Milk macronutrients and HMOs: Taking a closer look

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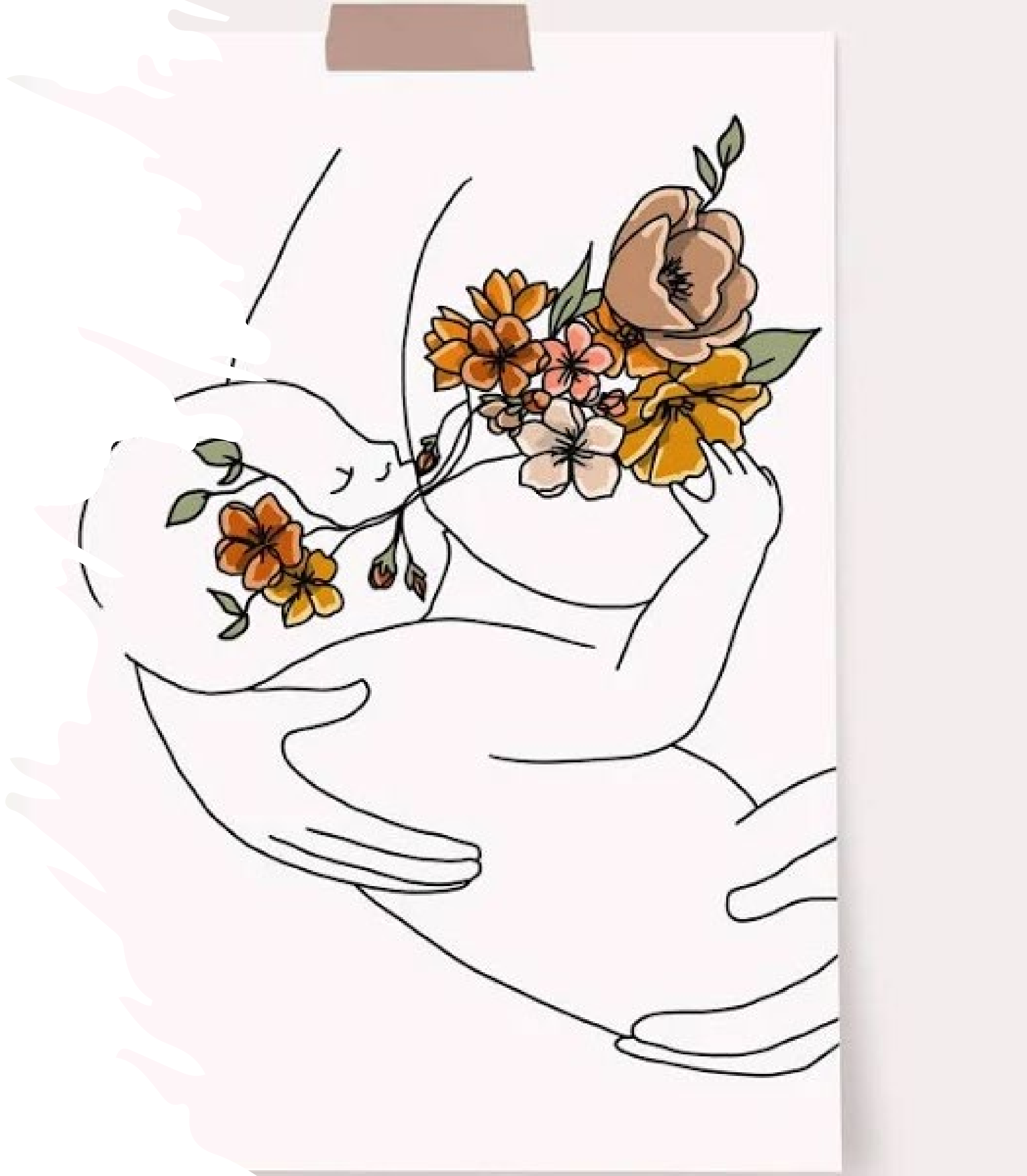


Disclosures

- No financial disclosures
- Comment: Human milk composition variation information is derived from breastfeeding studies of individuals who were cis-gendered females, and I will refer to participants in the studies variably as breastfeeding women, breastfeeding individuals, and mothers.
- However, there are increasing cases of successful lactation induction in transgender women. Breastmilk variation in transgender women, as well as in transgender men, is an important area of current research.

Objectives

Know	Recognize	Understand	Increase
Know current average reference values for milk macronutrients at different stages of lactation	Recognize that milk composition varies within and between women	Understand the factors that may play a role in shaping milk macronutrient composition	Increase awareness of role of human milk macronutrient and oligosaccharide (HMO) in infant health

Outline of this presentation

Review	New data	Hot topics
Review of the literature on causes and consequences of milk macronutrient concentrations	MILK Study data on individual-differences in milk composition in an exclusively breastfeeding cohort	Milk composition in transgender women Cannabis during breastfeeding Lab-grown milk substitute

Importance of breastfeeding to national health

Infant health: If 90% of US families had the opportunity to breastfeed exclusively for 6 months, the United States would save $13 billion/year and prevent an excess 911 deaths, almost all of which would be infant deaths

Maternal health: Low breastfeeding rates may cause as many as 5,000 cases of breast cancer, nearly 54,000 cases of hypertension and almost 14,000 heart attacks each year in the US.

Falling short: Only 37% of Minnesotan and 25% of US families meet these targets and their own breastfeeding goals, with significant disparities across race and ethnic groups

Sources: CDC 2022 Breastfeeding Report Card; Bartick et al, Matern Child Nutr. 2017 Jan;13(1): e12366

Breastfeeding and lactation: A socioecological model

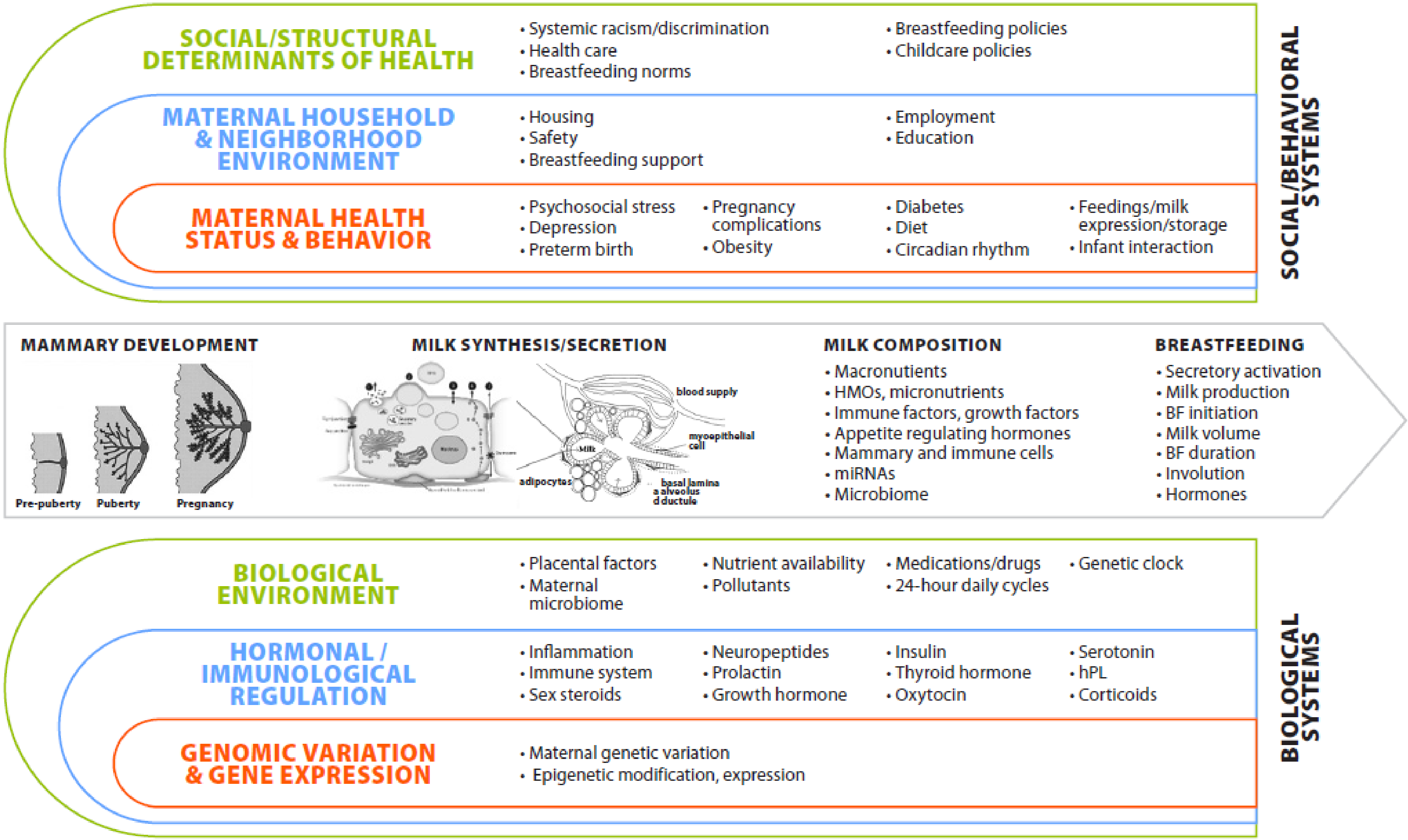
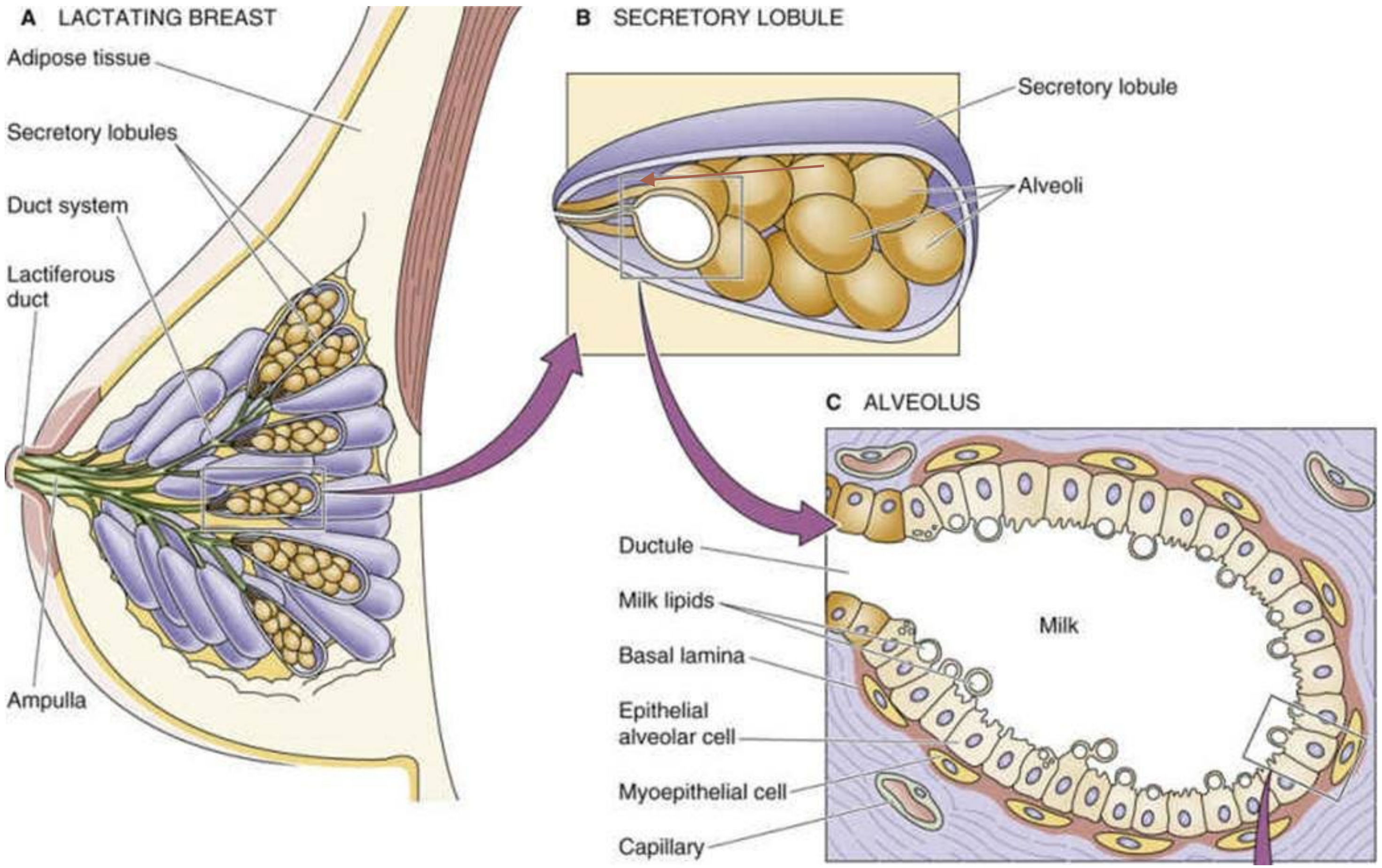
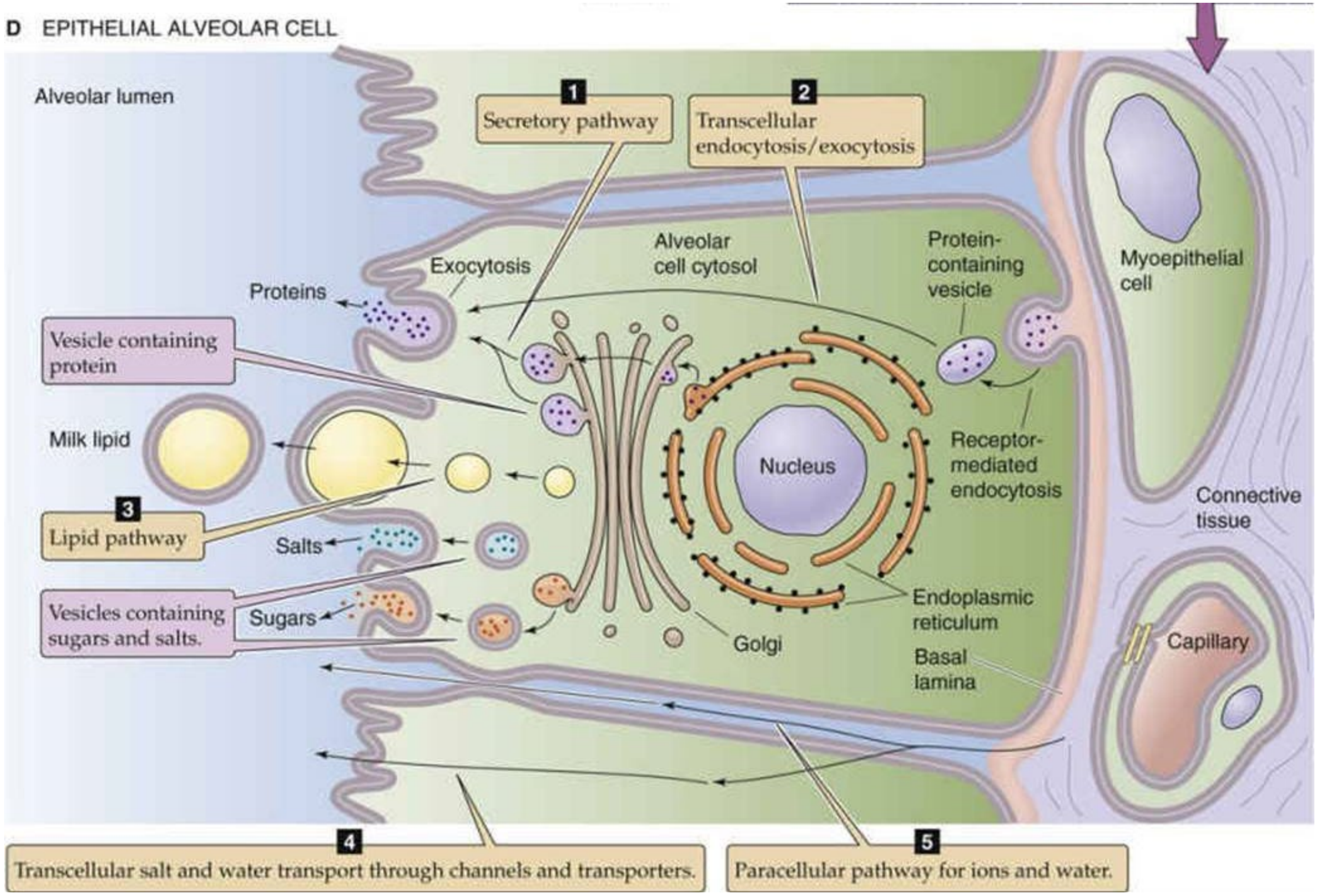


