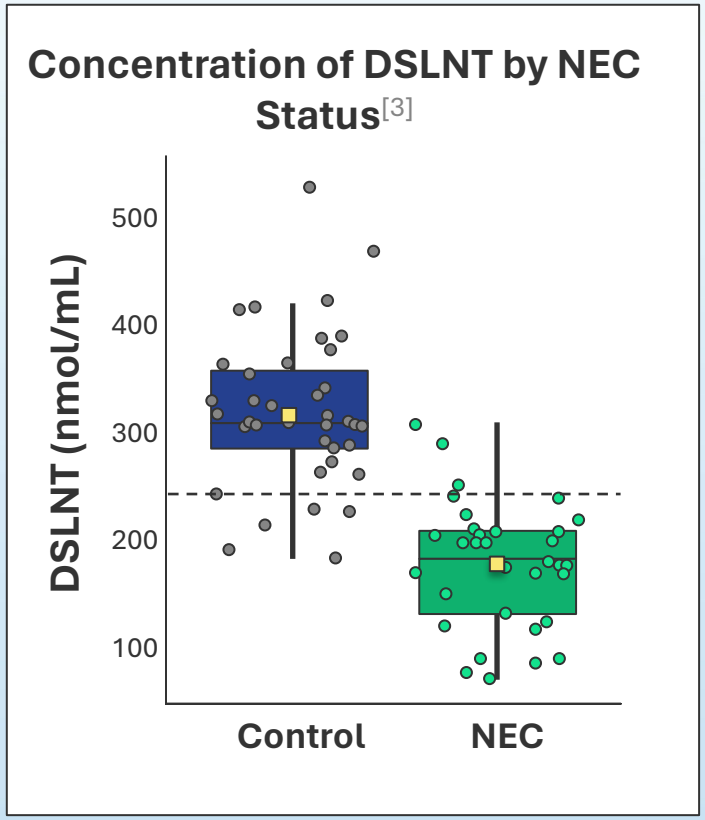
Time to reach full enteral feeding (FEF), full analysis set

	HMO (n = 38)	Placebo (n = 40)	Adjusted mean treatment difference[a] HMO–Placebo
Time from birth to FEF (days), LS means (95% CI)	12.15 (9.50, 14.81)	14.32 (11.71, 16.92)	-2.16[b] (-5.33, 1.00)
Min, Max	7, 3	5, 70	–
Q1, Q3	9, 14	8.5, 15.5	–

a. Adjusted estimates are based on an ANCOVA model adjusted for birth weight, study site, and sex of infant.
b. $P < .001$ for noninferiority analysis (ie, upper bound of 95% CI < 4 + days)

HMOs and Potential Protection Against NEC

- **Low HMO diversity** has been linked to **NEC** in ELBW infants[1]
- DSLNT concentration in MOM is predictive of NEC development[2]
  - DSLNT threshold level of 241 nmol/mL had a sensitivity and specificity of 0.9 for NEC
- **HMOs may mediate NEC** development via the **microbiome**[2]
  - Infants with NEC have lower relative abundance of *Bifidobacterium longum* and higher relative abundance of *Enterobacter cloacae*

