Bioactivity of Milk Fat Globule Membranes (MFGMs) and Their Impact on Infant Growth and Development

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Disclosures

Research Support	Arla Foods: Clinical area- infant nutrition Mead Johnson Nutrition: Clinical area- infant nutrition
Consultant	Albion: Clinical area- infant nutrition Biostime: Clinical area- infant nutrition Hero: Clinical area- infant nutrition HiPP: Clinical area- infant nutrition Nestle Nutrition: Clinical area- infant nutrition
Speakers Bureau	Mead Johnson Nutrition: Clinical area- infant nutrition Nestle Nutrition: Clinical area- infant nutrition

Learning Objectives

- Identify 2 key processes required for brain growth and development
- Explain how MFGM supports a developing central nervous system
- Use evidence-based research to develop a supplementation plan that best aligns with the nutrition provided in human milk

MFGM, milk fat globule membrane.

Breast-Fed vs Formula-Fed Infants

- Different growth patterns
- Less otitis media, gastrointestinal infections and possibly other infections
- Reduced risk for celiac disease
- Reduced risk for obesity
- Reduced risk for T2D
- Reduced risk for T1D
- Better cognitive performance

T2D, type 2 diabetes; T1D, type 1 diabetes.

Dutch State Institute for Nutrition and Health, Van Rossum CMT, et al. RIVM Report 350040001/2005; Ip S, et al. AHRQ Publication No. 07-E007. Rockville, MD: Agency for Healthcare Research and Quality. April 2007; ESPGHAN Committee on Nutrition, Agostoni C, et al. *J Pediatr Gastroenterol Nutr.* 2009;49:112-125; EFSA Panel on Dietetic Products, Nutrition and Allergies. *EFSA Journal.* 2009;7:1423.

Questions

- Are there factors in breast milk not present in infant formula today that are needed for optimal cognitive development and health?
- What factors?
- How can they be added to infant formula?

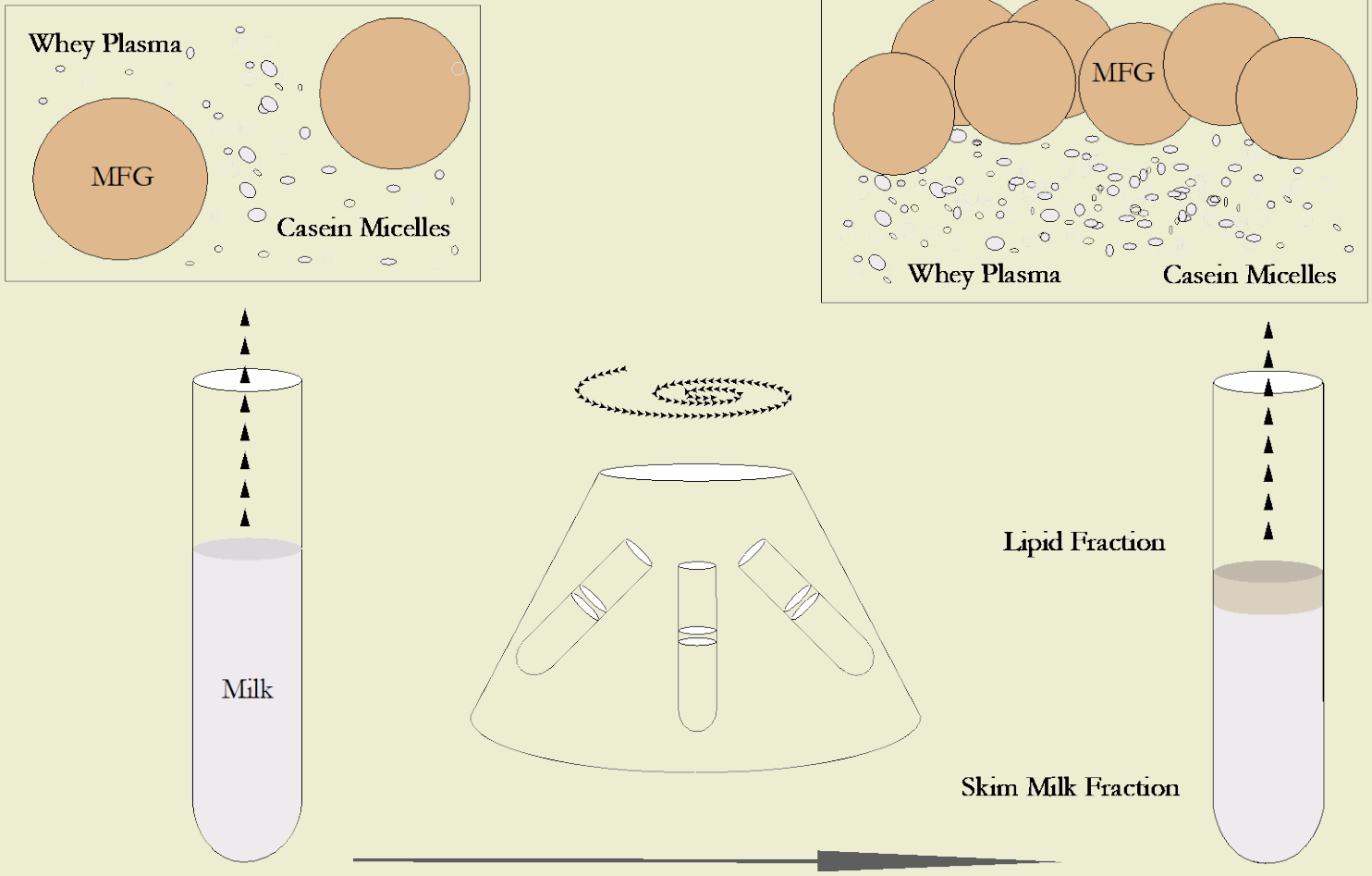
Protein Fractions in Breast Milk

- Whey proteins
- Casein
- Milk fat globule membrane (MFGM) proteins

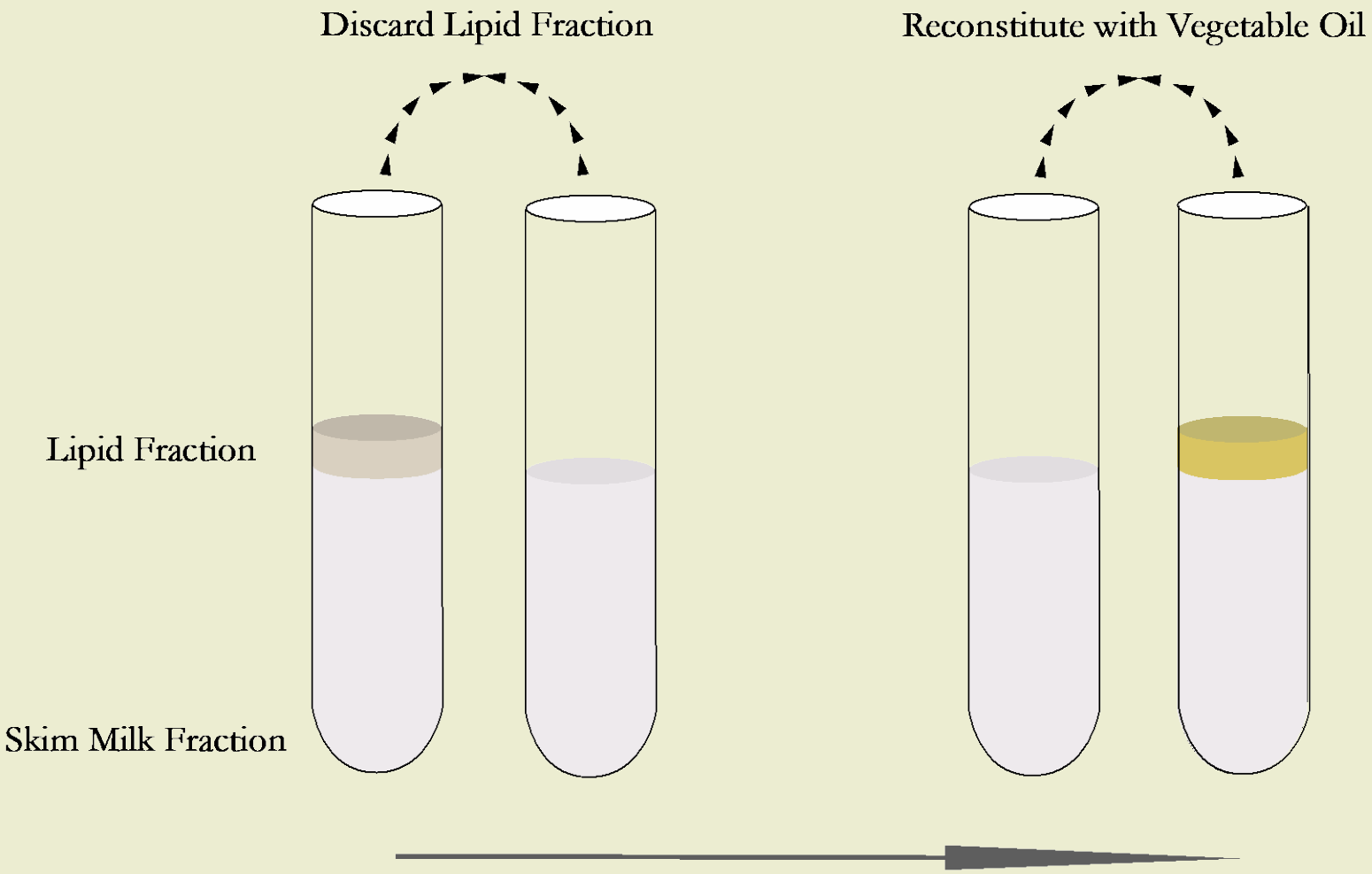
Infant formulas are made of whey and casein (skim milk)

Missing: MFGM !!