FIGURE 1. The Mammary system: inputs and outputs. MFG, milk fat globule; RER, rough endoplasmic reticulum; HMO, human milk oligosacchaide; hPL, human placental lactogen. *Diagram from Ellen Demerath.*

Mammary gland



Cellular processes involved in human milk synthesis

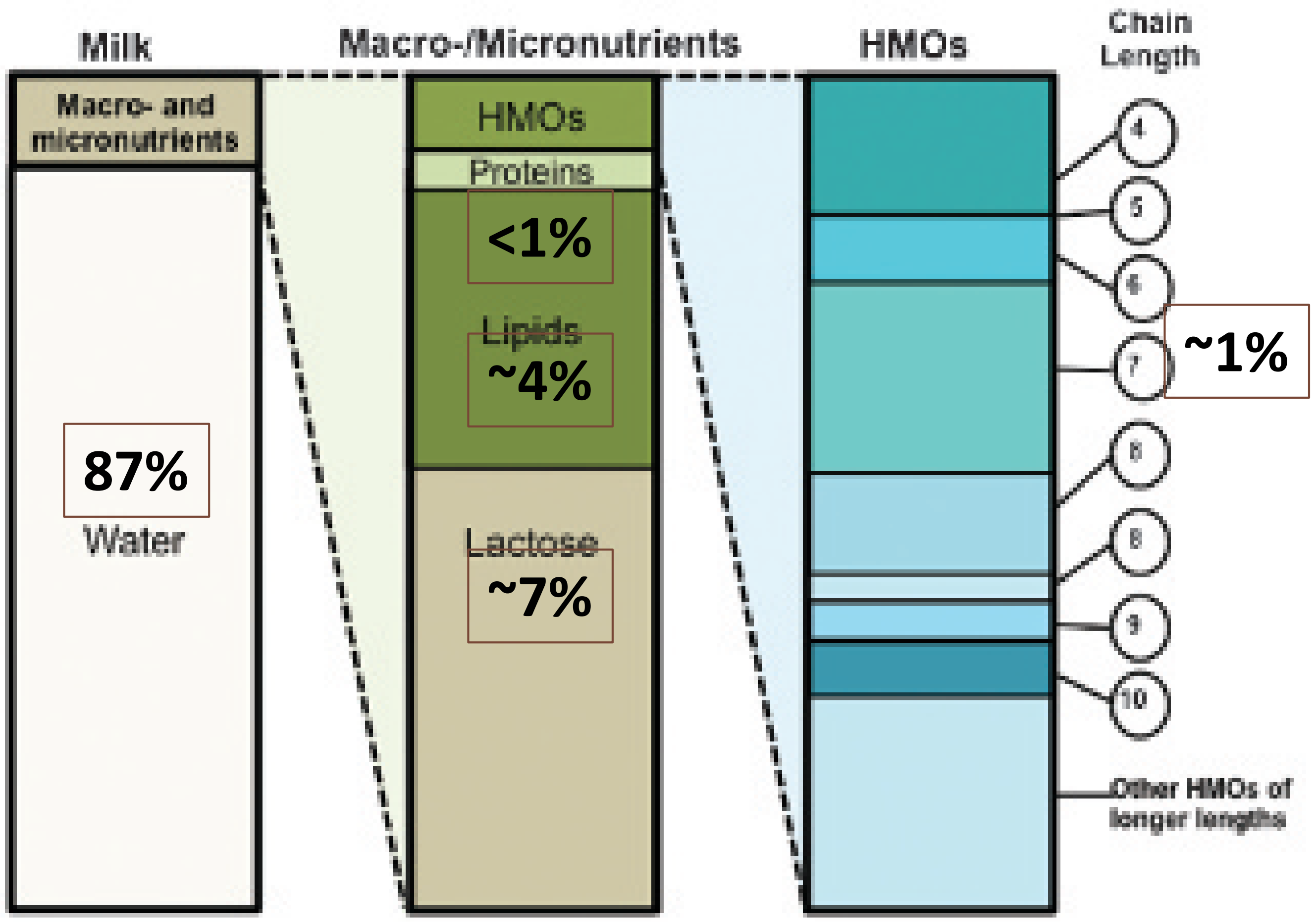


Human milk co-evolved with human physical development

111.—Skeleton of Man. 112.—Skeleton of Chimpanzee

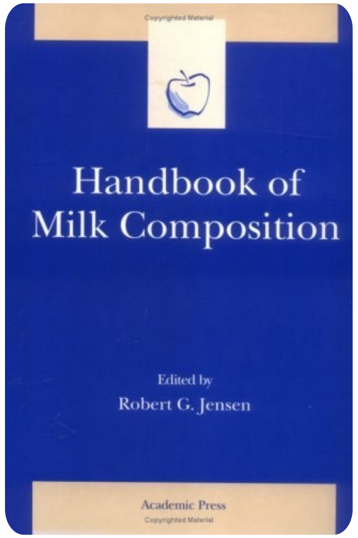
- Human milk is rich in carbohydrates but relatively poor in amino acids and fat compared to non-primate milk.
- High milk sugar supports large, rapidly growing infant brain
- Lower protein and calories supports slower human physical development

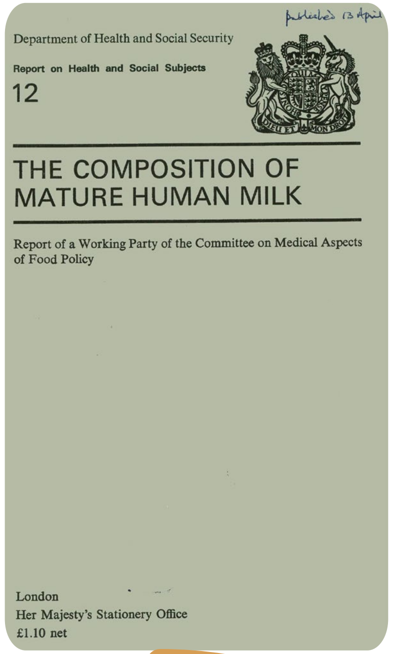
Mature milk composition: Major components



Macronutrient reference values: where do they come from?

- Nutritional analyses of human milk: 1950s–1980s
- Gold standard milk collection
  - 24-hour pooled sampling
- Gold-standard laboratory milk analysis methods
- Usually exclusively breastfeeding, highly educated participants





Elsie Widdowson, PhD
British Nutritionist

Milk Reference Value charts present just a few of studies

Macronutrient (g/dL) and energy (kcal/dL) composition of human milk from specified references

Author (year), n	Protein Mean (± 2 SD)	Fat Mean (± 2 SD)	Lactose Mean (± 2 SD)	Energy Mean (± 2 SD)
Term infants, 24-hour collection, mature milk				
Nommsen et al (1991), n=58	1.2 (0.9, 1.5)	3.6 (2.2, 5.0)	7.4 (7.2, 7.7)	70 (57, 83)
Donor human milk samples				
Wojcik et al (2009), n=415	1.2 (0.7, 1.7)	3.2 (1.2, 5.2)	7.8 (6.0, 9.6)	65 (43, 87)
Michaelsen et al (1990), n=2553	[a] 0.9 (0.6, 1.4)	[a] 3.6 (1.8, 8.9)	[a] 7.2 (6.4, 7.6)	[a] 67 (50,115)
Representative values of mature milk, term infants				
Reference standard	0.9	3.5	6.7	65 to 70
Preterm, 24-hour collection, first 8 weeks of life				
Bauer & Gerss (2011)				
Born <29 weeks, n=52	2.2 (1.3, 3.3)	4.4 (2.6, 6.2)	7.6 (6.4, 8.8)	78 (61, 94)
Born 32-33 weeks, n=20	1.9 (1.3, 2.5)	4.8 (2.8, 6.8)	7.5 (6.5, 8.5)	77 (64, 89)
Preterm donor milk				
Hartmann (2012), n=47	1.4 (0.8, 1.9)	4.2 (2.4, 5.9)	6.7 (5.5, 7.9)	70 (53, 87)

[a]

Nommsen et al 1991 values are among the most frequently cited

TABLE 2
Milk volume and composition*

	Month of lactation			
	3 mo (n = 58)	6 mo (n = 45)	9 mo (n = 28)	12 mo (n = 21)
Volume consumed by infant (g/d)	811 ± 133	780 ± 185	674 ± 236	514 ± 238
Volume produced (g/d)	895 ± 200	844 ± 237	750 ± 252	516 ± 232
Protein (g/L)	12.1 ± 1.5	11.4 ± 1.5	11.6 ± 1.8	12.4 ± 1.5
Lipid (g/L)	36.2 ± 7.0	37.7 ± 9.6	38.1 ± 8.0	37.2 ± 11.3
Lactose (g/L)	74.4 ± 1.5	74.4 ± 1.9	73.5 ± 2.9	74.0 ± 2.7
Gross energy (kcal/L)	697 ± 67	707 ± 92	709 ± 74	706 ± 110

* $\bar{x}$ ± SD.

A recent review of US milk studies show a decent amount of macronutrient variation depending on population characteristics and measurement method

Human milk *energy* in US and Canada 1980-2017 (kcal/100 ml)

Ref[1]	Energy 1–6 mo Mean	Energy 1–6 mo SD	Energy 7–12 mo Mean	Energy 7–12 mo SD
Nommsen et al. (35)	68.09	7.81	68.62	9.09
Butte et al. (111)	64.00	7.07	—	—
Gross et al. (92)	67.07	11.74	—	—
Butte et al. (90)	65.62	9.89	—	—
Ferris et al. (130)	75.43	10.71	—	—
Garza and Butte (64)[2]	70.47	9.35	—	—
Dewey and Lonnerdal (91)	73.59	14.04	—	—
Butte et al. (128)	64.50	8.79	—	—
Garza et al. (125)	55.87	3.98	—	—

[1]Ref, reference.
[2]24-h collection.