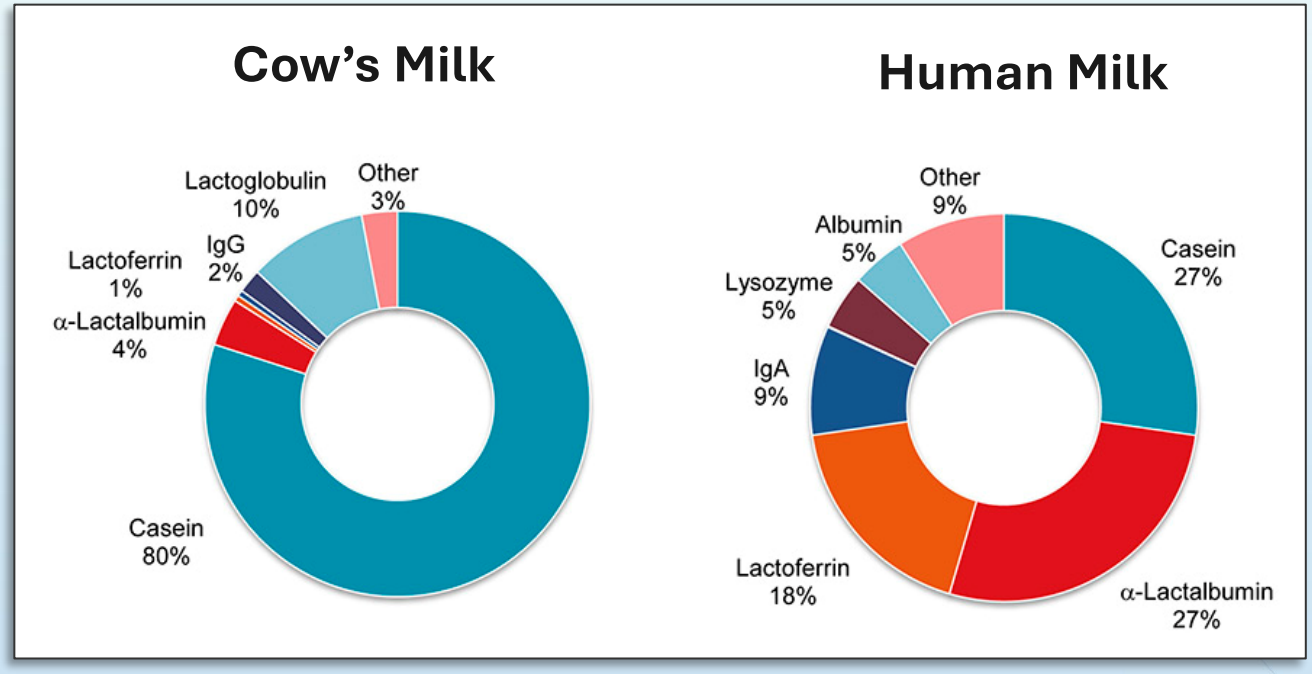


[1]. Wejryd E et al. *Nutrients*. 2018;10(10):1556. [2]. Masi AC, et al. *Gut*. 2020:gutjnl-2020-322771. [3]. Jantscher-Krenn E, et al. *Gut*. 2012;61(10):1417-1425.

Lactoferrin: a Human Milk Protein

- **Major whey protein** in human milk
  - Resistant to proteolytic digestion
  - Glycoprotein with iron-binding properties
- Key functions of lactoferrin include:
  - GI maturation and development
  - Immune modulation
  - Protection against infection

Relative Protein Concentrations[2]



[1]. Lönnerdal B. *J Paediatr Child Health*. 2013;49 Suppl 1:1-7. [2]. Hennet T et al. *Swiss Med Wkly*. 2014. 144:13927.

RCTs of Lactoferrin Supplementation: Effects on Morbidity and Neurodevelopment in Preterm Infants

- In a trial of 414 LBW infants, 8 weeks of bovine lactoferrin 200 mg/kg/d supplementation was compared with placebo[1]
  - **No significant difference** in late-onset sepsis (HR, 0.73; 95% CI, 0.42–1.26) or growth
  - Significantly less bronchiolitis with lactoferrin (RR, 0.34; 95% CI, 0.14–0.86)
- In a trial of 1542 LBW infants, bovine lactoferrin 200 mg/kg/d was compared with no supplement through 34 weeks' postmenstrual age[2]
  - **No difference** in in-hospital death or major mortality
- In a meta-analysis of 5609 preterm infants, lactoferrin supplementation was associated with a **21% decreased risk** of **late-onset sepsis**[2]

[1]. Ochoa TJ et al. *J Pediatr.* 2020;219:118-125.e115. [2]. Tarnow-Mordi et al. *Lancet Child & Adolesc Health.* 2020;4(6):444-454.