Skimming of Milk



Infant Formula Manufacture

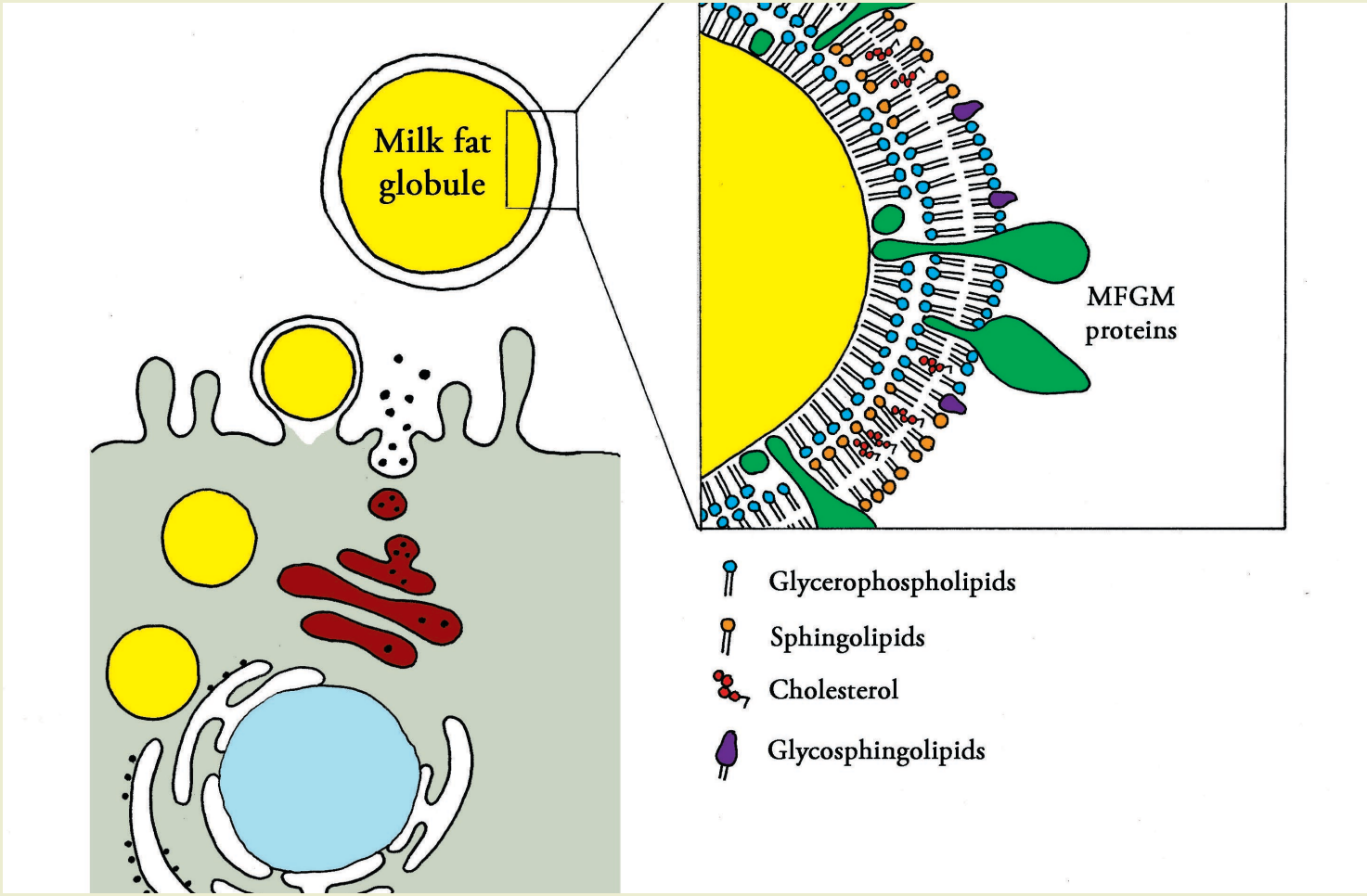


Milk Fat Globule Membranes

- Surround lipid droplets in milk
- ~60 % protein, ~30% lipid
- Minor protein component (1-2% of total protein)
- Proteomics reveal > 100 proteins
- Several proteins have antimicrobial activity and others are involved in nutrient absorption
- Rich in phospholipids (sphingomyelin), gangliosides, cholesterol and sialic acid

Liao Y, et al. *J Proteome Res*. 2011;10:3530-3541; Lönnerdal B. *Am J Clin Nutr.* 2014;(suppl):712S-717S.

Secretion of Milk Fat



MFGM, milk fat globule membrane.

Effect of MFGM on Infectious Disease

- A novel dairy fraction (Arla Food Ingredients, Denmark)
- Safety and acceptability
- Appropriate dose (breast milk level)
- Older infants (6-12 months of age)
- High prevalence of diarrhea

MFGM, milk fat globule membrane.

Zavaleta N, et al. *J Pediatr Gastroenterol Nutr*. 2011;53:561-568.

Efficacy of an MFGM-enriched Complementary Food in Diarrhea, Anemia, and Micronutrient Status in Infants

Nelly Zavaleta, Anne Staudt Kvistgaard, Gitte Graverholt, Graciela Respicio, Henry Guija, Norma Valencia, Bo Lönnerdal

Zavaleta N, et al. *J Pediatr Gastroenterol Nutr*. 2011;53:561-568.

Study Design

- Double-blind RCT in Lima, Peru
- Healthy 6 to 12-month-old infants
- Complementary food, 2X daily with MFGM (Lacprodan® MFGM-10, Arla Foods Ingredients, Viby, Denmark) or placebo (skim milk protein) for 6 months
- 1 RDA of all micronutrients added
- Anthropometry, morbidity, bacteriology/virology, nutritional status (Fe, Zn, vitamin A, vitamin B_{12}, folate)

Fe, iron; MFGM, milk fat globule membrane; RCT, randomized controlled trial; RDA, recommended dietary allowance; Zn, zinc

Zavaleta N, et al. *J Pediatr Gastroenterol Nutr*. 2011;53:561-568.

Results

- Primary outcome: diarrhea morbidity
  - Incidence: no difference
  - Longitudinal prevalence: lower in the MFGM group (3.84% vs. 4.37%, *P*<0.05)
- Multivariate model adjusted for initial anemia and potable water facilities
  - Incidence of bloody diarrhea lower in the MFGM group, adjusted OR 0.54 (95%CI 0.31-0.93, *P*=0.025)

MFGM, milk fat globule membrane; OR, odds ratio.

Zavaleta N, et al. *J Pediatr Gastroenterol Nutr*. 2011;53:561-568.

Effect of Milk Fat Globule Membranes (MFGM) Added to Infant Formula on Infection and Cognitive Development in Infants

Timby N, et al

Department of Clinical Sciences, Umeå University, Sweden

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

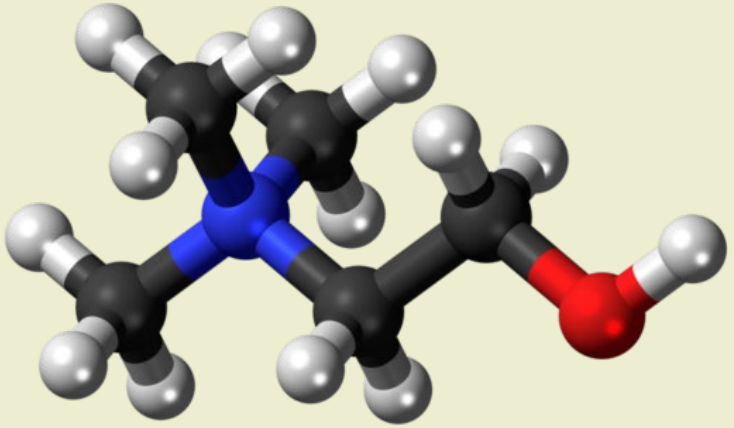
Hypotheses

- Supplementation of formula with bovine milk fat globule membranes would result in:
  - Improved cognitive development
  - Reduced infection frequency
    - (ie, more similar to breast-fed infants)

Timby N, et al. *Am J Clin Nutr*. 2014;99:860-868

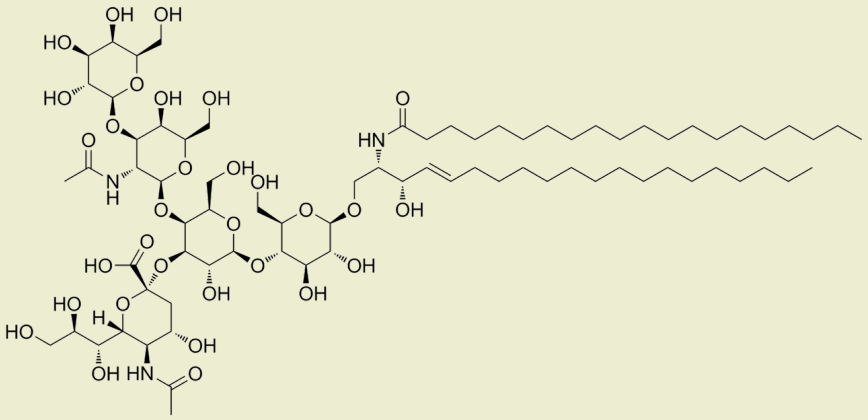
Choline/Phosphatidylcholine

- Facilitates memory and learning functions in rodents (Zeisel SH, 2006)
- When given as supplement to pregnant women – no effect on offspring (breast-fed) (Cheatham CL et al, 2012)



Zeisel SH. *Annu Rev Nutr*. 2006;26:229–250; Cheatham CL, et al. *Am J Clin Nutr.* 2012;96:1465–1472.

Gangliosides

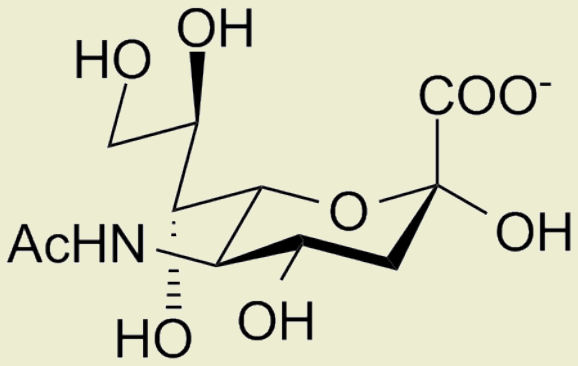


- Involved in neuronal growth, migration and maturation, neuritogenesis, synaptogenesis and myelination

- Dietary gangliosides improve brain development during early life and optimal brain function throughout life in animal models

McJarrow P, et al. *Nutr Rev*. 2009;67:451-63; Palmano K, et al. *Nutrients*. 2015;7:3891-3913.

Sialic Acid

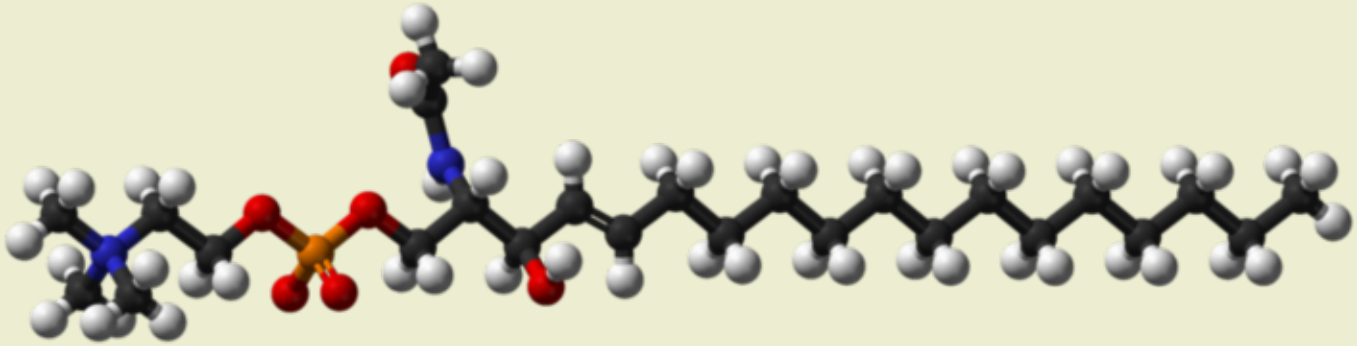


- In a piglet model, sialic acid supplementation improved memory and learning
- Formula-fed infants have lower concentrations of sialic acid in saliva compared to breast-fed infants
- Sialic acid has been suggested as a breast milk component supporting optimal neural function

Wang B, et al. *Eur J Clin Nutr*. 2003;57:1351-1369; Wang B, et al. *Am J Clin Nutr*. 2007;85:561-569; Tram TH, et al. *Arch Dis Child*. 1997;77:315-318.