Source: Wu X. Human Milk Nutrient Composition in the United States: Curr Dev Nutr. 2018 May 31;2(7):nzy025.

US milk composition data: In need of updates

- 2018 USDA review of US milk composition data (Wu et al., 2018)
- **Only 28 US and Canadian milk compositional studies**
- **Out of date**: Only 6 studies since 2000
- **Small: 26/28 had N< 60**
- Variety of methods of milk collection and analysis
- **Gaps:**
  - **Need method-specific reference values**
  - **Need greater diversity and participants reflecting typical US body weight and diet**
  - **Need info on lactation > 7-12 months**

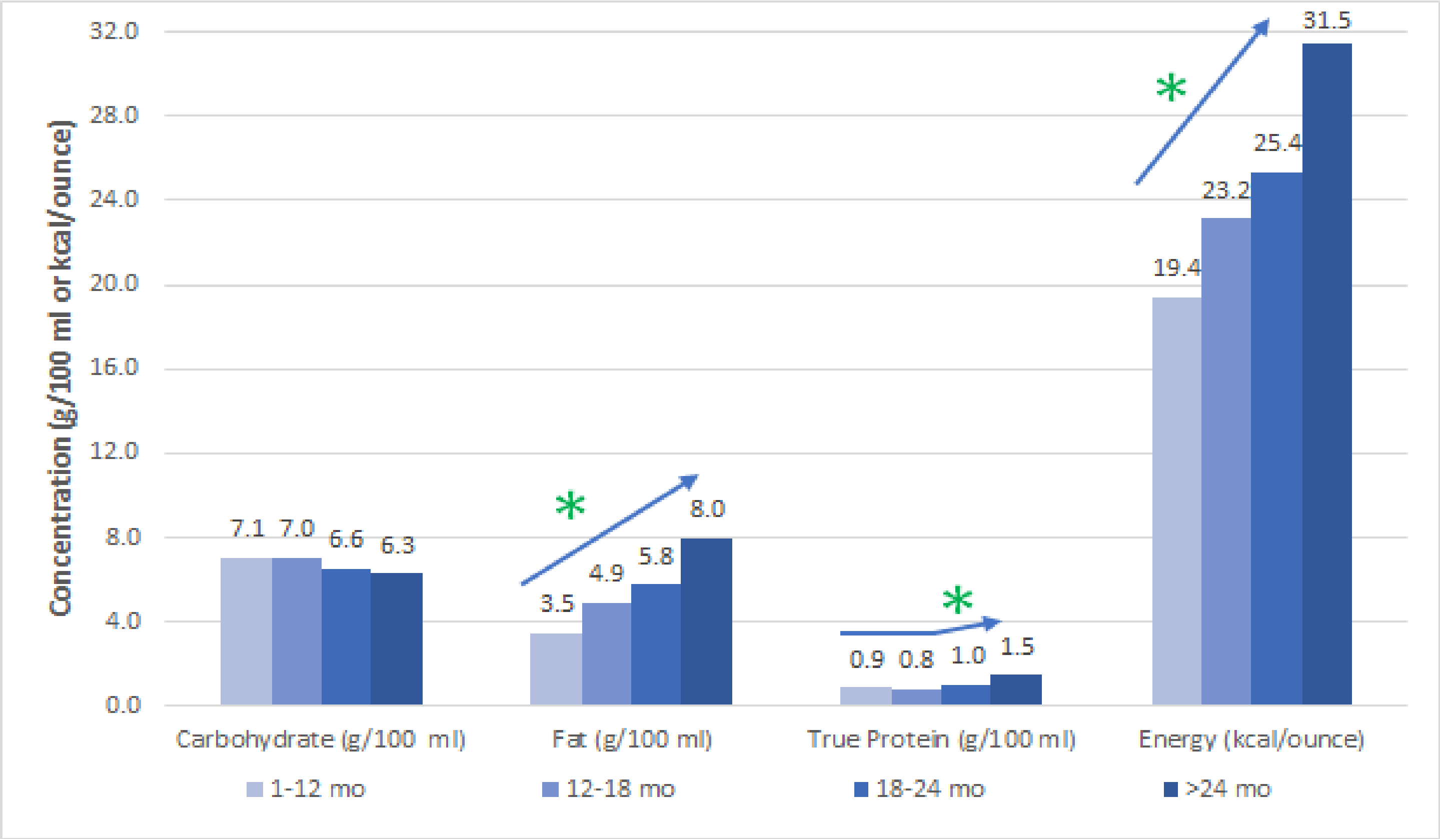
Source: Wu et al (2018) Curr Dev Nutr 2018;2:nzy025.

Best to refer to large systematic reviews and meta-analysis rather than single studies

Term	Energy (kcal)	Protein (g)	Fat (g)
1st week	60 (44–77)	1.8 (0.4-3.2)	2.2 (0.7-3.7)
2nd week	67 (47–86)	1.3 (0.8-1.8)	3.0 (1.2-4.8)
Week 3/4	66 (48–85)	1.2 (0.8-1.6)	3.3 (1.6-5.1)
Week 10/12	68 (50–86)	0.9 (0.6-1.2)	3.4 (1.6-5.2)

Source: Gidrewicz and Fenton BMC Pediatrics 2014; 14:216

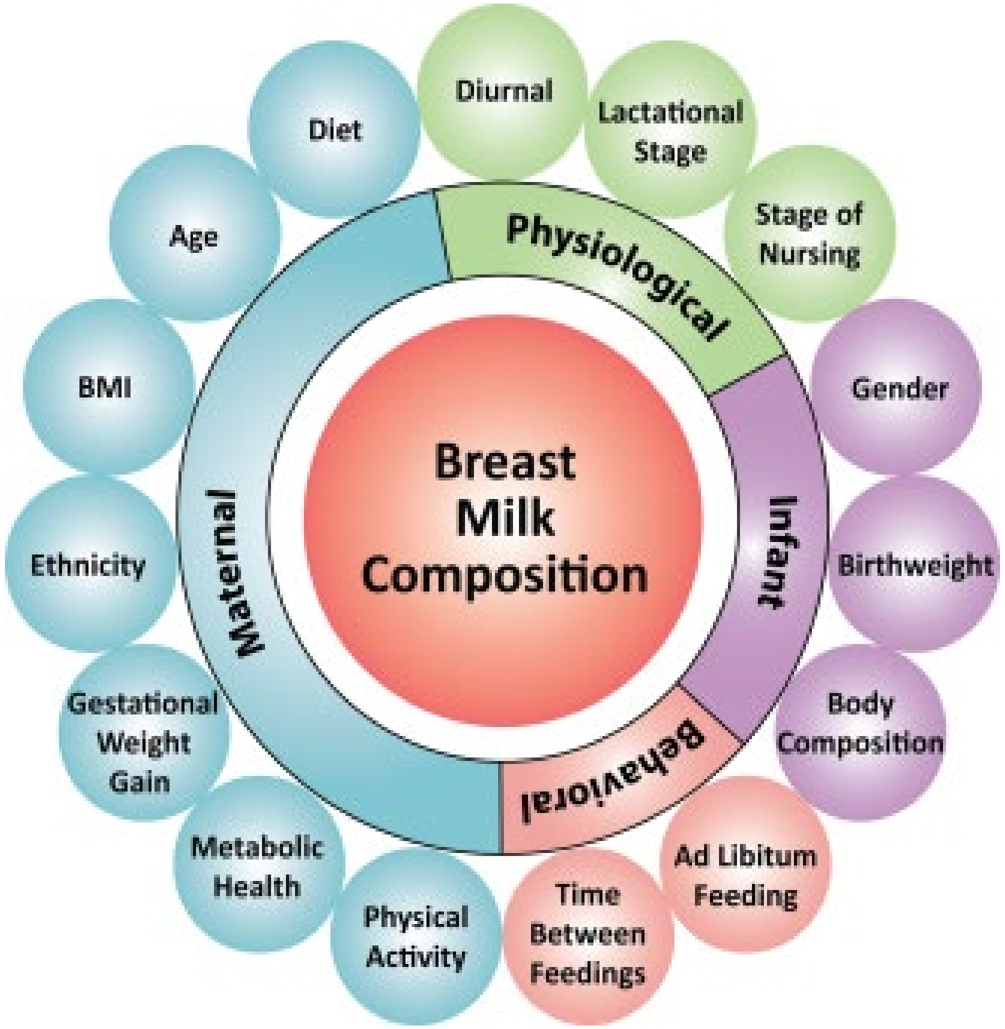
Macronutrient changes during prolonged lactation: Beyond 12 mo



Method = Miris; Total N = 136; Poland

Source: Czosnykowska-Łukacka et al. Nutrients. 2018 Dec 3;10(12):1893.

What explains differences in milk composition?



From Fields et al. (2016) Obesity. 24 (6): 1213–1221

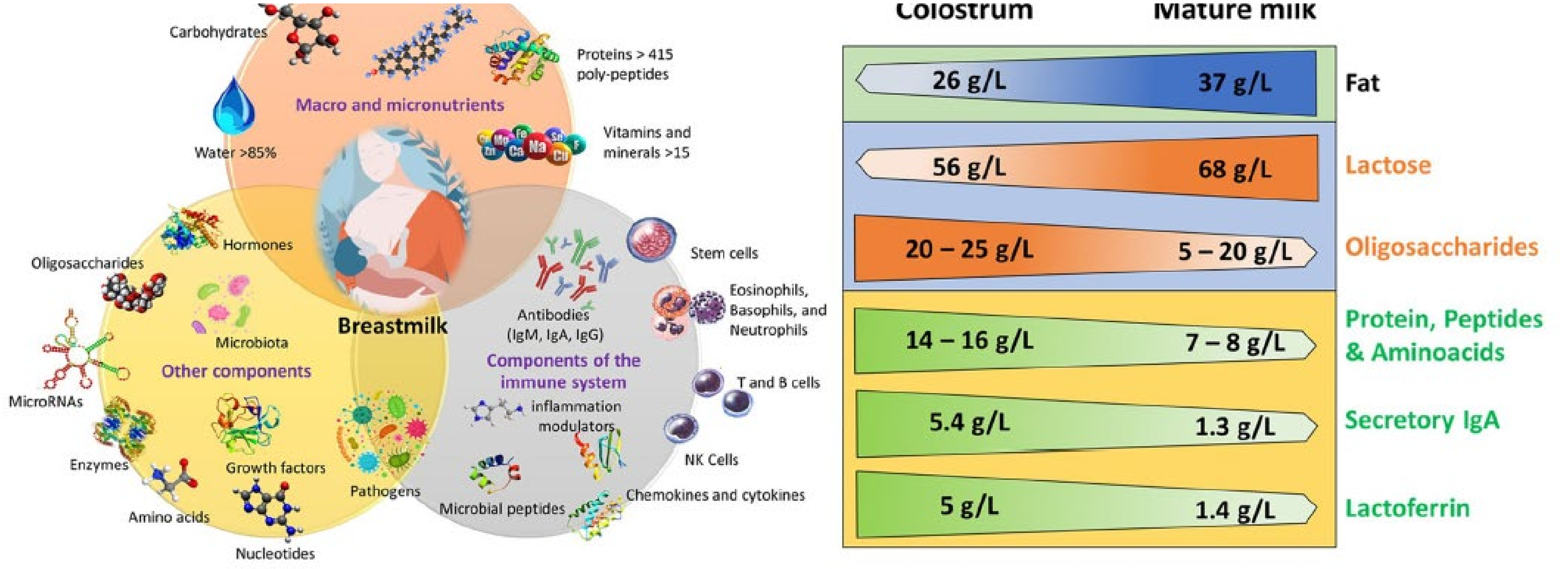


FIGURE 1 | Components of human milk and differences between colostrum and mature milk. **(A)** Macro and micronutrients, components of the immune system, and

Components of human milk and colostrum vs mature milk

Source: Caba-Flores (2022) Front. Nutr., 12 May 2022; Volume 9 – 2022. https://doi.org/10.3389/fnut.2022.867507

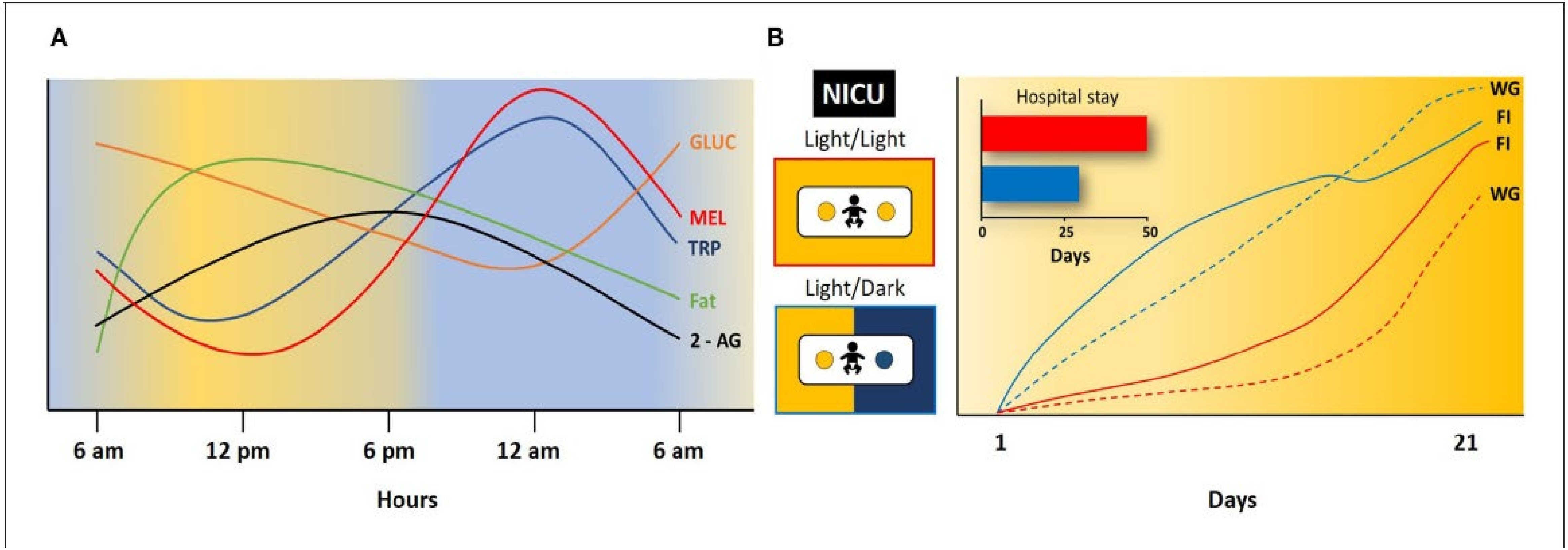
Table 2. FACTORS INFLUENCING HUMAN MILK FAT CONTENT AND COMPOSITION

Factor	Influence
Duration of gestation	Shortened gestation increases the long-chain polyunsaturated fatty acids secreted.
Stage of lactation	Phospholipid and cholesterol contents are highest in early lactation.
Parity	High parity is associated with reduced endogenous fatty acid synthesis.
Volume	High volume is associated with low milk fat content.
Feeding	Human milk fat content progressively increases during a single nursing.
Maternal diet	A diet low in fat increases endogenous synthesis of medium chain fatty acids (C6 to C10).
Maternal energy status	A high weight gain in pregnancy is associated with increased milk fat.