Lactoferrin Combined With MFGM in Term Infants: a Randomized Controlled Trial

- Randomized **291 healthy term infants** to receive standard cow's milk formula or cow's milk formula with added bovine lactoferrin and MFGM through 1 year of age[1]
- At ages 12 and 18 months, infants receiving lactoferrin/MFGM had significantly **improved cognitive, language**, and **motor development scores**[1]
- Neurodevelopmental benefits persisted in several cognitive domains at 5.5 years of age (n = 116)[2]

Wechsler Preschool & Primary Scale of Intelligence 4th Edition Composite Scores (mean ± standard error) at 5.5 y of age

	ANOVA			ANCOVA[b]		
WPPSI-IV composite scores[a]	**Control**	**MFGM + LF**	***P***	**Control**	**MFGM + LF**	***P***
Verbal Comprehension Index	93.5 ± 1.4	96.4 ± 1.4	.139	92.3 ± 2.7	94.3 ± 2.9	.287
Visual Spatial Index	95.3 ± 1.7	100.6 ± 1.7	.027	92.3 ± 3.4	98.2 ± 3.6	.014
Fluid Reasoning Index	97.5 ± 1.4	101.1 ± 1.4	.067	94.0 ± 2.8	97.3 ± 3.0	.094
Working Memory Index	101.4 ± 1.7	102.0 ± 1.7	.820	102.6 ± 3.5	103.2 ± 3.8	.831
Processing Speed Index	100.0 ± 1.4	107.1 ± 1.4	<.001	98.6 ± 2.8	105.4 ± 3.0	<.001
FSIQ	93.5 ± 1.5	98.7 ± 1.4	.012	90.9 ± 2.9	95.6 ± 3.0	.020

ANCOVA, analysis of covariance; ANOVA, analysis of variance, FSIQ, full-scale IQ; MFGM + LF, milk fat globule membrane + lactoferrin.

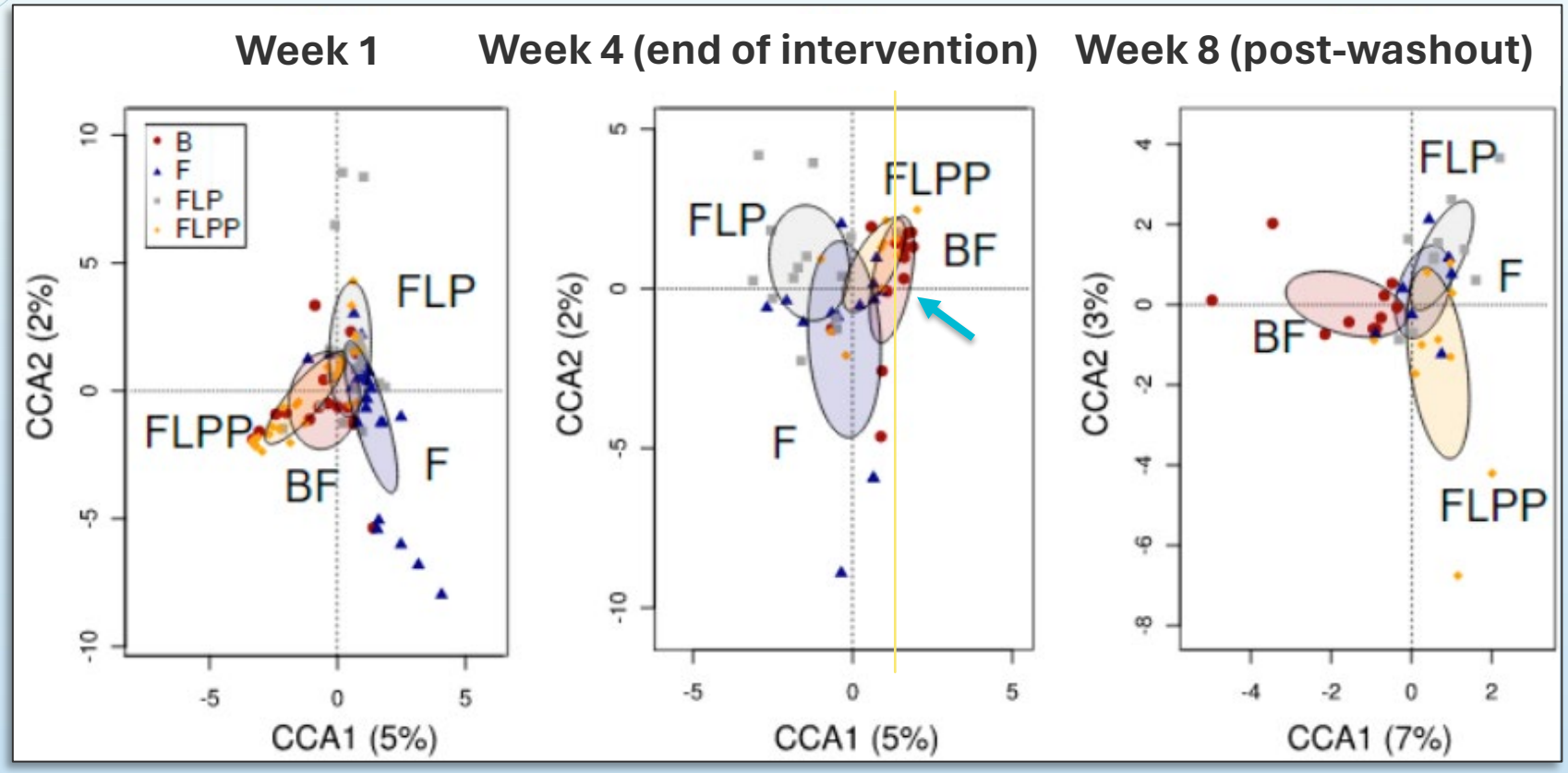
a. Control, n = 59 and MFGM + LF, n = 57.