Sphingomyelin

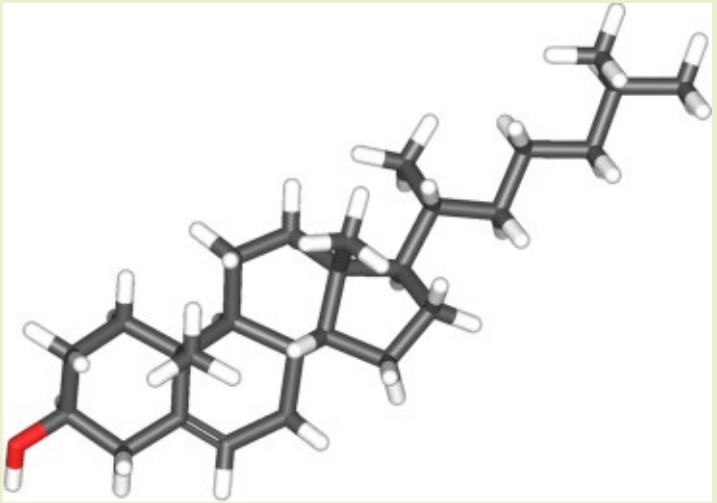
- Supplementation to rats accelerates myelination of cortex (Oshida K et al, 2003)
- Supplementation to VLBW infants improves neurobehavioral development (pilot study) (Tanaka K et al, 2013)



Oshida K, et al. *Pediatr Res*. 2003;53:589-593; Tanaka K, et al. *Brain Dev.* 2013;35:45-52.

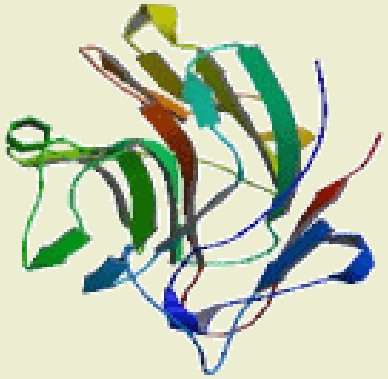
Cholesterol

- Supplementation in animal models increases brain myelination and exploratory behavior (Haque ZU et al, 1992; Schoknecht PA et al, 1994)
- Total serum cholesterol linked to cognitive function in elderly (Elias PK et al, 2005)



Haque Zu, et al. *Biosci Biotechnol Biochem.* 1992;56:1351-1354; Schoknecht PA, et al. *J Nutr*. 1994;124:305-314; Elias PK, et al. *Psychosom Med*. 2005;67:24-30.

The Proteome

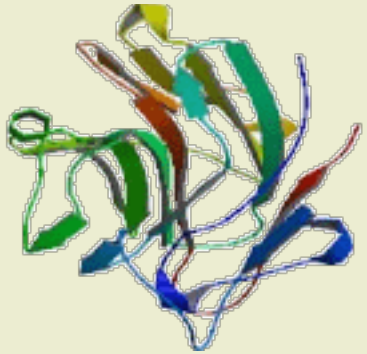


- Human MFGM: 191 proteins
- Bovine MFGM concentrates: 133-244 proteins
- 1-4% of total milk protein
- 50% membrane/protein trafficking or cell signaling functions

MFGM, milk fat globule membrane.

Liao Y, et al. *J Proteome Res*. 2011;10:3530-3541; Reinhardt TA, et al. *J Dairy Res*. 2006;73:406-416.

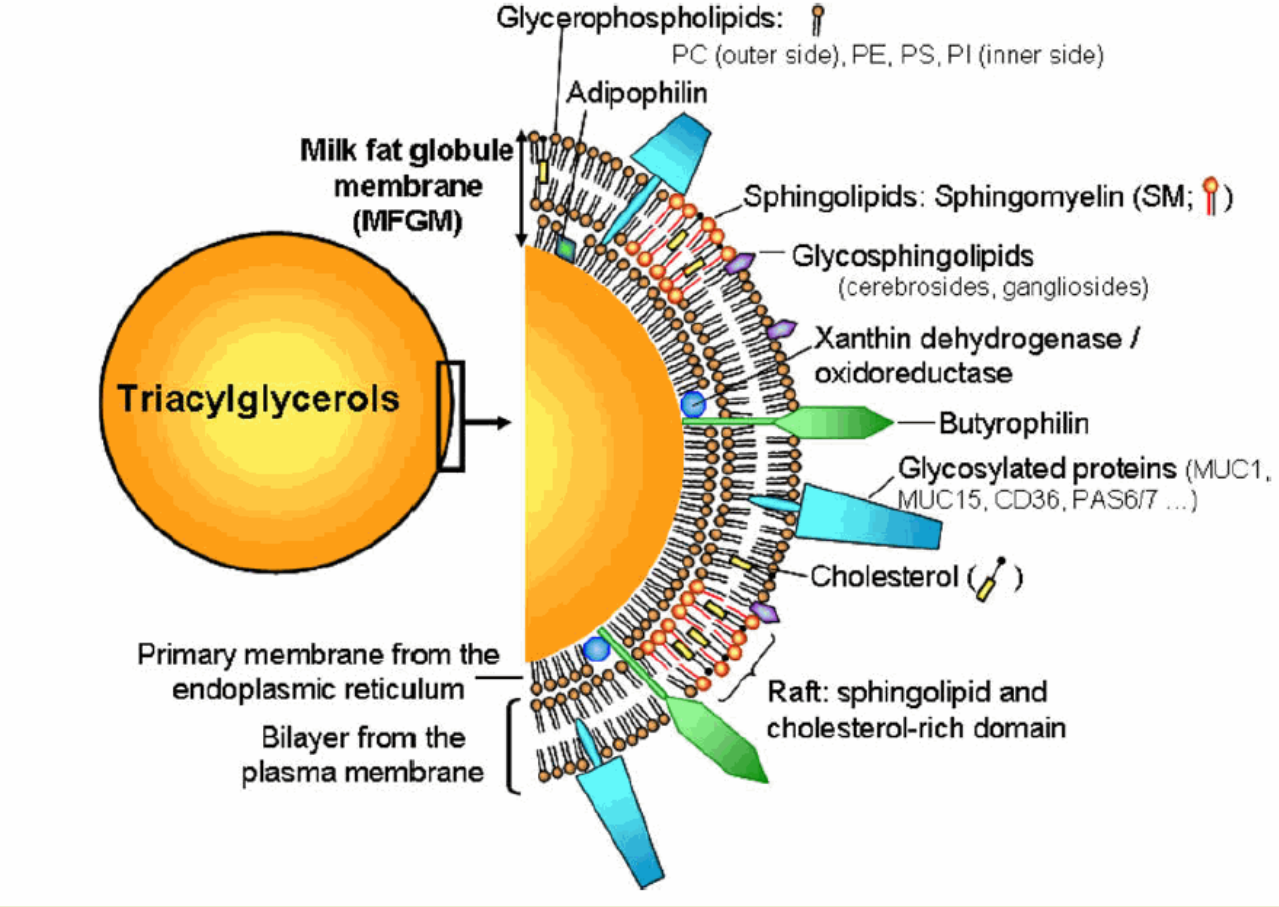
The Proteome



- Antimicrobial effects
  - *Helicobacter pylori* -inhibitor, butyrophilin, MUC1, PAS6/7 (lactadherin), CD14, toll-like receptor 1 and 4, xanthine oxidase, lactoferrin
- Anti-inflammatory effects
  - Butyrophilin

Reinhardt TA, et al. *J Dairy Res*. 2006;73:406-416; Spitsberg VL. *J Dairy Sci*. 2005;88:2289-2294;
Clare DA, et al. *J Food Prot*. 2008;71:126-133; Cavaletto M, et al. *Adv Exp Med Biol*. 2008;606:129-141.