Determinants of human milk fat variation

Source: Picciano, Ped Clinics N. Amer. 2001; 48(1):53-67, 2001

Time of day effects Chronobiology of Milk



Source: Caba-Flores (2022) Front. Nutr., 12 May 2022; Volume 9 - 2022
https://doi.org/10.3389/fnut.2022.867507

Storage effects

- In unpasteurized milk, storage can result in a reduction in antioxidant activity and an increase in lipolysis even when frozen at -20 C
- A study comparing paired samples of raw milk and frozen milk (at -20 C) found:
  - Clinically significant decrease in total lipids and calories
    - 3% reduction after one week
    - 9% reduction after 3 months
  - Lactose and protein relatively stable

Source: Garcia-Lara et al. Breastfeed Med. 2012 Aug; 7(4): 295–301.

Maternal weight status effects

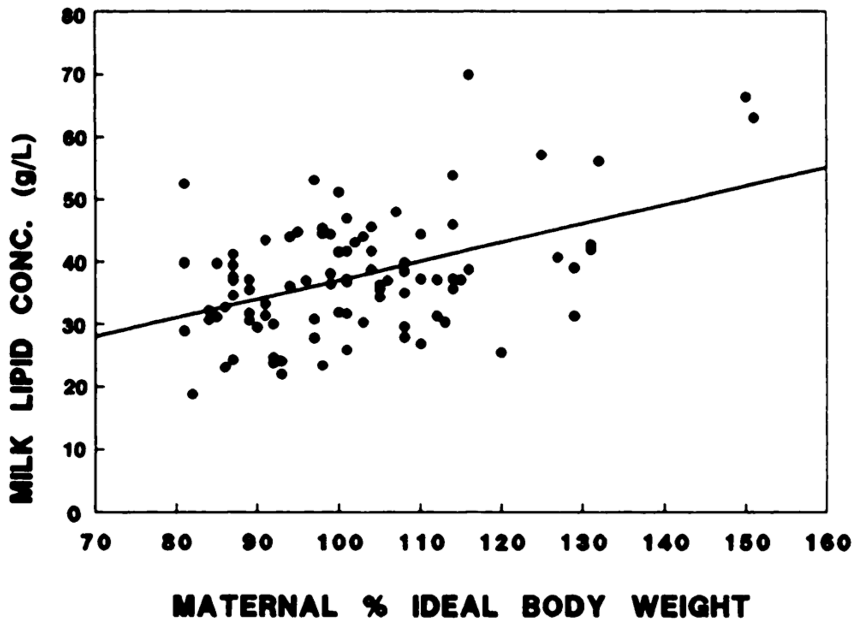


FIG 1. Milk lipid concentration and maternal percent of ideal body weight; data from 6, 9, and 12 mo combined. $r = 0.46$, $P < 0.01$, $y = 0.3x + 7.0$.

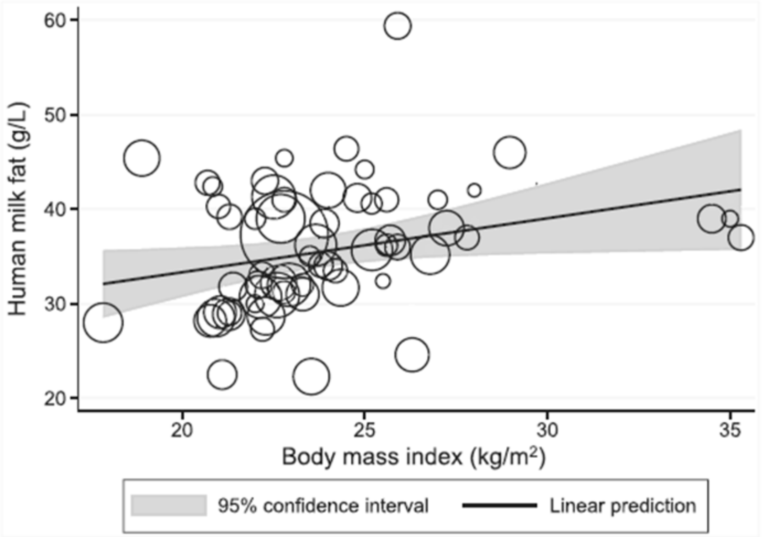


FIGURE 3 Meta-regression of the relation between maternal BMI and human-milk fat. The bubble sizes in this meta-regression are proportional to the inverse of the study-level SE for human-milk fat. The solid line represents the linear prediction for the means of human-milk fat as a function of the mean BMI observed at the study level (β: 0.56 g/L; 95% CI: 0.034, 1.1; $P = 0.04$, $I^2 = 93.7\%$, $n = 63$ datapoints).

Source: Nommsen et al Am J Clin Nutr 1991; 53:457-651.

Daniels et al Am J Clin Nutr 202; 113:1009-1022.

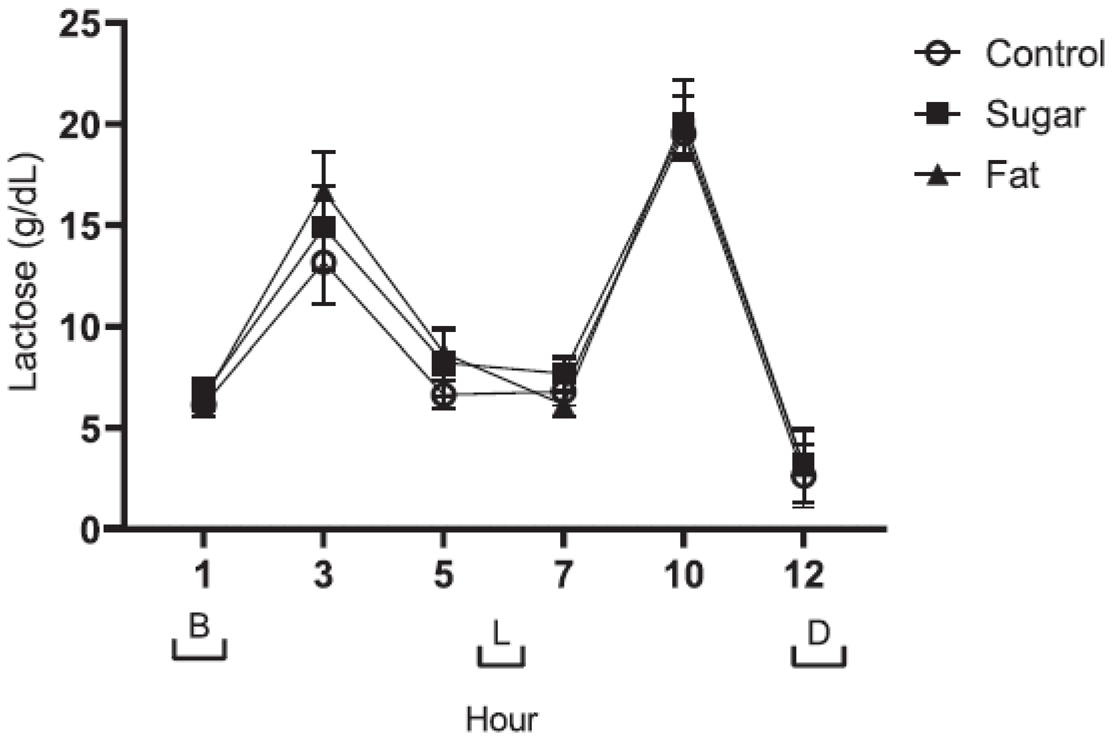
Effects of maternal dietary intervention

DIETARY INTERVENTION	MILK COMPOSITION
High dairy fat vs Low dairy fat intake	+ Total milk lipid
Fish Oil supplement	+ DHA, EPA
Alph lineoleic acid (ALA) supplement	+ALA
DHA supplement	+DHA
High Fat diet vs isocaloric High CHO diet*	+13% total milk fat and energy (no effect on lactose, protein)*

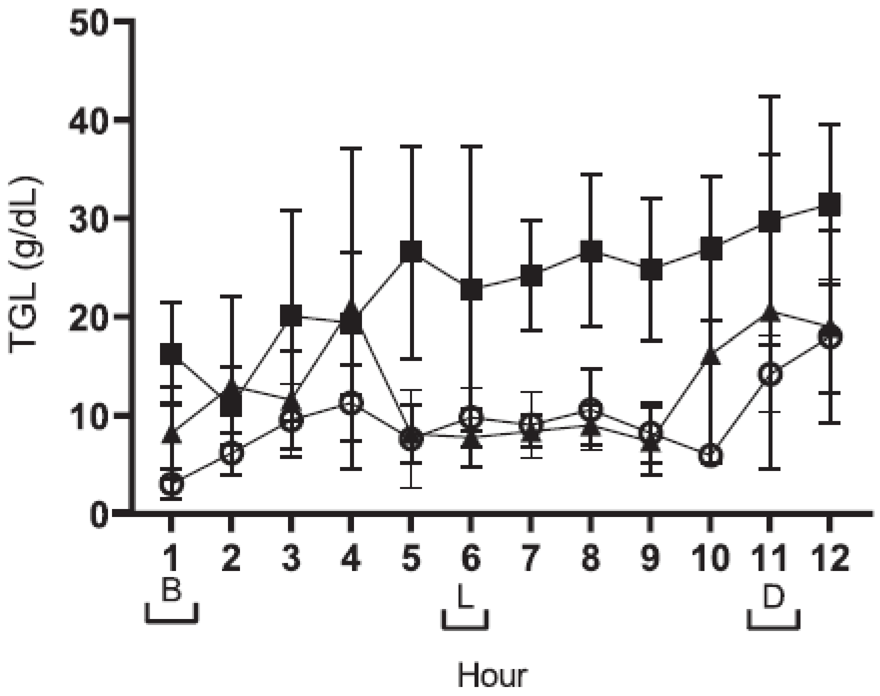
Sources: Keikha et al Breastfeed Med. 2017; 12(9): 517-27
*Mahmoud et al. AJCN 2009 89(6): 1821-7

Rapid changes in milk composition over 12 hours on a high carb or high sugar controlled diet