b. Additional demographic variables included: number of family members in household, monthly average family income, mother and father's highest education, years early education completed before primary school, and exposure to smoking in the home.

[1]. Li F et al. *J Pediatr.* 2019;215:24-31.e8. [2]. Colombo J et al. *J Pediatr.* 2023;261:113483

Combined Bioactives in Preterm Infants: an RCT

Microbial Diversity Over Time



At 4 weeks, infants receiving formula with lactoferrin and oligosaccharides (FLPP) had overlapping microbial populations with breastfed infants.

BF, breast fed; F, formula fed; FLP, formula with lactoferrin and probiotics; FLPP, formula with lactoferrin, probiotics, and prebiotics (bovine oligosaccharides).

Bovine Colostrum: a Source Rich in Bioactives

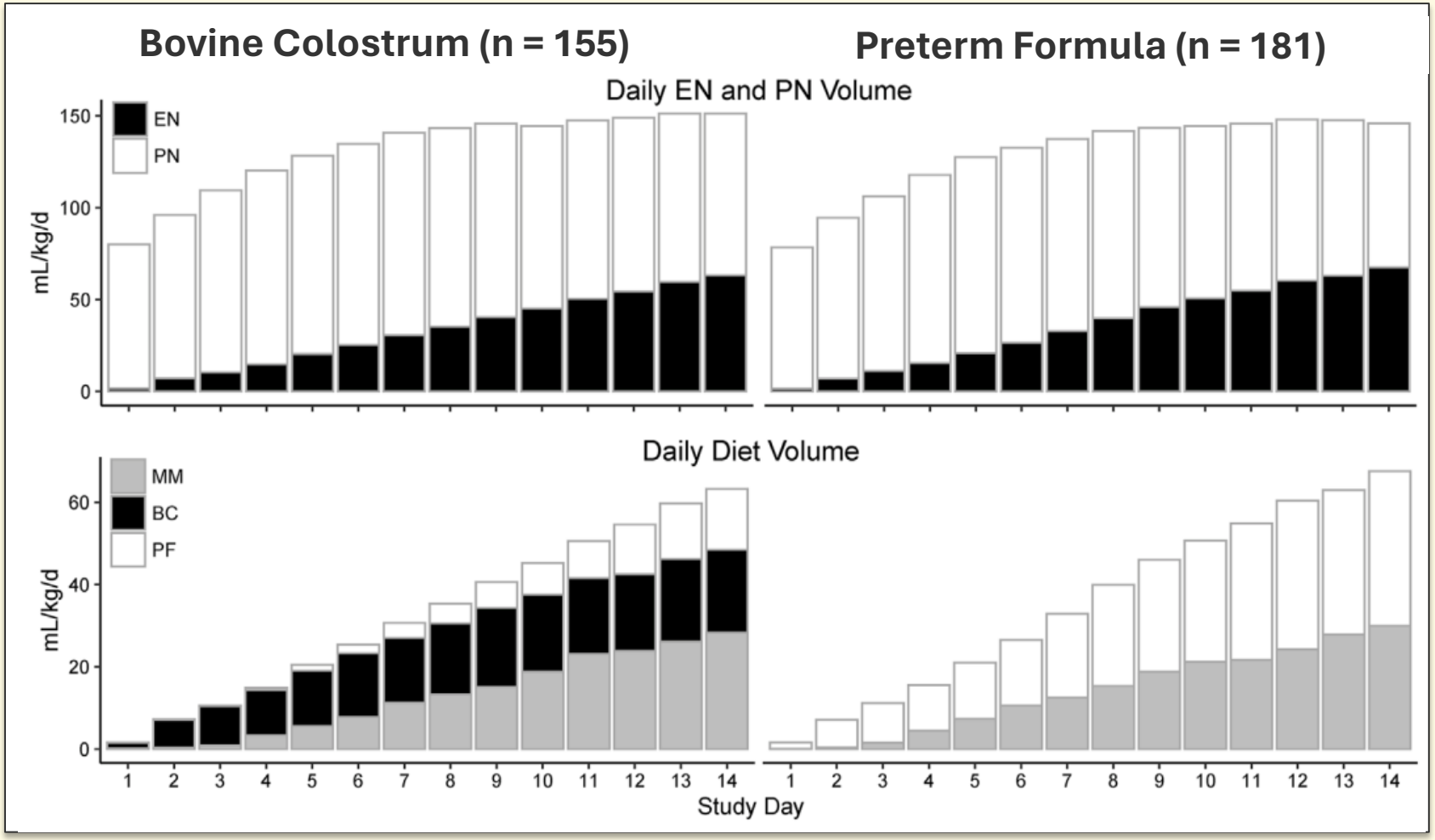
- First milk produced within the first days following birth
- More than **250** functional compounds
  - Rich in immune proteins, lactoferrin, oligosaccharides, lipids, minerals, and vitamins
- Increasing interest in the potential health benefits of colostrum for infants
  - Also entering the adult supplement industry