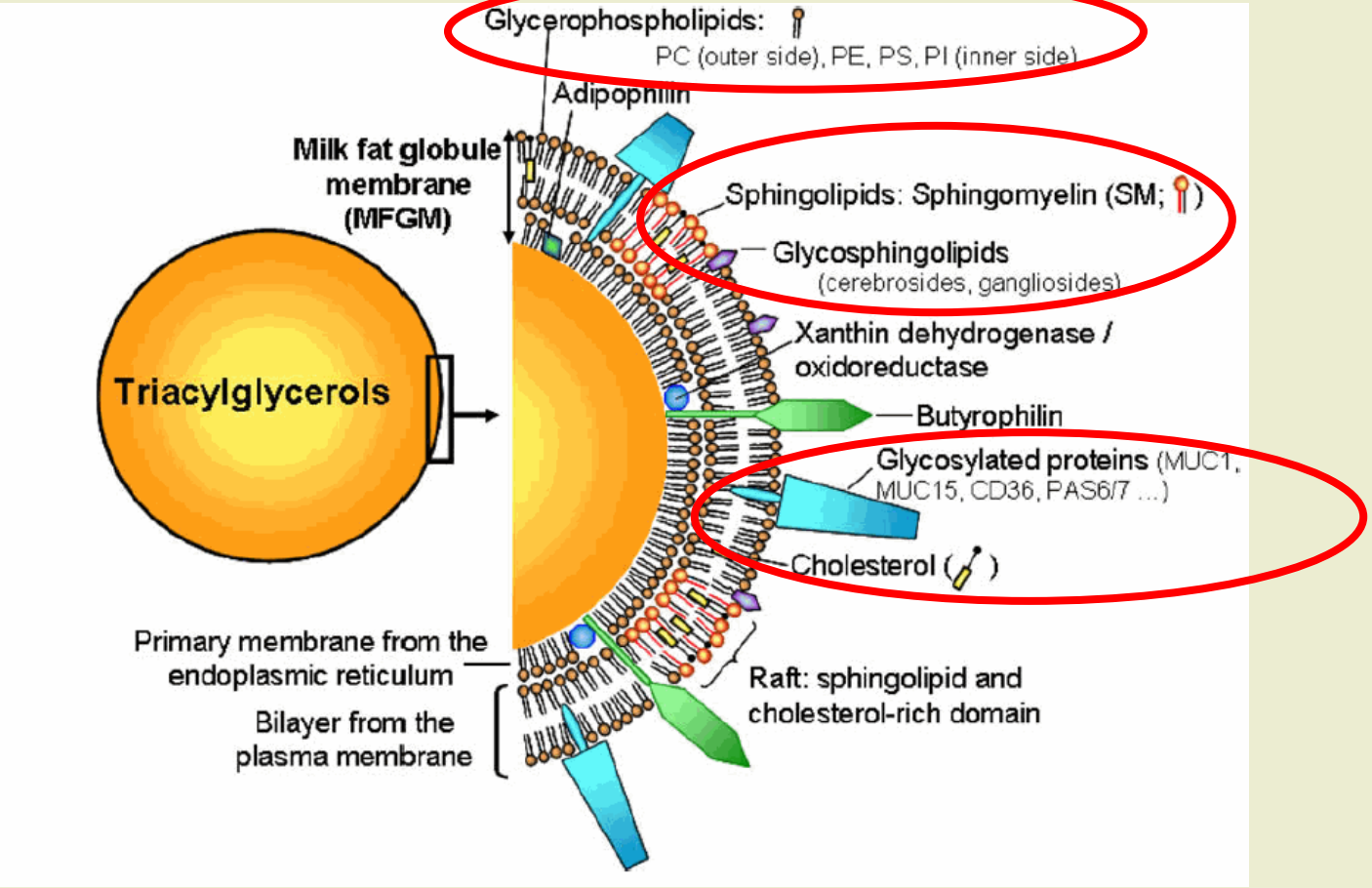
Milk Fat Globule Membrane



Factors possibly associated with improved neurologic and immunologic development, and defense against infections

http://www.dairymoos.com/health-benefits-of-the-milk-fat-globule-membrane/

Milk Fat Globule Membrane



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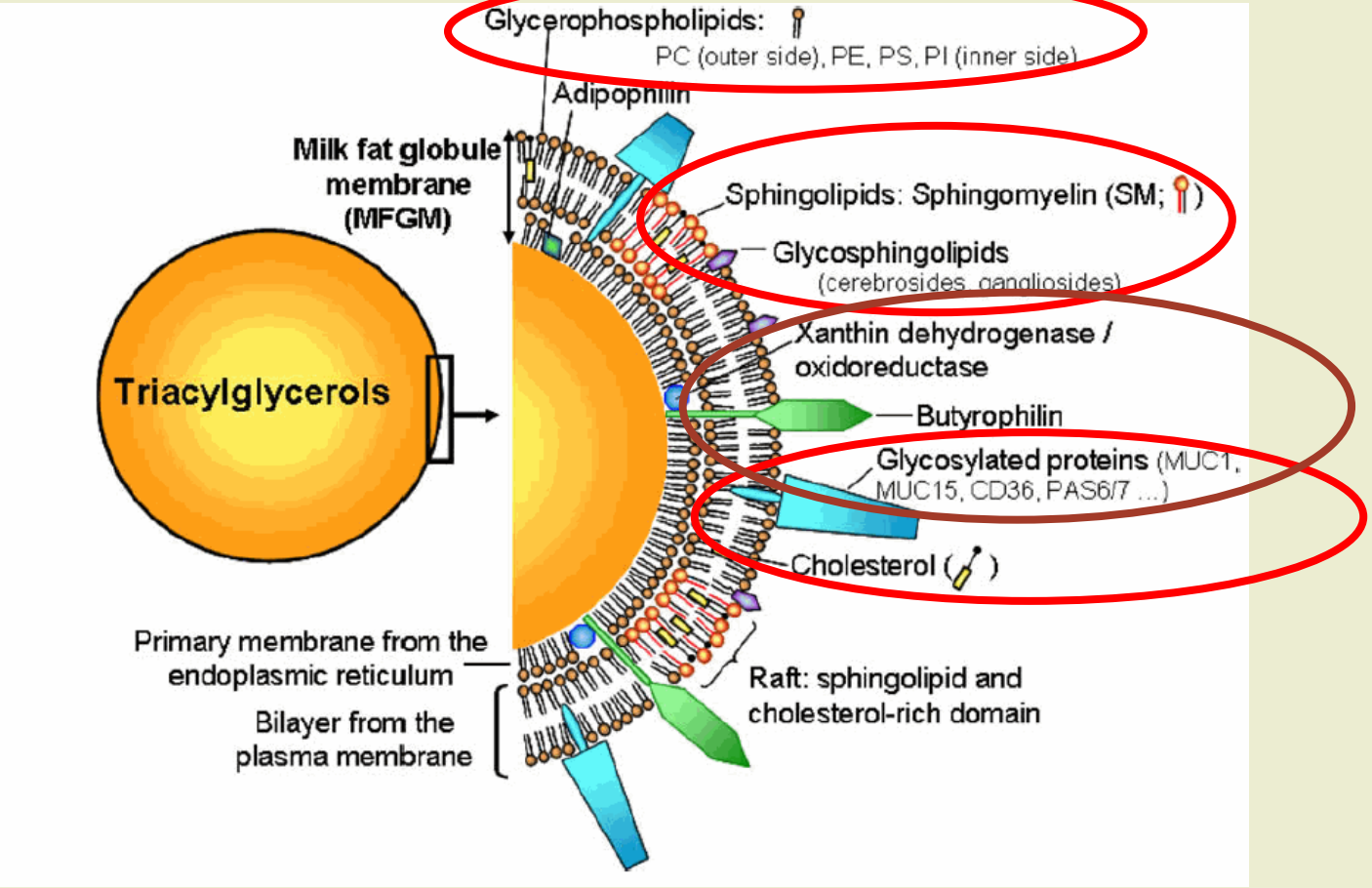


Factors possibly associated with improved neurologic and immunologic development, and defense against infections

http://www.dairymoos.com/health-benefits-of-the-milk-fat-globule-membrane/

Infant Formulas Studied

- Standard formula (SF): BabySemp
- Experimental formula (EF): modified from BabySemp, supplemented with Lacprodan® MFGM-10 (Arla Foods, Denmark)

Timby N, et al. *Am J Clin Nutr*. 2014;99:860-868.

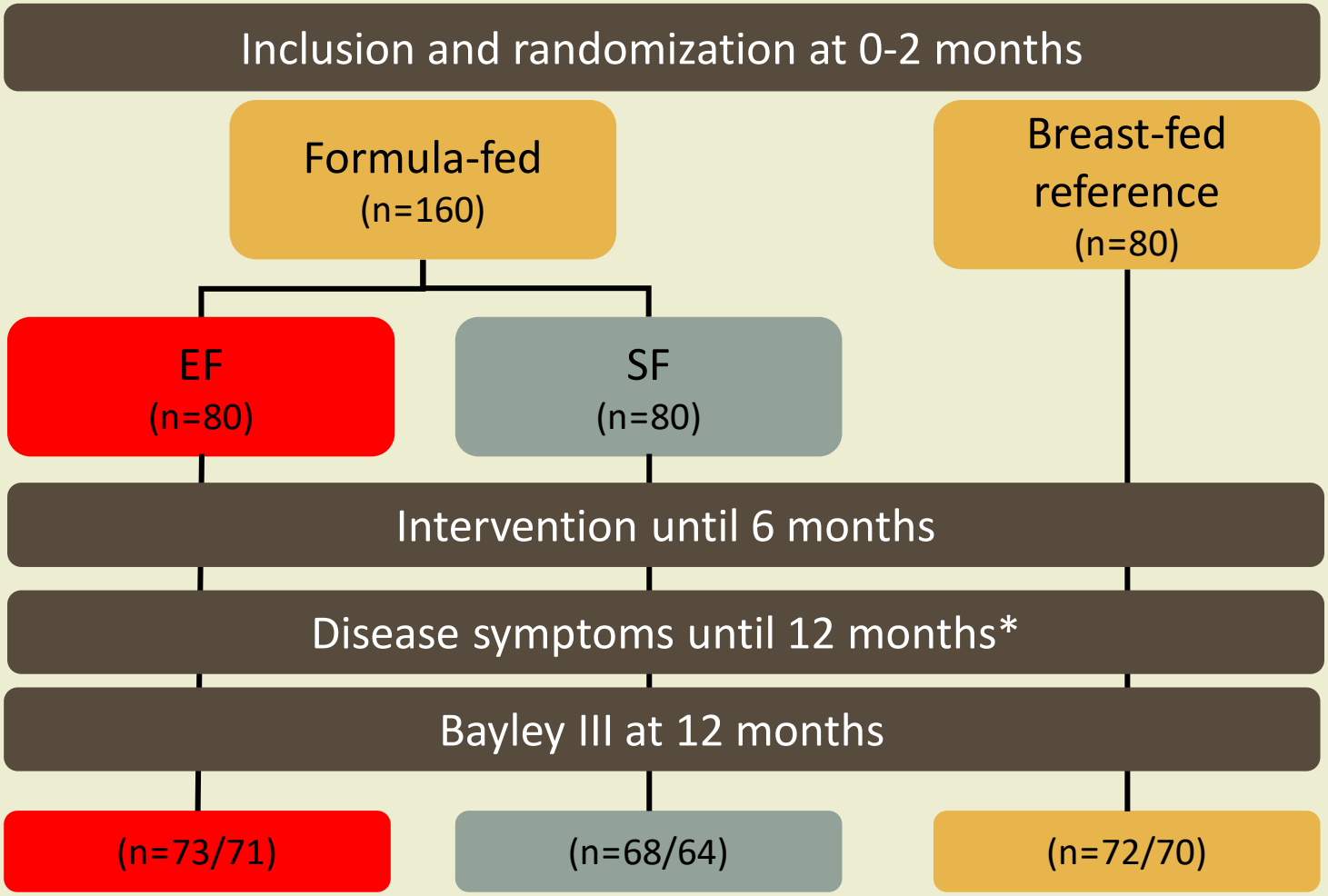
Macronutrient Contents

Per 100 ml	EF	SF
Energy (kcal)	60	66
Proteins (g)	1.20	1.27
Casein (g)	0.35	0.50
Whey (g)	0.85	0.80
Carbohydrates/lactose (g)	6.0	7.4
Lipids (g)	3.5	3.5
Saturated FA (g)	1.35	1.30
Monounsaturated fatty acids (g)	1.35	1.40
Polyunsaturated fatty acids (g)	0.60	0.60
Linoleic acid (mg)	460	460
α-linolenic acid (mg)	70	70
Arachidonic acid (mg)	15	15
Docosahexaenoic acid (mg)	9	9
Cholesterol (mg)	8	4
Phospholipids (mg)	70	30

EF, experimental formula; SF, standard formula.

Timby N, et al. *Am J Clin Nutr*. 2014;99:860-868.

Study Design



* Disease symptoms included any infection-related symptoms (fever, coughing, breathing difficulties, or rash), stool frequency and consistency, as well as medication use and medical visits.