Lactose increases after meals

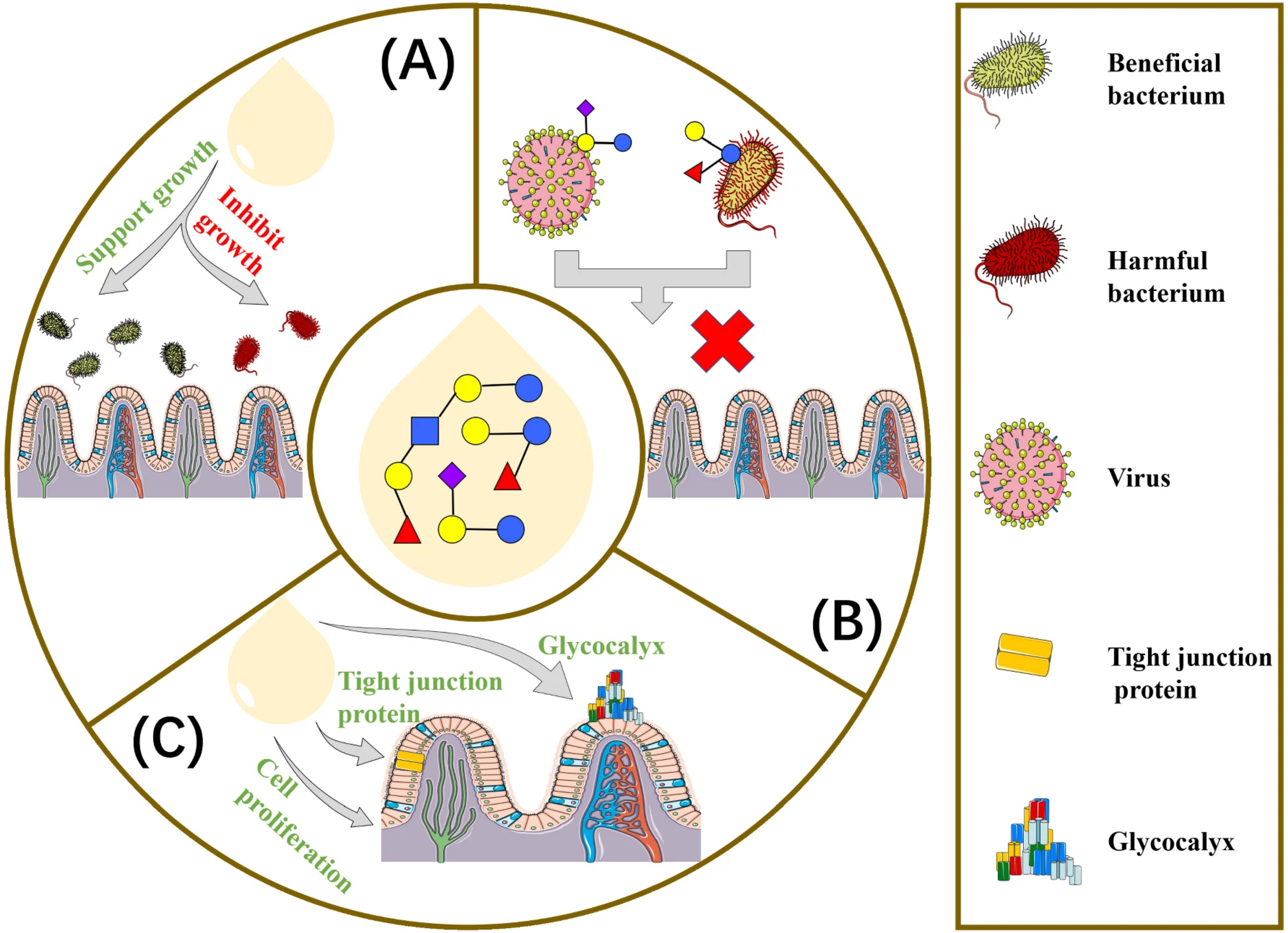


High carb diet results in higher milk fat



Source: Ward et al Matern Child Nutr. 2021;17:e13168.

The importance of HMOs for infant health



HMOs and preterm infant necrotizing enterocolitis (NEC)

- NEC is the most common, serious gastrointestinal disease affecting newborn infants, and is a medical emergency. It is most commonly seen in premature infants.
- Preterm infants fed human milk have far lower risk of NEC than those fed formula
- Particular HMOs in human milk likely explain this benefit:
  - Infants with NEC had lower relative abundance of *Bifidobacterium longum* and higher relative abundance of *Enterobacter cloacae* than controls
  - Breastfed infants and infants fed formula with 2'FL had 29-83% lower concentrations of plasma inflammatory cytokines and TNF-a than infants fed control formula

Sources: Puccio et al Pd Gast Nutr 2017; 64: 624-31; Masi et al. Gut. 2021 Dec; 70(12):2273-2282;. Goerring et al J Nutr 2016; 146: 2559-66

Milk macronutrient concentrations and infant growth and adiposity

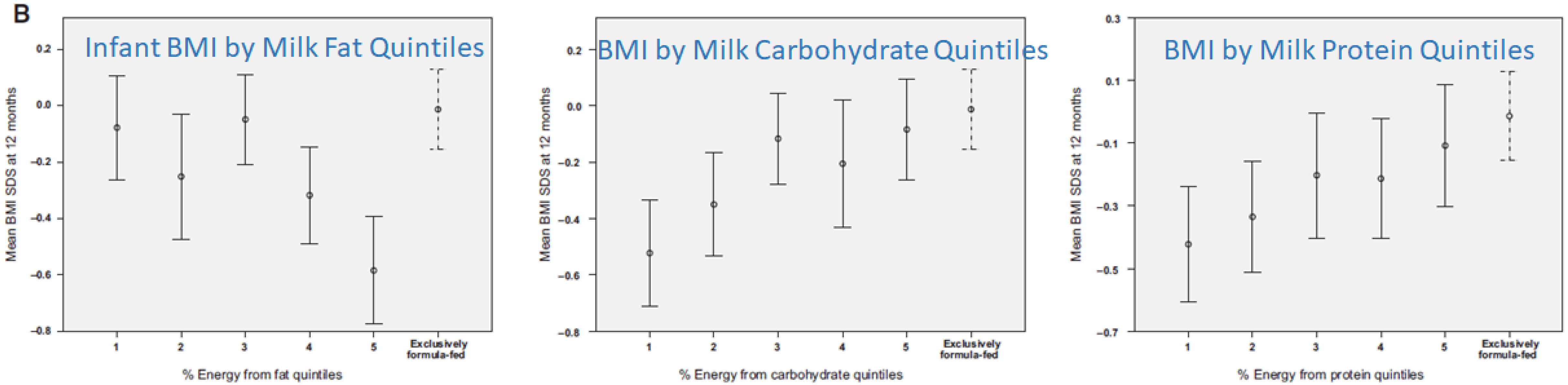
Milk composition and Infant Growth:

Few studies; Little consensus

Milk Factor	Direction of effect on infant weight
Protein	+0.3
Lactose	+0.2
n6/n3 fatty acid ratio	+0.1
HMO: 3'SL	+0.05
HMO:SSLNT	+0.05
HMO: LNFPIII	0.05
HMO: SLNNT	-0.05
HMO: LNFPI	-0.05
HMO diversity	-0.10
Total fat (lipids)	-0.42

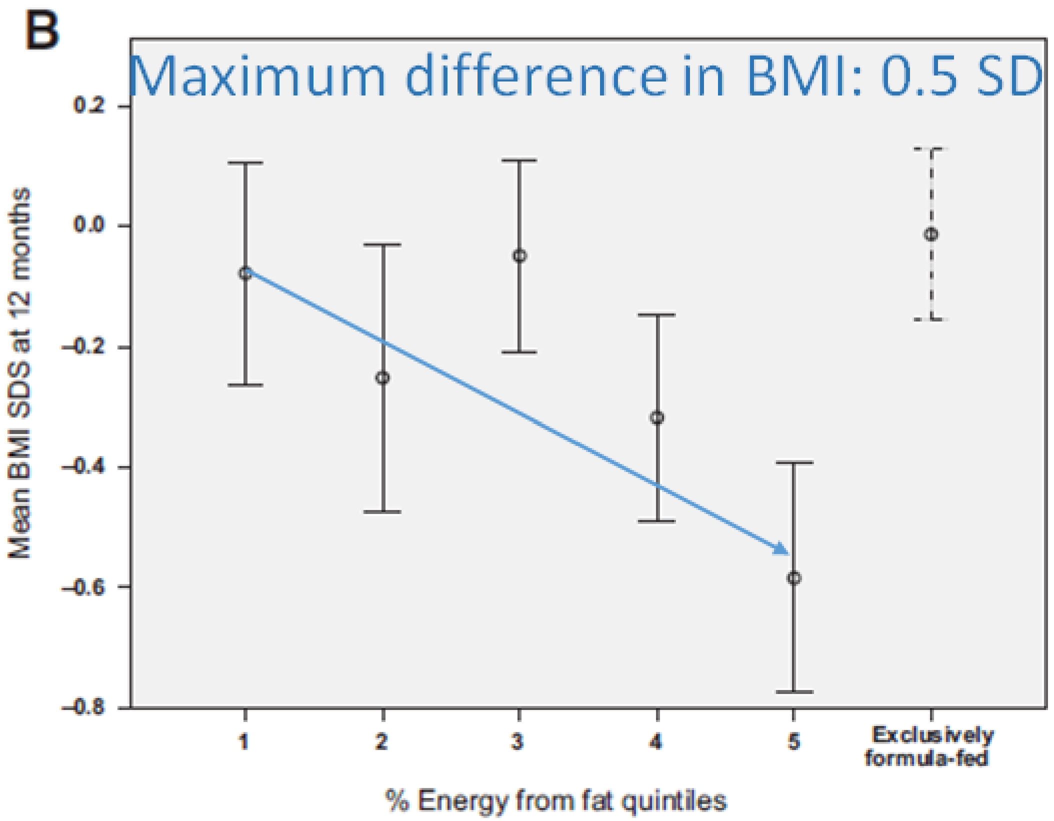
Sources: Puccio et al Pd Gast Ntur 2017; 64: 624-31; Masi et al. Gut. 2021 Dec; 70(12):2273-2282;. Goerring et al J Nutr 2016; 146: 2559-66

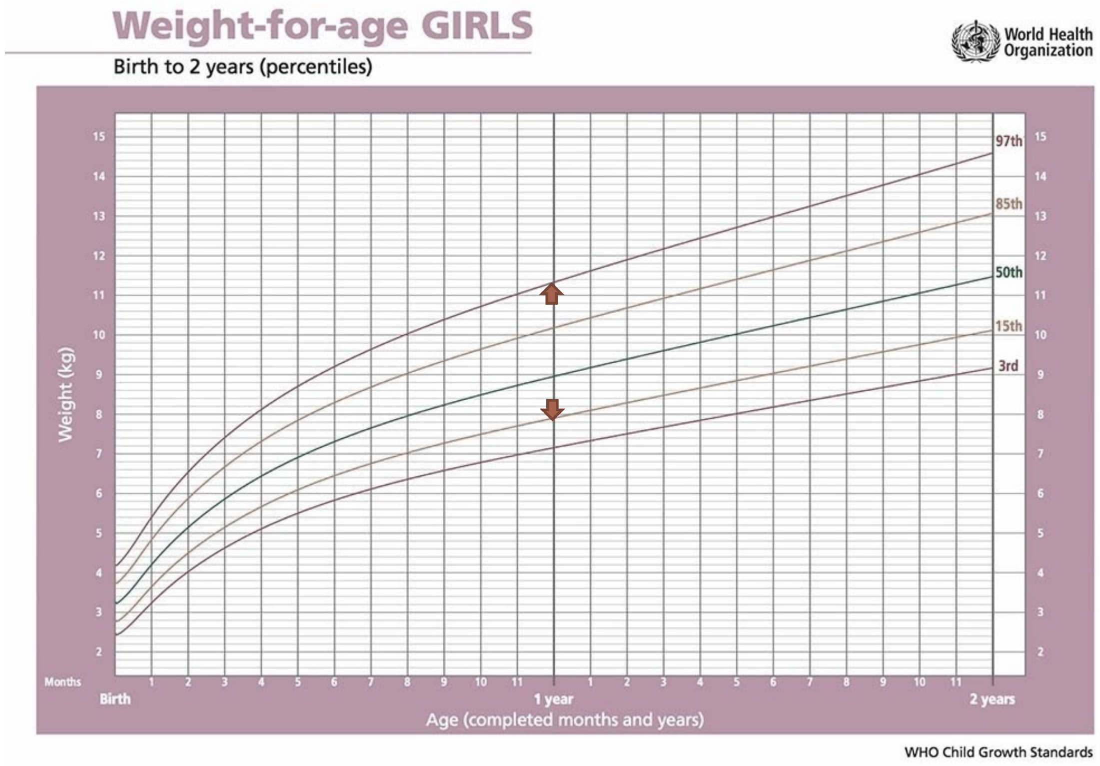
Milk composition and infant weight (BMI) at 12 months



Source: Prentice et al., Acta Pædiatrica 2016 105, pp. 641–647

Effects of milk macronutrient concentration on breastfed infant growth are fairly modest





Source: Prentice et al., Acta Pædiatrica 2016 105, pp. 641–647

Summary: What do we know about human milk macronutrients?