Poonia A, Shiva. *Food Prod Process Nutr*. 2022;4(1):26.

Bovine Colostrum to Supplement MOM for First 14 Days: the PreColos Randomized Trial

PreColos Randomized Trial

- Enrolled preterm infants (<32 weeks' gestation) to receive supplementation with bovine colostrum or preterm formula for the first 14 days in addition to MOM
- **No** difference reported for time to full enteral feeding, growth, or morbidity



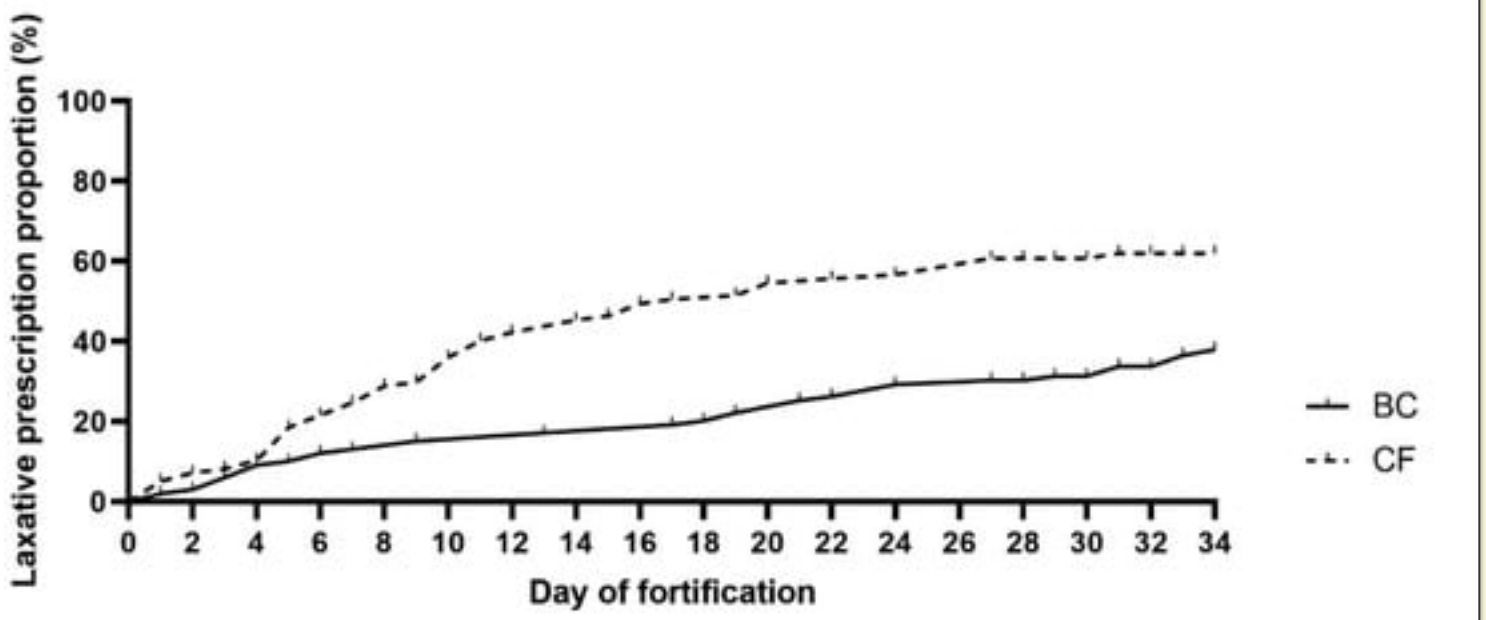
Yan X et al. *Clin Nutr*. 2023;42(8):1408-1417.