EF, experimental formula; SF, standard formula.

Timby N, et al. *Am J Clin Nutr*. 2014;99:860-868.

Randomized, Controlled Intervention Trial

- Intention to breast-feed exclusively until 6 months
- Recommendation to give no or only small amounts of complementary foods between 4-6 months of age
- At each visit:
  - Weight, length, head circumference
  - Blood sample for insulin, glucose, cholesterol, BUN, amino acids, etc.
  - 3-day food diary (every month)
  - Symptom diary daily during intervention and between 6-12 months noting any disease symptoms, medication or hospitalization
  - Visits were at inclusion (<2 months), 4, 6, and 12 months of age

BUN, blood urea nitrogen.

Timby N, et al. *Am J Clin Nutr.* 2014;99:860-868.

Background Characteristics

	EF	SF	BFR
Maternal age	29.9 ± 5.5	29.2 ± 5.5	31.4 ± 4.0
Maternal education (years after 7)	13.6 ± 2.7	13.4 ± 2.2	15.9 ± 2.6
Maternal smoking	8 (11%)	7 (10%)	1 (1%)
Paternal age	32.9 ± 5.8	32.1 ± 5.8	34.3 ± 4.1
Paternal education (years after 7)	12.7 ± 2.2	12.9 ± 2.2	15.2 ± 2.8
Paternal smoking	9 (13%)	8 (12%)	2 (3%)

BFR, breast-fed reference; EF, experimental formula; SF, standard formula; mean ± SD; [n (%)]

Timby N, et al. *Am J Clin Nutr.* 2014;99:860-868.

Infant Characteristics

	EF	SF	BFR	*P* value (EF vs SF)	*P* value (EF+SF vs BFR)
Gestational age (wk)	39.6 ± 1.4	39.7 ± 1.3	40.0 ± 1.2	.85	.033
Birth weight (kg)	3.53 ± 0.40	3.44 ± 0.47	3.61 ± 0.37	.26	.036

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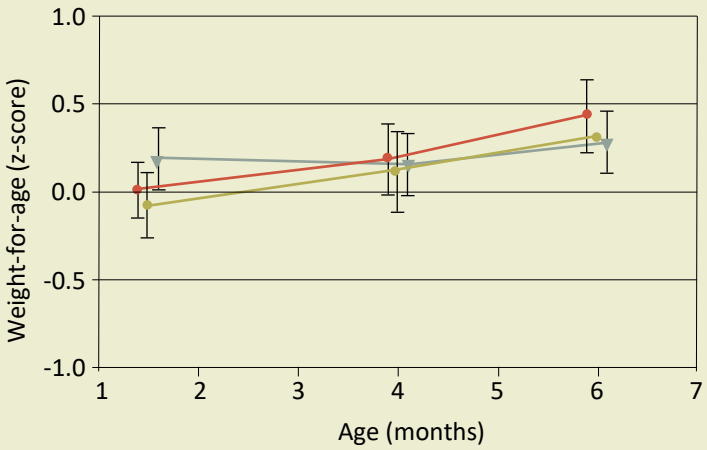
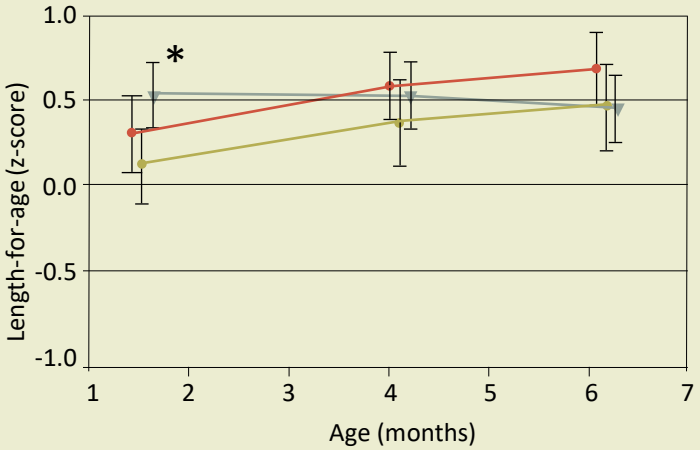
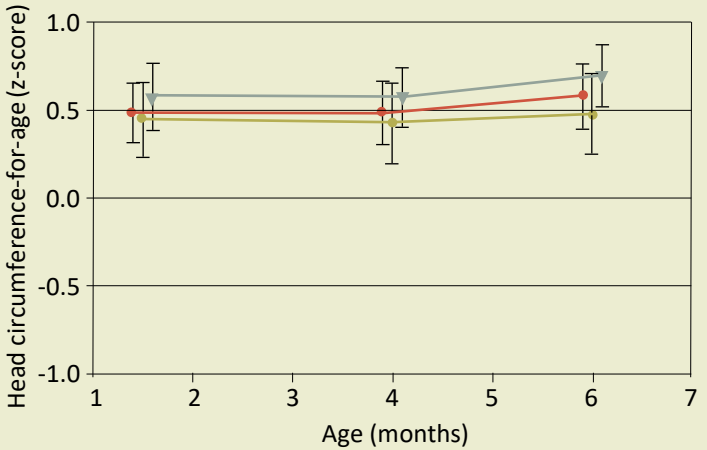
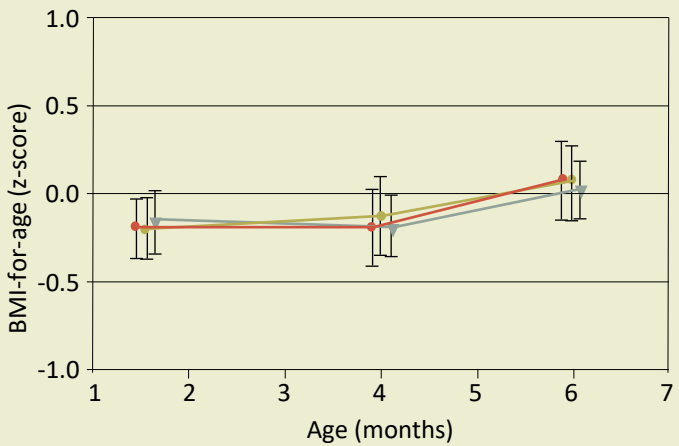
Timby N, et al. *Am J Clin Nutr.* 2014;99:860-868.

Growth: No Difference EF vs SF







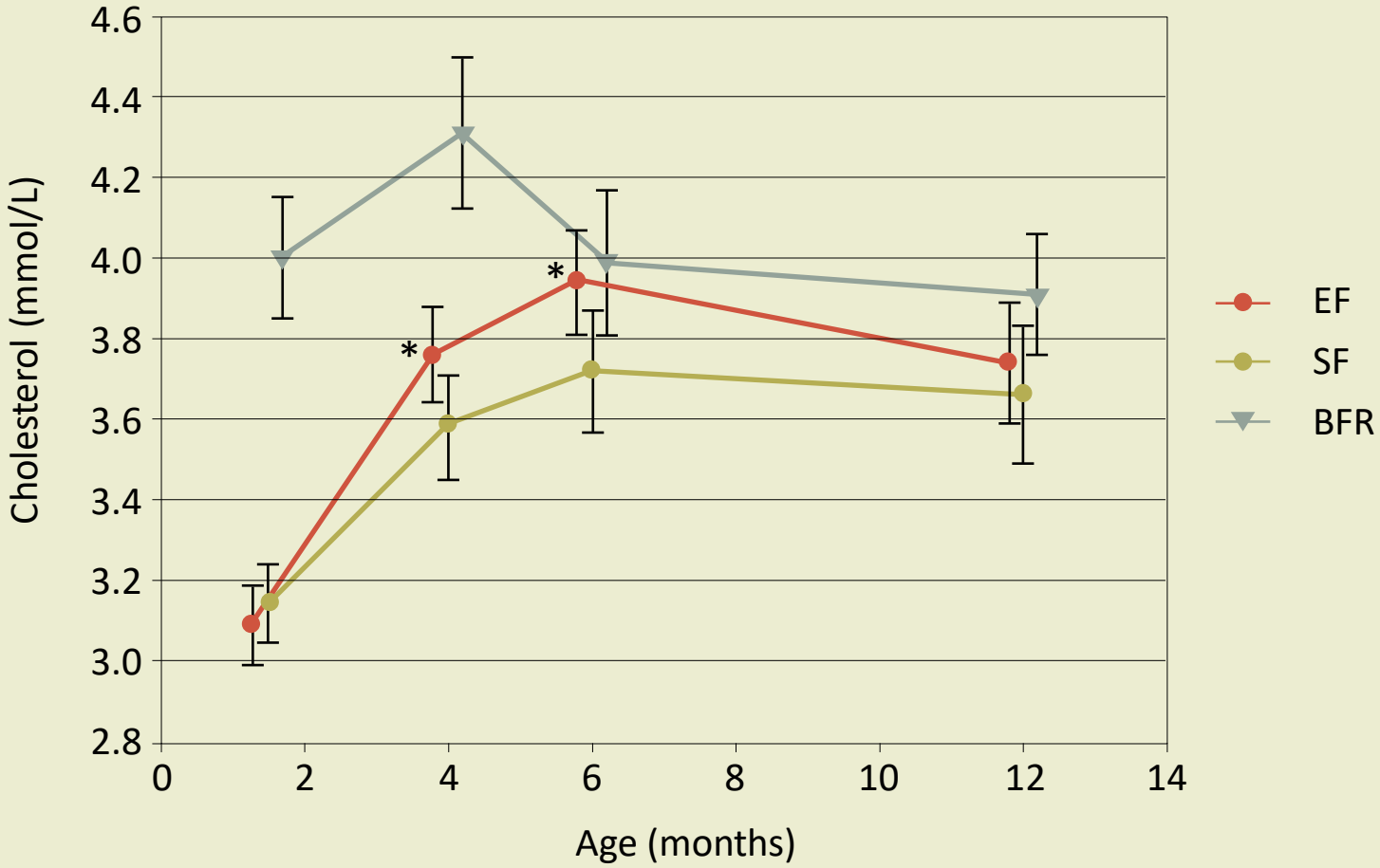


EF
SF
BFR

BFR, breast-fed reference; EF, experimental formula; SF, standard formula. * BFR significantly different from EF + SF, $P < .05$.

Timby N, et al. *Am J Clin Nutr*. 2014;99:860-868.