- No single reference value for milk macronutrients gives you the full picture of normal variation in human milk; US data are old and larger updated studies are needed, with meta-analysis for US population
- Prolonged lactation may be associated with higher concentrations of fat, energy, and protein, probably due to lower volume.
- We know some of the technical and biological reasons why milk macronutrients vary (maternal diet, time of day of expression, stage of lactation, storage and method of measurement)
- Yet, there is much we do not know about what causes differences in HMOs and other bioactive elements of human milk
- Further, there is a major gap in knowledge on the consequences of inter-individual differences in milk composition for the breastfed infant
  - Studies on infant growth and adiposity dominate
  - Effects tend to be modest, studies can rarely estimate actual intake (versus concentration), little replication/consensus
  - Infant microbiome, gut development and cognitive studies are needed

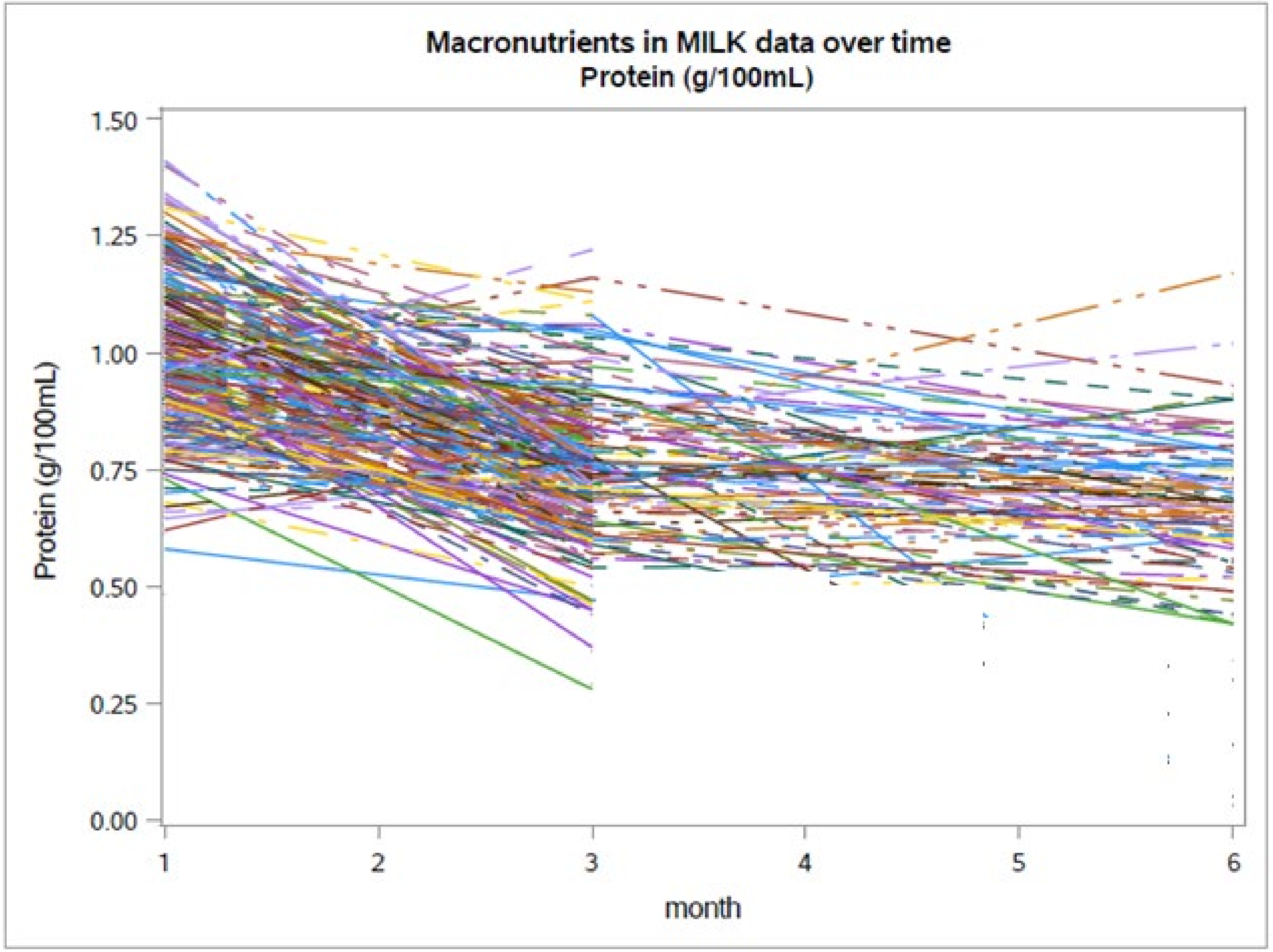
The MILk Study

- **N=550 mother-infant dyads followed from third trimester of pregnancy to 6 months post-partum for serial milk sampling and infant body composition assessment**
  - Oklahoma City and Twin Cities MN
  - Non-smoking, alcohol-abstaining mothers who intended to exclusively breastfeed, and their term, AGA, singleton infants
  - 100% exclusively breastfeeding at 1 month postpartum by inclusion criterion
  - 75% still exclusively breastfeeding at 6 mos.
  - Mostly White and Non-Hispanic, college educated; variation in income
  - Focused on non-nutritive milk bioactives

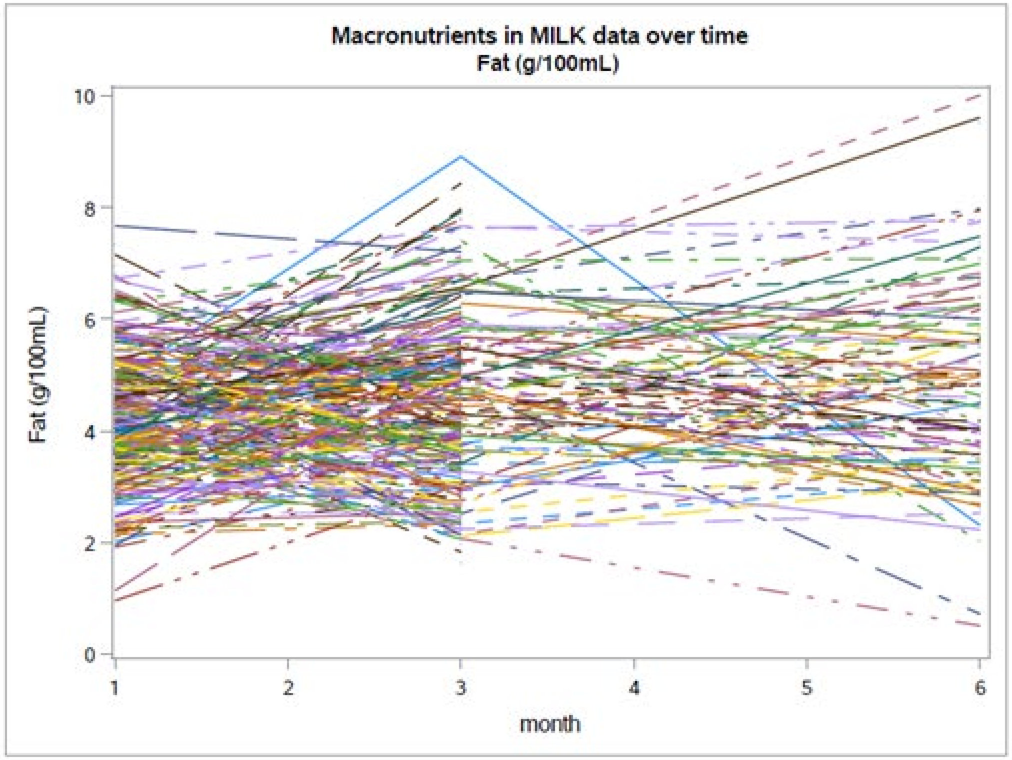
Milk collection protocol

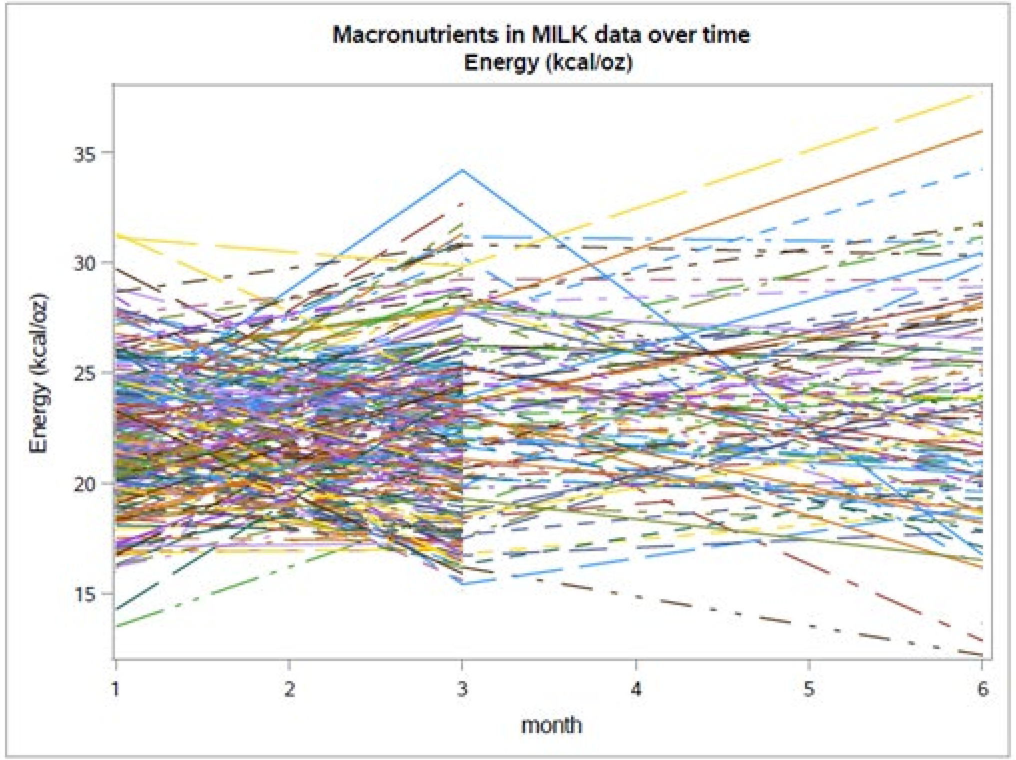
- Study visits at 1, 2, 3, and 6 months postpartum
- Arrive 8 am – 11 am
- Nurse infant as usual upon arrival
- Single full breast milk expression
  - 2 hours after infant feeding (range 9:30 am – 2:00 pm)
  - Medela Symphony breast pump with various sized breast shields
  - Instructed to pump until milk no longer copiously flowing
- 30-180 ml obtained
- Milk gently mixed, volume and weight measured, and aliquoted into cryotubes for storage at -80°C
- **Macronutrients measured using the Miris HMA**

Individual Milk protein concentrations from 1 to 6 months (MILk Study)



Individual Milk fat concentrations and energy content from 1 to 6 months (MILk Study)





Relationship of *milk protein* to maternal and infant factors (MILK Study)

Clinical characteristic	Effect	p-value
Infant weight for length gain 1-6 months	0.0645	***
Infant weight gain 1-6 months	0.0502	***
Maternal diet: Saturated fats	0.0105	*
Volume of breastmilk expresed	0.0103	*
GDM status (Yes vs No)	0.0037	
Maternal BMI	0.0033	
Time of day beginning of milk expression (continu	0.0018	
Maternal diet: Overall quality	0.0003	
Maternal age	0.0001	
Maternal diet: Refined Grains	0.0001	
Maternal diet: Sodium	0.0000	
Maternal diet: fatty acids	-0.0005	
Gestational age at birth	-0.0013	
Infant sex (Male vs Female)	-0.0017	
Infant birth weight	-0.0023	
Infant body fat % at 6 months	-0.0029	
Delivery mode (Cesarean vs Vaginal)	-0.0034	
Maternal diet: Added sugars	-0.0049	
Infant body fat % at 3 months	-0.0081	*
Gestational weight gain (kg)	-0.0170	*
Breastmilk volume ingested at test feed	-0.0264	**
Infant birth order (2+ vs 1)	-0.0372	***
Pea pod fat percent at 1 month	-0.0476	***

Relationship of *milk carbohydrate* to maternal and infant factors (MILK Study)

Clinical characteristic	Effect estimate	p-value
Getational weight gain (kg)	0.0164	**
Gestational age at birth	0.0146	**
Added sugars HEI	0.0116	*
Pea pod fat percent at 1 month	0.0116	*
Infant sex (ref=Female)	0.0079	*
Maternal diet: Overall quality	0.0059	
Maternal diet: Refined Grains	0.0051	
Maternal diet: Fatty acids	0.0048	
Milk volume ingested at test feed	0.0028	
Infant body fat % at 3 months	0.0023	
Infant body fat% at 6 months	0.0019	
Breast milk expression volume	0.0016	
Maternal diet: Sodium	0.0008	
Delivery mode (Cesarean vs Vaginal)	0.0000	
Infant birth order (2+ vs 1)	0.0000	
Maternal diet: Saturated fats	-0.0004	
Infant birthweight	-0.0023	
Time of day beginning of milk expression (continu	-0.0025	
Maternal age	-0.0083	*
Maternal BMI	-0.0105	*
Infant weight gain 1-6 months	-0.0118	*
Infant weight for length gain 1-6 months	-0.0221	**
GDM status (Yes vs No)	-0.0259	***

Hot topics in milk composition

Milk macronutrients in a transgender woman

Table 2. Milk Macronutrients and Calories, Compared to Standard by Days Relative to Due Date.

	Days Relative to Due Date (DD)				Standard Term Milk*
	DD+22	DD+70	DD+93	DD+117	70-94 days after delivery
Protein (g/dL)	1.2	1.1	1.0	1.0	0.9
Fat (g/dL)	4.1	5.6	5.9	6.2	3.4
Lactose (g/dL)	6.9	7.6	7.3	7.4	6.7
Calories (kcal/30 mL)	21	25	25	26	20.4

Note. Standard term milk (Gidrewicz & Fenton, 2014).

Patient produced 150 ml milk per day after undergoing lactation induction (estradiol, progesterone, domperidone)

Source: Weimer. Journal of Human Lactation. 2023. 39(3): 488-94.