Bovine Colostrum Fortifier: the FortiColos Randomized Trial

FortiColos Randomized Trial

- Enrolled preterm infants (26–31 weeks' gestation) to receive bovine colostrum fortification or BMBF[1]
- **No** difference reported for growth or morbidities[1]
- **Higher protein** intake with bovine colostrum[1]
- **Delayed need** for laxatives with bovine colostrum[2]
- **Greater improvement** in stomach appearance scores[1]

Time to Laxative Prescription[2]



[1]. Ahnfeldt AM et al. *Clin Nutr.* 2023;42(5):773-783. [2]. Kappel SS et al. *Nutrients*. 2022;14(22):4756.

Donor Milk and Bioactive Properties

- Compared with MOM, DM has fewer bioactives due to pasteurization, processing, and handling[1]
- Evidence-based strategies for restoring or improving bioactive properties in DM include:
  - Supplementation with HMBF to restore lactoferrin (better than BMBF or unfortified DM)[1]
  - Using (or selecting manufacturers who use) vat pasteurization over retort sterilization or ultra-high temperature processing[2]

[1]. Philip RK et al. *Breastfeed Med.* 2023;18(4):279-290. [2]. Liang N et al. *Front Nutr.* 2022;9:926814.

Considerations for Your Clinical Practice

- Bioactives are an exciting development in infant nutrition:
  - Supplementation with HMOs and/or lactoferrin may reduce morbidity, improve growth, and improve stool consistency
  - Supplementation with lactoferrin and MFGM may improve neurodevelopmental outcomes
- More studies evaluating individual and combined bioactive supplementation in preterm infants are eagerly awaited

Key Takeaways

Donor Milk Fortification

- Use of DM requires mineral supplementation (eg, sodium, zinc).
- After optimizing volume, fortification, and mineral supplementation, monitor growth over the next 4–6 weeks, and adjust feeding approach (including consideration of formula) if growth faltering persists.
- When considering the discontinuation of DM and transition to formula, evaluate DM availability and the infant's individualized risk for NEC (eg, based on gestational age and postnatal age).

KEY TAKEAWAYS

The Microbiome & Probiotics

- Milk source (ie, DM vs MOM) has a greater influence on preterm infant microbial diversity than fortifier type (ie, BMBF vs HMBF).
- When used as a preventative intervention, probiotics may reduce the risk of severe NEC in preterm infants.
- Follow ESPGHAN safety guidelines for probiotic use, including maintaining hygienic workflows and explaining risks and benefits of probiotics to parents using simple language.

KEY TAKEAWAYS

Fortifier Types & Strategies

- When choosing fortifier type, displacement is a more important consideration for MOM than for DM.
- “Aggressive” feeding advancements with either DM or MOM do not increase NEC risk and may reduce sepsis risk.
- Evidence suggests that stable infants may experience growth benefits with early BMBF or HMBF fortification.

KEY TAKEAWAYS

Emerging Solutions

- **Supplementation with HMOs and/or lactoferrin may reduce morbidity, improve growth, and improve stool consistency.**
- **Supplementation with lactoferrin and MFGM may improve neurodevelopmental outcomes.**