Cholesterol: EF Closer to BFR



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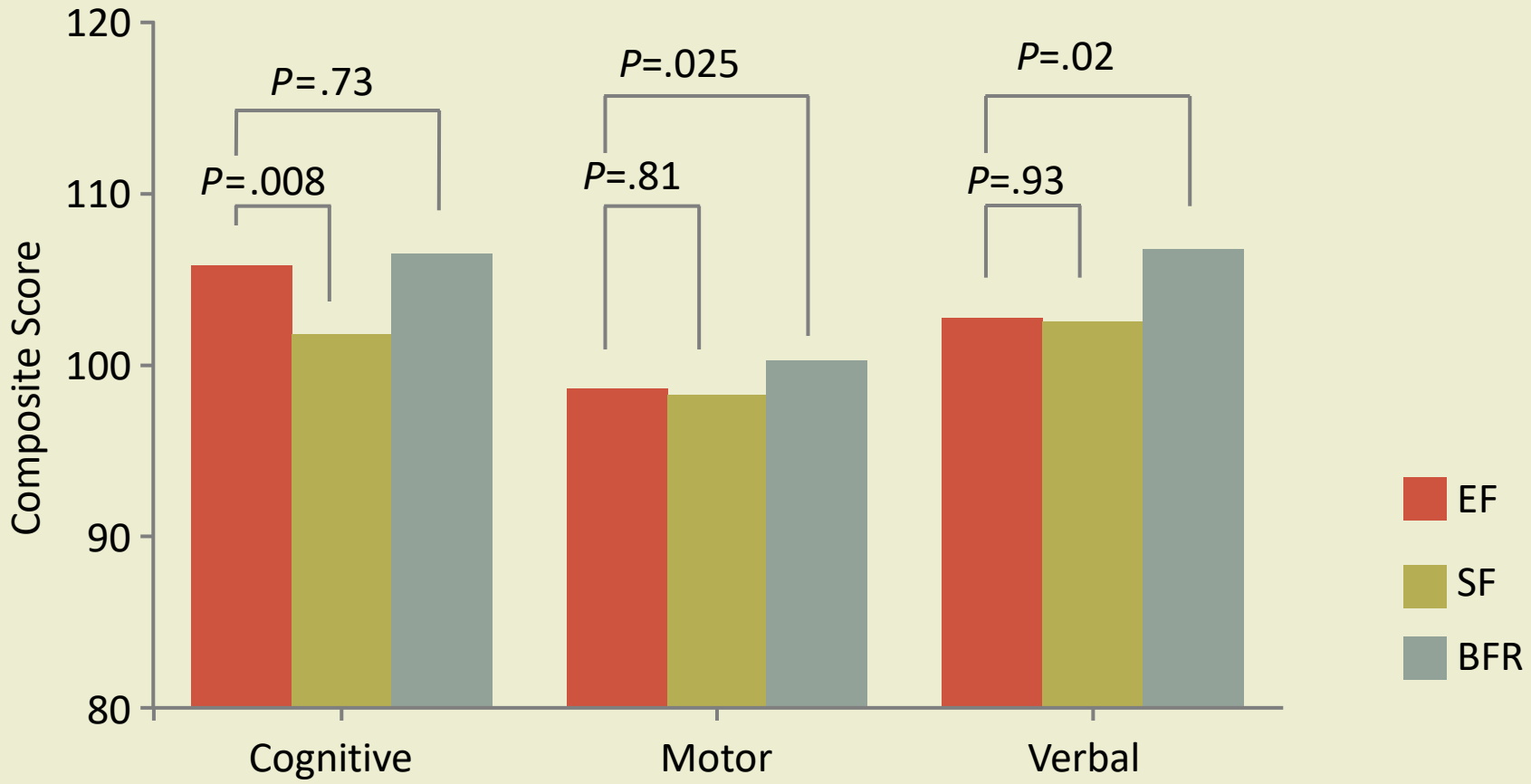
Cognitive Function: EF > SF and Same Level as BFR at 1 Year of Age



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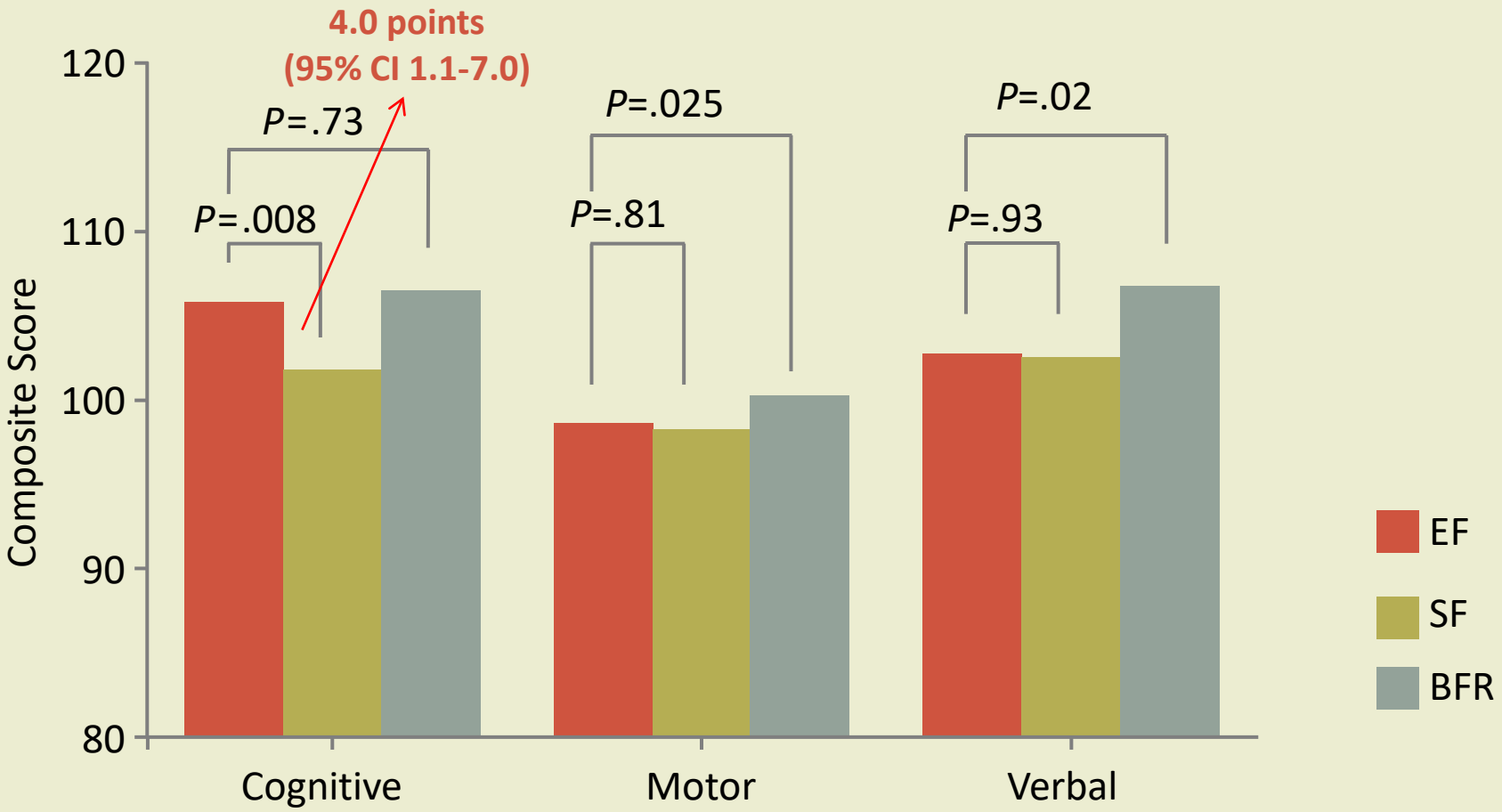
Results: Bayley III

12 Months of Age



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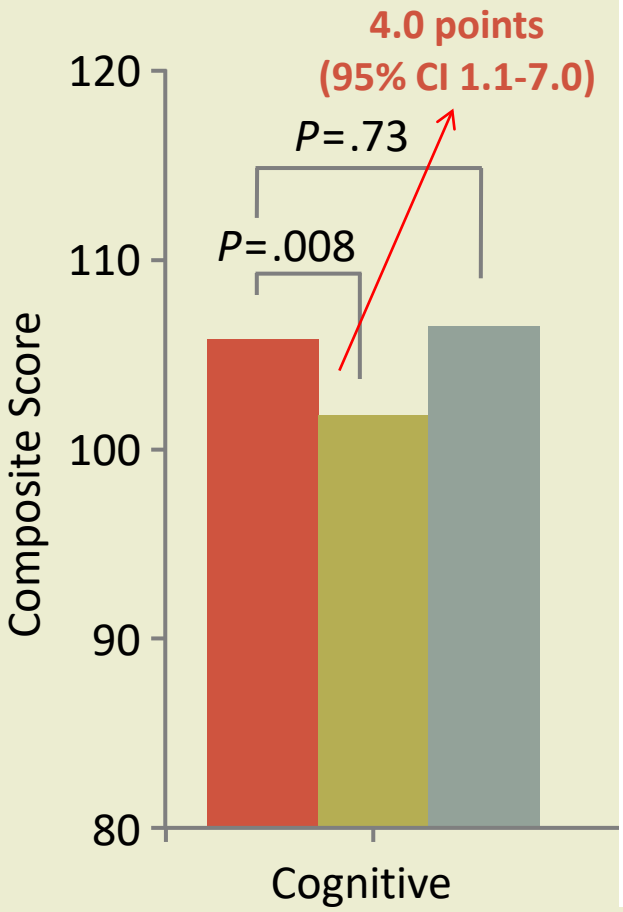
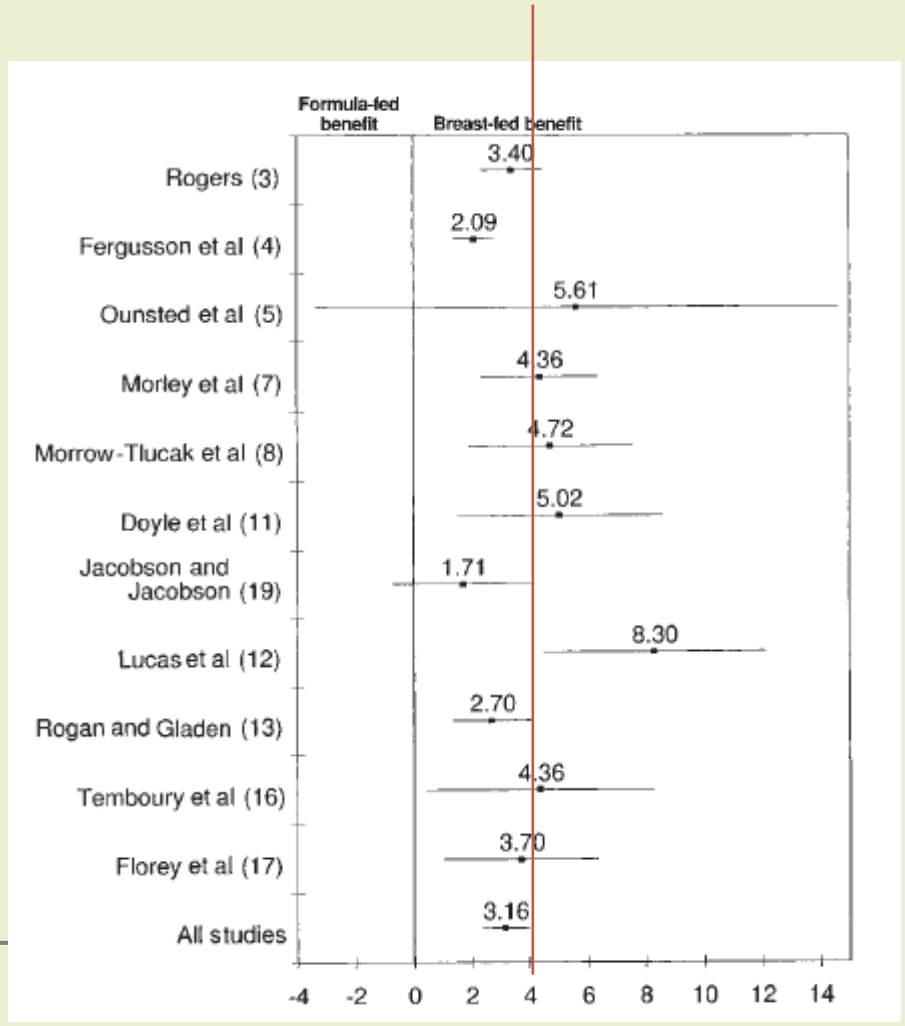