Cannabis during breastfeeding

Cannabis use increasing in women before and during pregnancy

Delta-9 and other THCs detected in milk days to weeks after use (half life 17 days)

THC concentrates in breast milk relative to maternal plasma (6:1)

Milk concentration peaks 1-2 hours after inhalation

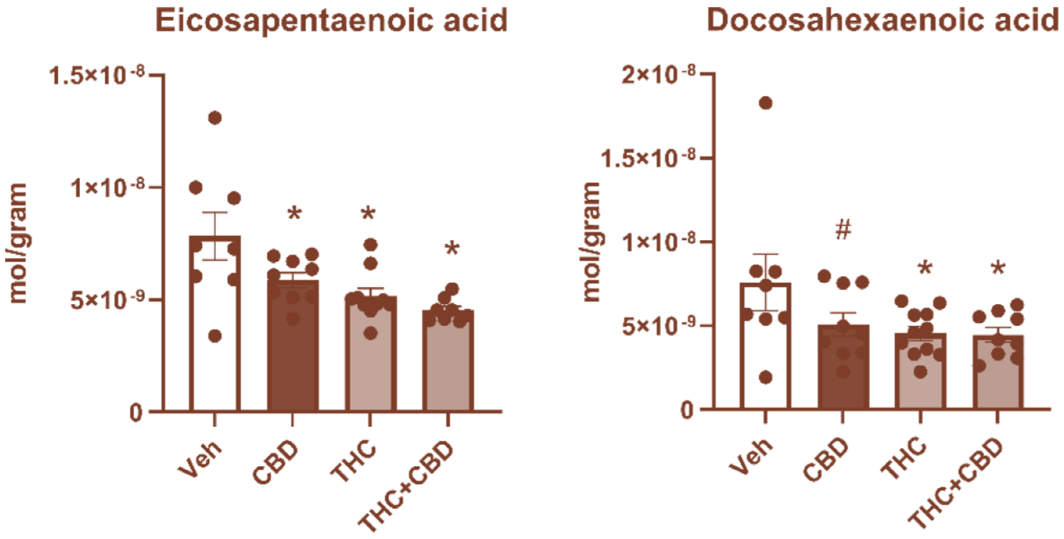
Insufficient current evidence linking infant exposure through milk to infant outcomes

No amount of cannabis is known to be safe during pregnancy or breastfeeding

Sources: Young-Wolff et al 2019 JAMA Netw Open. 2(7):e196471; Wymore et al JAMA Peds 2021);Moss et al. Pediatr Res. 2021 Oct;90(4):861-868; Baker et al., 2018 Obstetrics & Gynecology 131(5):p 783-788. Tennes et al., 1985. NIDA Res Monogr 59:48-60

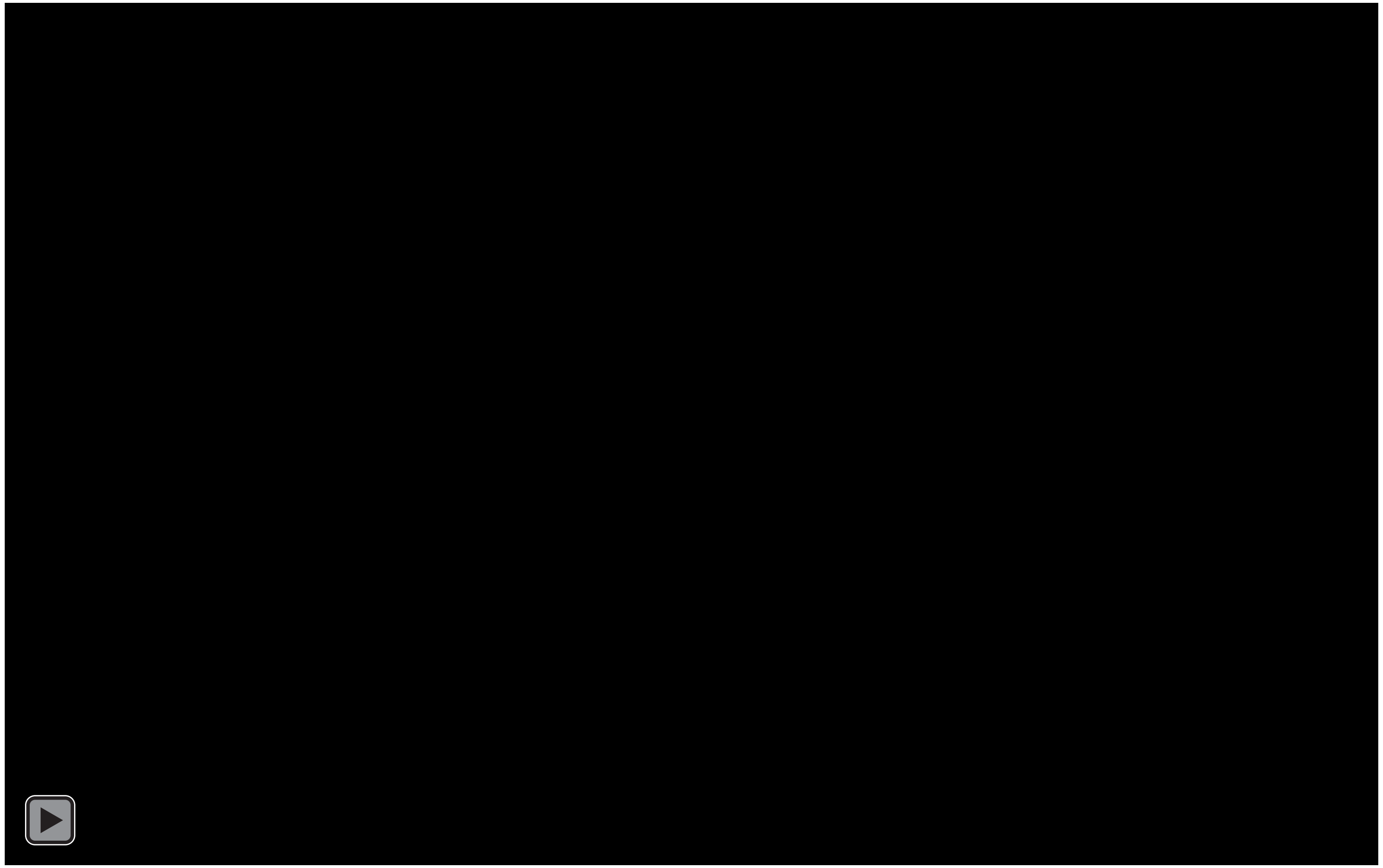
Impact of cannabis on milk composition?

- **Impact on human milk:** Relative to non-users (*n* = 17), lactose levels were higher and IgA levels were significantly lower in the milk of subjects who used cannabis during lactation (*n* = 14) (Josan et al., 2023)
- **Impact on milk composition in a rodent model**: Chronic CBD, THC and CBD + THC treatment of lactating mouse dams resulted in broad reductions in milk lipids and fatty acids



Source: Johnson et al BBA Advances 2 (2022) 10005

Lab-grown human milk substitute: A superior "formula"?



Acknowledgments

Team

- Katy Duncan, Tipper Gallagher, and all MILK study staff and participants
- Investigators David Fields, Ran Blekhman, Frank Albert, Cheryl Gale
- Post-doctoral fellows Kelsey Johnson, Emily Nagel, Marie Swanson, Kara Whitaker, Yuni Choi

Resources

- University of Minnesota Genomics Center (https://genomics.umn.edu)
- Minnesota Supercomputing Institute (https://www.msi.umn.edu/)
- Center for Neurobehavioral Development (https://midb.umn.edu/research/cnbd)

Special thanks to Tipper Gallagher for her many contributions to the MILK Study!

Tipper Gallagher, BA IBCLC
Study Coordinator
IRB wrangler
Artist in Residence

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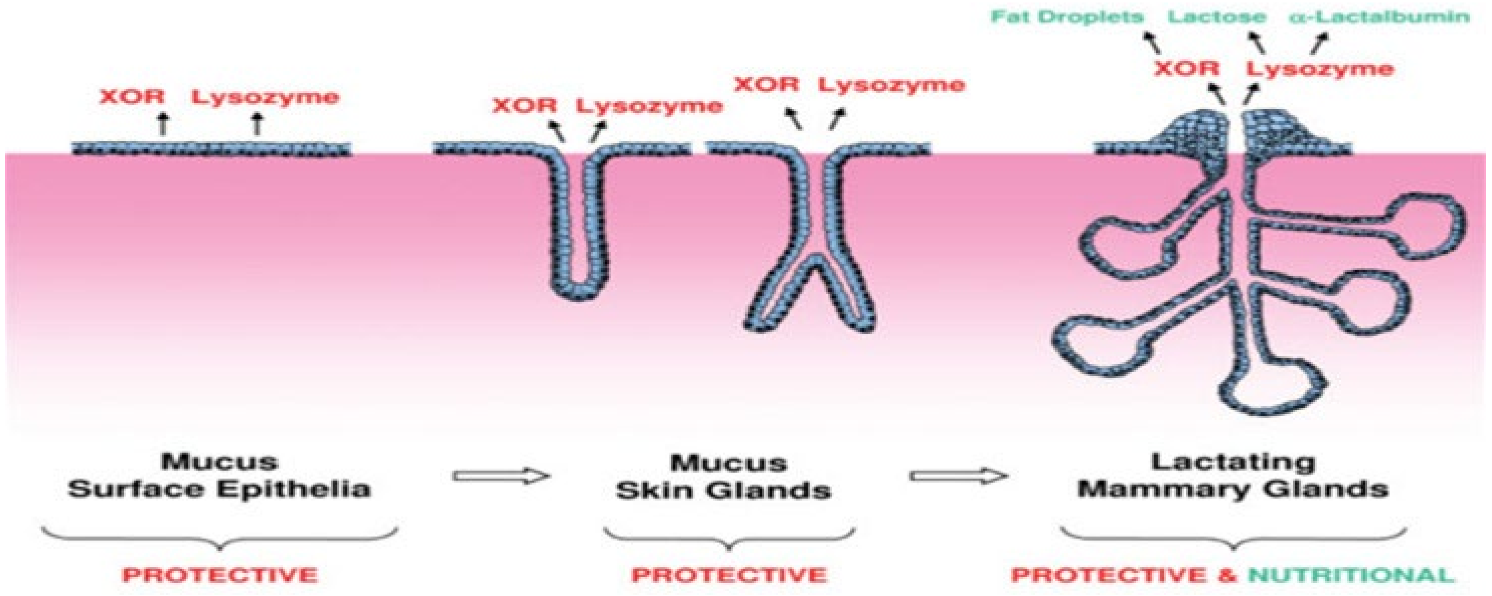
Questions and Answers

Evolution of the mammary gland: From protecting the skin to feeding the infant



Sources: Goldman. Evolution of immune functions of the mammary gland. Breastfeeding Medicine Vol 7 (3) 132-42 and Oftedal (2012) Animal 6:3,pp355–368

How does the Miris Human Milk Analyzer work?

- FDA approved diagnostic device for measuring breast milk nutrients
- Broadly used in the dairy industry and now in human clinical practice
- Quick and easy to use
- Repeatable and reproducible results
- Validated against benchmark laboratory methods with high inter-method correlation of 0.85-0.99 and low bias
  - Fat: +6%
  - Crude protein: -6%
  - Carbohydrate: -3%
  - Energy: +0.3%
- How it works: Mid-infrared spectrometry
  - Measures the absorption of infrared radiation by different functional groups of macronutrients
  - Height of absorption intensity provides relative concentrations

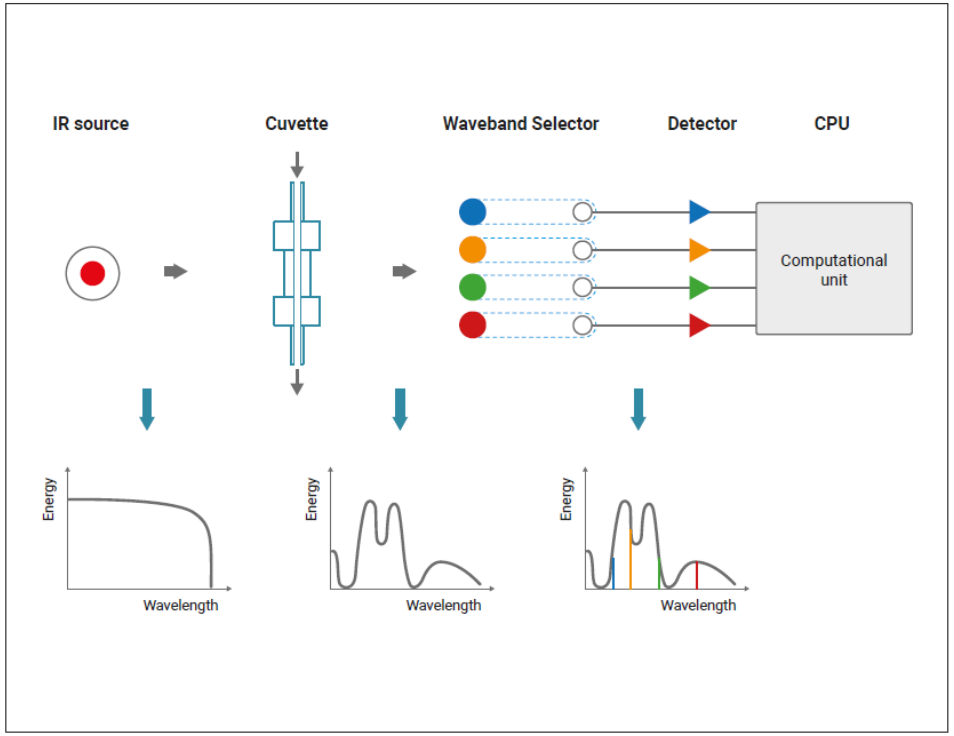


Figure 33. Working principle of the Miris HMA™.