EF

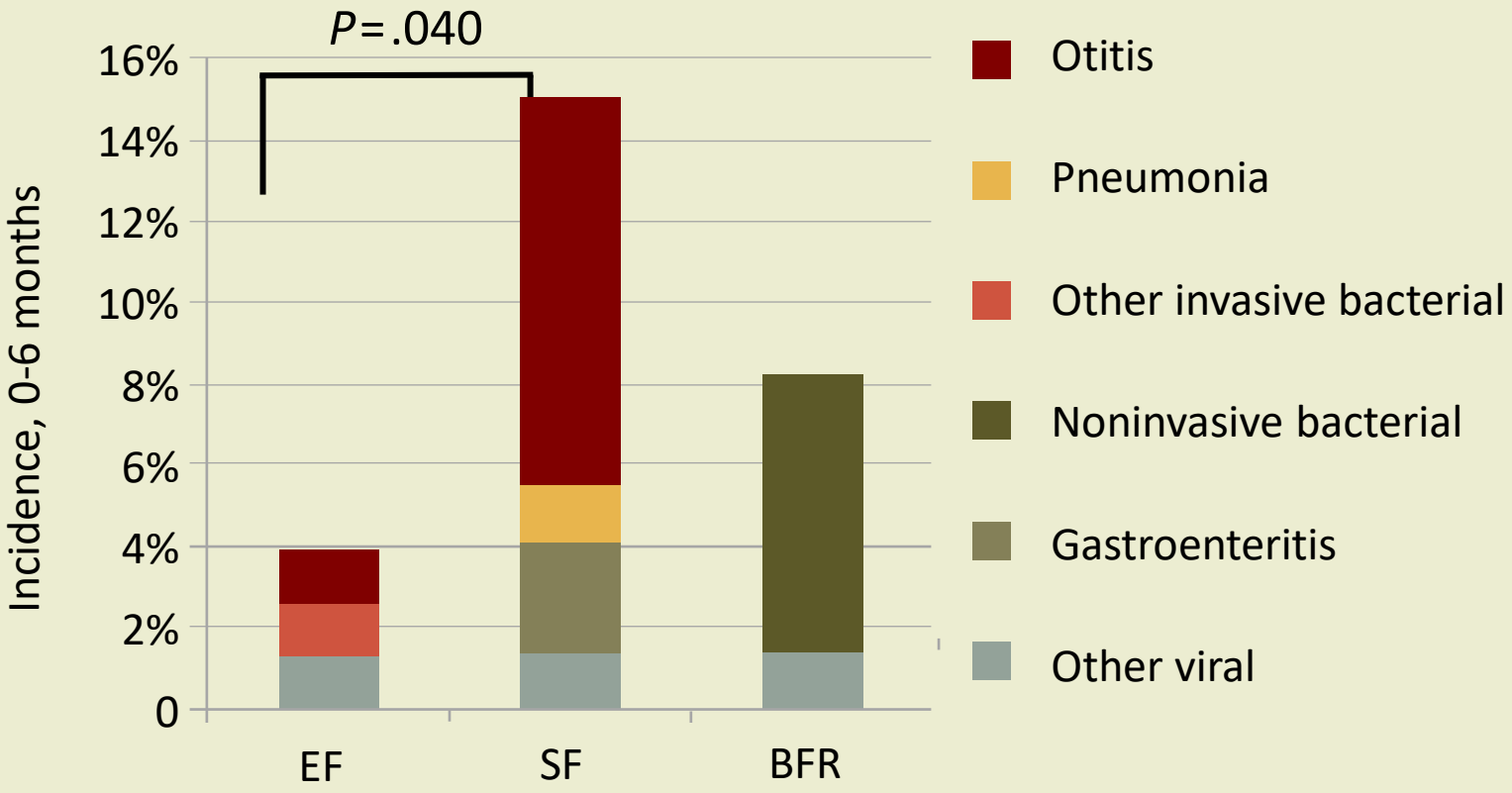
SF

BFR

BFR, breast-fed reference; EF, experimental formula; SF, standard formula.

Timby N, et al. *Am J Clin Nutr*. 2014;99:860-868.

Infections Treated with Antibiotics or Requiring Hospitalization



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Timby N, et al. *J Pediatr Gastroenterol Nutr*. 2015;60:384-389.

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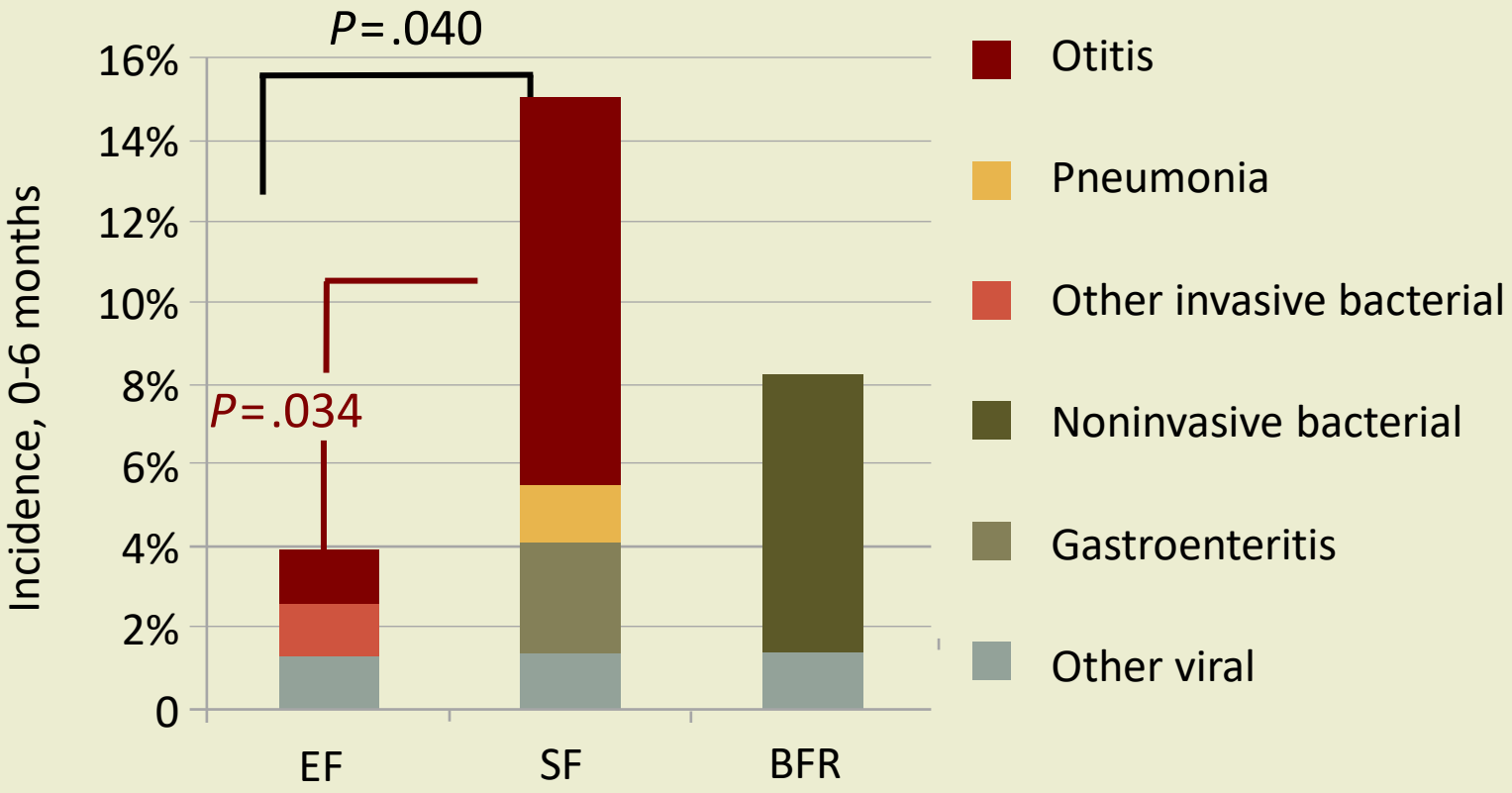

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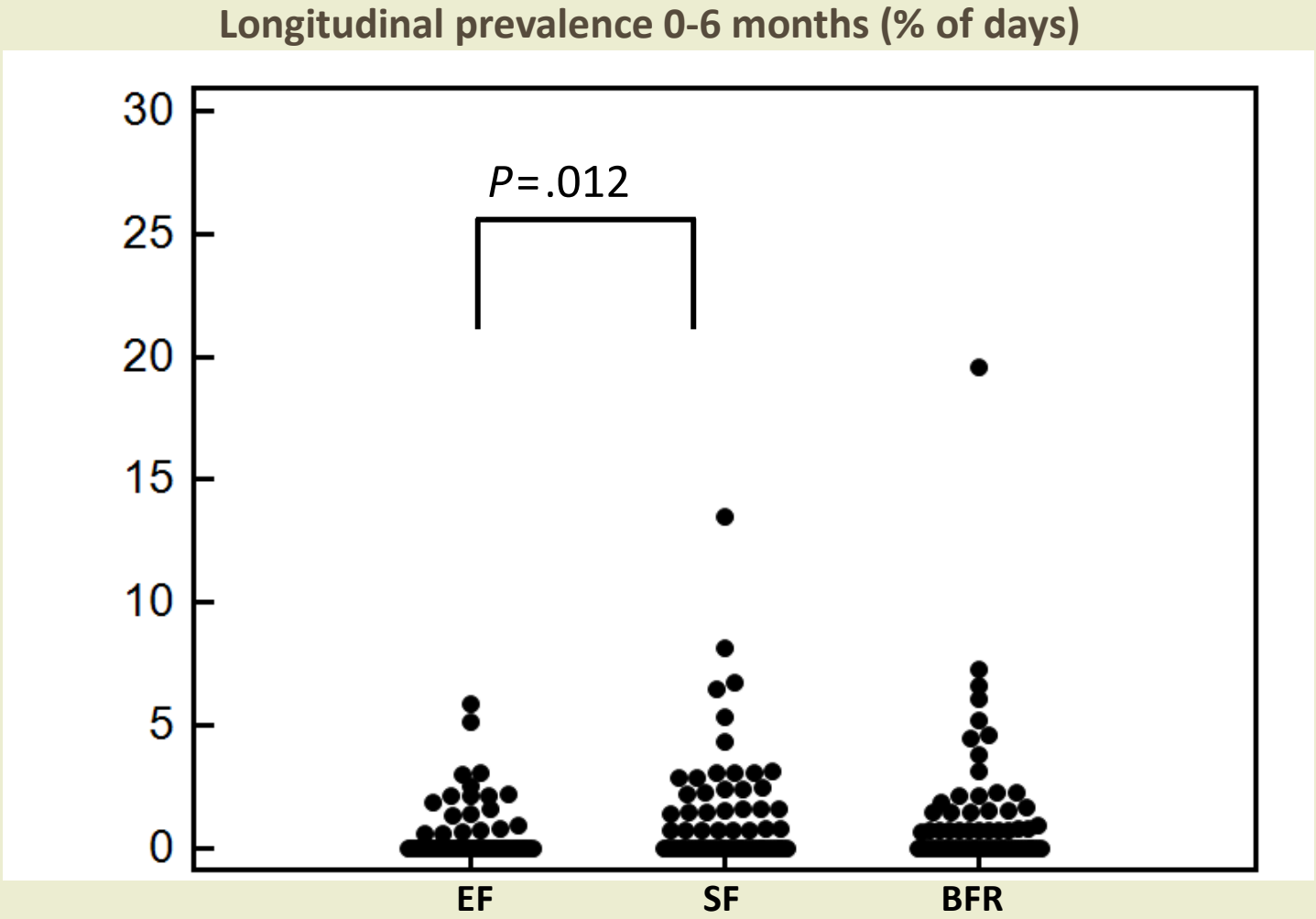
Use of Antipyretics



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Conclusions From the Tumme Study

- MFGM supplementation reduces the risk of AOM and use of antipyretics at 6 months of age
- MFGM improves cognitive function at 12 months
- MFGM in breast milk may thus explain part of the observed differences in infection frequency and cognitive development between breast-fed and formula-fed infants

AOM, acute otitis media; MFGM, milk fat globule membrane.

Timby N, et al. *J Pediatr Gastroenterol Nutr*. 2015;60:384-389.

Disclosures

- Funding: Sweden´s Innovation Agency (Vinnova), the Västerbotten County Council and Semper AB
- Bo Lönnerdal and Olle Hernell are members of the SAB of Semper AB

Other Studies on Feeding MFGM to Infants and Children

MFGM, milk fat globule membrane.

Adverse Events in Infants Fed Formula Supplemented with MFGM

	Control (n=57)	MFGM-l (n=70)	MFGM-p (n=72)
	n (%)		
Conjunctivitis	4 (7.0)	3 (4.3)	6 (8.3)
Constipation	2 (3.5)	8 (11.4)	7 (9.7)
Diarrhea	4 (7.0)	4 (5.7)	7 (9.7)
Infantile colic	5 (8.8)	5 (7.1)	5 (6.9)
Regurgitation	8 (14.0)	7 (10.0)	5 (6.9)
Vomiting	4 (7.0)	2 (2.9)	3 (4.2)
Pyrexia	4 (7.0)	5 (7.1)	6 (8.3)
Bronchiolitis	11 (19.3)	9 (12.9)	7 (9.7)
Bronchitis	3 (5.3)	4 (5.7)	8 (11.1)
Ear infection	10 (17.5)	3 (4.3)	6 (8.3)
Gastroenteritis	5 (8.8)	8 (11.4)	5 (6.9)
Nasopharyngitis	15 (26.3)	18 (25.7)	15 (20.8)
Oral candidiasis	5 (8.8)	5 (7.1)	1 (1.4)
Rhinitis	2 (3.5)	5 (7.1)	5 (6.9)
Eczema*	2 (3.5)	1 (1.4)	10 (13.9)

Note: *$P = .001$ for *post hoc* global comparison across all groups. Randomized controlled trial of healthy, term infants 14 d - 4 mo (day 112). The MFGM-enriched formulas contained different MFGM fractions providing different levels of phospholipids while maintaining an overall similar nutrient profile. The MFGM-p formula was the same as that used in the Timby et al study.

MFGM, milk fat globule membrane; MFGM-l, lipid-rich MFGM fraction; MFGM-p, protein-rich MFGM fraction.

Billeaud C, et al. *Clin Med Insights Pediatr*. 2014;8:51-60; Timby N, et al. *Am J Clin Nutr*. 2014;99:860-868.

Rash in the Tumme Study

- Skin reactions assessed by prospective parental diary each day when the infant had a rash during the intervention (until 6 months)

Rash	EF	SF	*P* value	BFR
Incidence rash (%)	17	26	.22	30
Longitudinal prevalence (median) (% of days)	0.0	0.8		4.1
Longitudinal prevalence 95th percentile (% of days)	4.8	15.6	.21	36

BFR, breast-fed reference; EF, experimental formula; SF, standard formula.

Timby N, et al. *Clin Med Insights Pediatr*. 2015;9:63-64.

Griffith Scale Scores at 6 Months in Infants Fed Ganglioside-Supplemented or Control Formula From 2-8 Weeks of Age

Griffith Scale	*P* value	Treatment group[a] (n=29)	Control group[a] (n=30)	Breast-fed group[b] (n=32)
Locomotor IQ	.225	120.0 (114.3–123.2)	117.2 (111.1–123.2)	113.7 (110.9–116.5)
Personal–social IQ	.368	121.2 (115.1–127.4)	119.0 (112.5–125.5)	115.4 (112.0–118.8)
Hearing and speech IQ	.114	120.3 (114.7–126.0)	116.7 (110.7–122.7)	115.1 (112.1–118.1)
Hand and eye coordination IQ	.006	129.5 (123.0–136.0)	122.0 (115.1–128.9)	123.9 (120.3–127.6)
Performance IQ	.001	131.1 (125.7–136.5)	123.2 (117.5–128.9)	127.8 (124.9–130.8)
General IQ	.041	125.4 (119.7–131.1)	120.6 (114.6–126.7)	120.0 (116.8–123.2)

DBRCT in which infants received the treatment or control product from 2 to 8 weeks of age until 24 weeks of age. The control group (n=30) received standard infant formula and the treatment group (n=29) received the same formula supplemented with complex milk lipids to increase the ganglioside content to approximately 11-12 µg/ml.

[a]Results based on analysis of variance for the effect of treatment group after adjustment for age, Hb, and TIBC at baseline, as well as socioeconomic factors such as family size, mother's education and occupation, and father's occupation.
[b]Raw means.

DBRCT, double-blind, randomized controlled trial; Hb, hemoglobin; TIBC, total iron binding capacity. Gurnida DA. et al. *Early Hum Dev*. 2012;88:595-601.

MFGM-Enriched Formula Drink Decreases Febrile Periods and May Improve Behavior in Children

- Prospective, double-blind RCT in healthy 2.5 to 6-year-old children (4.4+0.9 y); 182/253 (72%) completed the study
- 4-month daily intake of 200 mL formula with or without enrichment with MFGM (INPULSE, Bullinger SA, Belgium, 9-fold enrichment in phospholipids)
- Data collected from parental diaries
- Primary endpoints: Days with fever, diarrhea, coughing, or constipation
- Secondary endpoints: Doctor visits, medication intake, number of missed school days, acceptability of the test drinks, and safety

MFGM, milk fat globule membrane; RCT, randomized controlled trial.

Veereman-Wauters G, et al. *Nutrition*. 2012;28:749-752.

MFGM Reduced Number of Days With Fever

Supplement	Fever	Diarrhea	Constipation	Coughing	Doctor visit	Medication	School missed
Placebo							
Mean	2.60	1.72	0.42	14.41	1.14	16.88	3.09
SD	3.06	2.83	1.68	13.46	1.42	21.40	3.89
IMPULSE							
Mean	1.71	1.51	0.39	14.89	1.11	14.32	2.47
SD	2.47	2.40	1.77	17.37	1.38	18.82	3.30
Total							
Mean	2.18	1.62	0.41	14.64	1.13	15.68	2.80
SD	2.83	2.63	1.72	15.37	1.40	20.22	3.63
Difference (IMPULSE versus placebo)	-0.89	-0.22	-0.03	0.48	-0.04	-2.56	-0.62
Difference versus placebo (%)	-34.34	-12.53	-8.15	3.34	-3.36	-15.16	-20.12
P*	**.028**	.890	.844	.445	.965	.516	.431

*Days of fever (>38.5˚C) and short febrile periods (<3 d) were the only outcome variables (primary and secondary) that were significantly different

MFGM, milk fat globule membrane.

Veereman-Wauters G, et al. *Nutrition*. 2012;28:749-752.

MFGM Improved Behavior?

- Child Behavior Check List (CBCL) 1.5-5 y and the Achenbach System of Empirically Based Assessment (ASEBA, standardized questionnaires referring to emotions and behavior) for 6-18 y
- Completed for 169/253 (67%) of the children
- Significant differences in internal ($P<.003$), external ($P<.004$), and total ($P<.002$) problem scores in favor of the intervention group
  - Between-subjects effects were highly correlated (internal, $P<.003$; external, $P<.005$; total, $P<.002$; one-way analysis of variance)
- Teachers returned 105 valid questionnaires. Based on their answers, there were no significant differences in internal, external, and total problem scores
- ***No evaluation at baseline!***

MFGM, milk fat globule membrane.

Veereman-Wauters G, et al. *Nutrition*. 2012;28:749-752.

General Conclusions

- MFGM is not a well-defined fraction
- Different MFGM fractions have been used in published studies
  - Lipid-enriched, phospholipid-enriched, protein-enriched, ganglioside-enriched
- Studies are not comparable
- Positive outcomes for cognitive development (Bayley III at 12 mo), IQ (Griffith at 6 mo), infections (AOM 1.5-6 mo), days of fever (2.5-6 y), diarrhea (6-11 mo)
- One study showed increased risk of eczema with a protein-enriched fraction. Results not convincing
- More studies needed with comparable fractions

AOM, acute otitis media; CBQ, child behavior questionnaire; MFGM, milk fat globule membrane.

Milk Fat Globule Membrane: A Case of Throwing the Baby Out With the Bathwater?

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Questions & Answers