Human milk *fat* in US and Canada 1980-2017 (kcal/100 ml)

Ref[1]	Lipid 1–6 mo Mean	Lipid 1–6 mo SD	Lipid 7–12 mo Mean	Lipid 7–12 mo SD
Clark et al. (88)	4.49	0.53	—	—
Perrin et al. (98)	—	—	3.83	1.94
Dewey et al. (36)	4.09	2.02	3.63	2.21
Nommsen et al. (35)	3.58	0.82	3.65	0.95
Butte et al. (111)	3.08	0.81	—	—
Stuff and Nichols (131)	3.04	0.83	3.32	1.15
Gross et al. (92)	3.83	1.37	—	—
Butte et al. (90)	4.26	1.14	—	—
Ferris et al. (130)	4.78	1.11	—	—
Dewey and Lonnerdal (91)	4.42	1.53	—	—
Butte et al. (128)	3.44	0.84	—	—
Garza et al. (125)	3.49	0.78	—	—
Clark et al. (126)	4.49	0.55	—	—
Glew et al. (96)	3.27	2.27	—	—
Glew et al. (122)	4.53	1.84	—	—

[1] Ref, reference.

Source: Wu X. Human Milk Nutrient Composition in the United States: Curr Dev Nutr. 2018 May 31;2(7):nzy025.

Human milk *lactose* in US and Canada 1980-2017 (kcal/100 ml)

Source: Wu X. Human Milk Nutrient Composition in the United States: Curr Dev Nutr. 2018 May 31;2(7):nzy025.

Ref[1]	Lactose			
	1–6 mo		7–12 mo	
	Mean	SD	Mean	SD
Perrin et al. (98)	—	—	5.67	0.73
Dewey et al. (36)	7.56	0.29	7.49	0.51
Nommsen et al. (35)	7.22	0.17	7.15	0.27
Butte et al. (111)	6.51	0.20	—	—
Gross et al. (92)	6.97	0.61	—	—
Ferris et al. (130)	6.67	0.67	—	—
Dewey and Lonnerdal (91)	7.17	0.51	—	—
Butte et al. (128)	6.59	0.24	—	—

[1]Ref, reference.

Human milk *total protein* in US and Canada 1980-2017 (kcal/100 ml)

	Protein			
	1–6 mo		7–12 mo	
Ref	Mean	SD	Mean	SD
Perrin et al. (98)	—	—	1.55	0.19
Dewey et al. (36)	1.29	0.37	1.35	0.37
Nommsen et al. (35)	1.14	0.15	1.16	0.16
Butte et al. (111)	0.92	0.21	—	—
Stuff and Nichols (131)	0.79	0.13	0.78	0.13
Gross et al. (92)	1.40	0.18	—	—
Butte et al. (90)	0.95	0.15	—	—
Dewey and Lonnerdal (91)	1.28	0.22	—	—
Butte et al. (128)	0.89	0.12	—	—
Garza et al. (125)	0.82	0.04	—	—

[1]BCA, bicinchoninic acid assay; Ref, reference.

Source: Wu X. Human Milk Nutrient Composition in the United States: Curr Dev Nutr. 2018 May 31;2(7):nzy025.

Human Milk Storage Guidelines

TYPE OF BREAST MILK	*STORAGE LOCATIONS AND TEMPERATURES* **Countertop** 77°F (25°C) or colder *(room temperature)*	**Refrigerator** 40 °F (4°C)	**Freezer** 0°F (-18°C) or colder
Freshly Expressed or Pumped	Up to **4 Hours**	Up to **4 Days**	Within **6 months** is best Up to **12 months** is acceptable
Thawed, Previously Frozen	**1–2 Hours**	Up to **1 Day** *(24 hours)*	**NEVER** refreeze human milk after it has been thawed
Leftover from a Feeding *(baby did not finish the bottle)*	Use within **2 hours** after the baby is finished feeding		

These guidelines are for healthy full-term babies and may vary for premature or sick babies. Check with your health care provider.

Find more breastfeeding resources at: WICBreastfeeding.fns.usda.gov
www.cdc.gov/breastfeeding/

CS311955-B

Milk maturity is the strongest driver of macronutrient differences

Table 7 The milk maturity effect: Comparison of colostrum versus mature milk

	Energy (measured)		Protein (true protein)		Fat		Lactose	
	Preterm	Term	Preterm	Term	Preterm	Term	Preterm	Term
Colostrum	49	54	2.7	2.0	2.2	1.8	5.1	5.6
Mature milk	73	63	1.1	1.0	3.3	3.4	6.2	6.5
Difference	49%	16%	−61%	−52%	50%	93%	21%	16%
p-value	<0.00001*	<0.00001*	<0.00001*	<0.00001*	<0.00001*	<0.00001*	<0.00001*	<0.00001*

- Fat increases by 50-93%, lactose increases by ~20% and true protein decreases by 50-60% from colostrum to mature milk
- Magnitude of change in fat differs for preterm and term milk

Outline

- Infant outcomes related to milk macro concentrations-----
- MILK Study
  - Maternal Determinants (heat maps) (2 slides)
  - Infant growth and body comp (heat map?) (1 slides)

Reliability statistics for Miris (MILK Study)

N= 57	Correlation P value	R-Square	CV%	Paired T-test. p-value
Fat (gm/100ml)	<.0001	0.98	3.2%	0.41
Carbohydrates (gm/100ml)	<.0001	0.92	0.8%	0.12
Total Sugar (gm/100ml)	0.99	0.98	1.0%	0.26
True protein (gm/100ml)	<.0001	0.91	7.2%	0.39
Crude protein (gm/100ml)	<.0001	0.91	6.8%	0.45
Energy (kcal/100ml)	<.0001	0.98	1.7%	0.34

Protein and Lactose in term (—) and preterm (----) milk

* Relatively low variability between individuals
* Relatively similar between term and preterm after 3 weeks

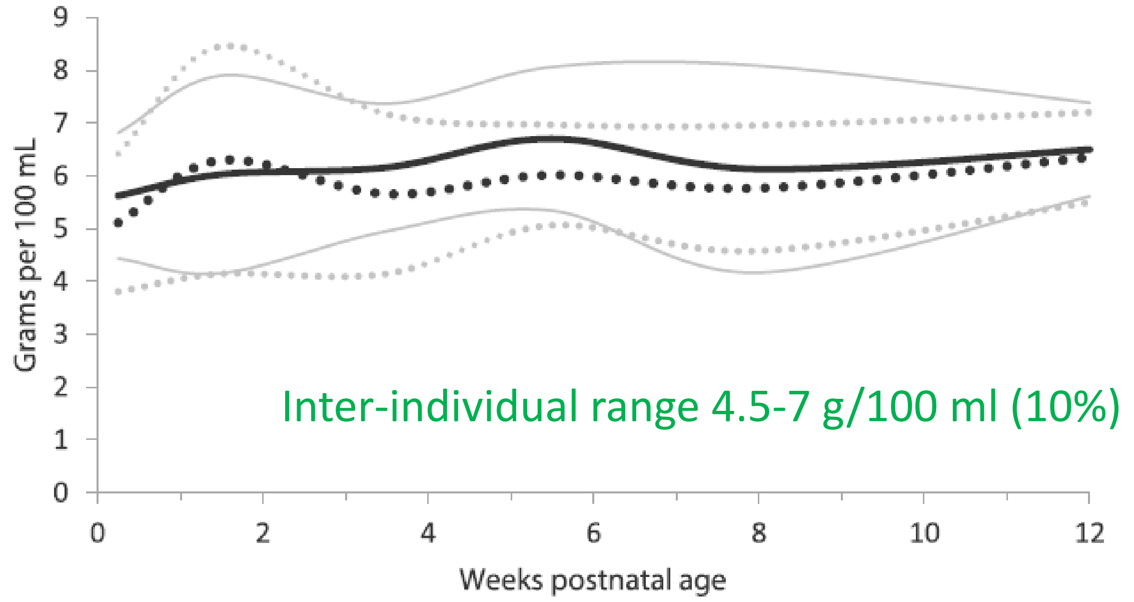


Figure 6 Lactose content distribution of preterm and term breast milk by postnatal age over the first 12 weeks of lactation, weighted mean and 95% reference interval. Preterm milk ···· Term milk —— : mean +/- 2 standard deviations.

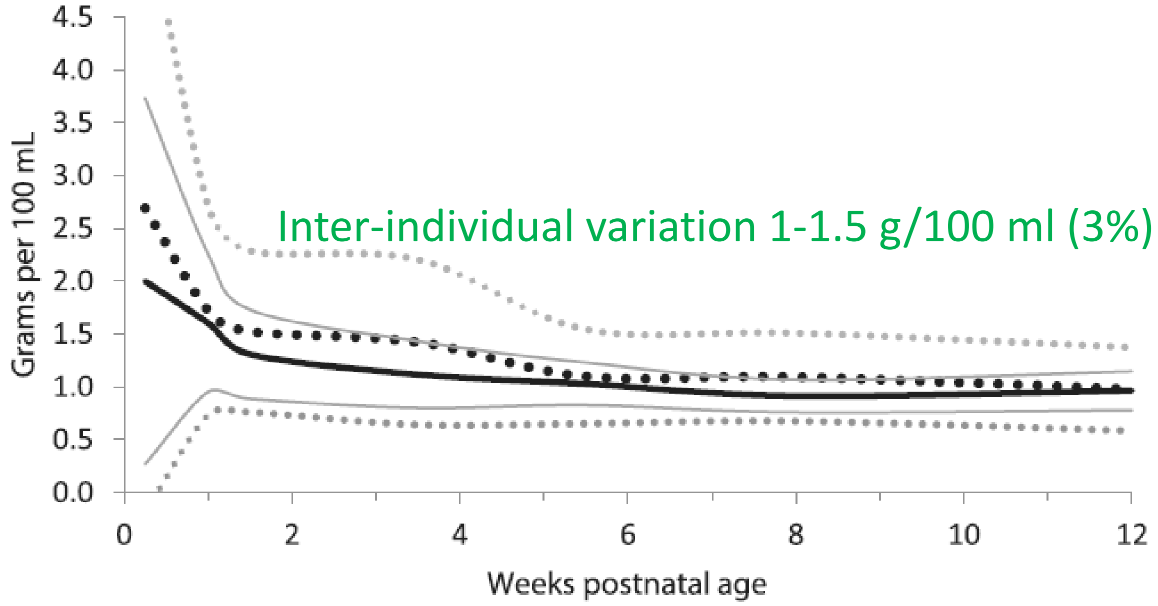


Figure 4 True Protein content distribution of preterm and term breast milk in by postnatal age over the first 12 weeks of lactation, weighted mean and 95% reference interval. Preterm milk ···· Term milk —— : mean +/- 2 standard deviations.

Fat in term (——) and preterm (-----) milk

* Relatively high variability between individuals
* Relatively similar between term and preterm

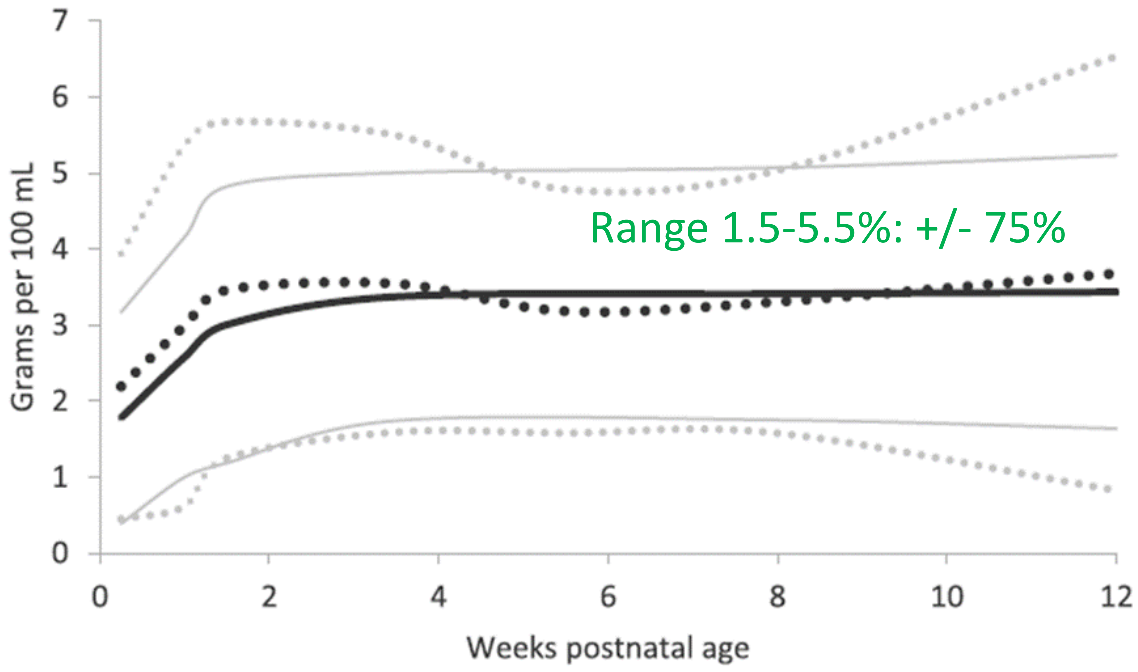
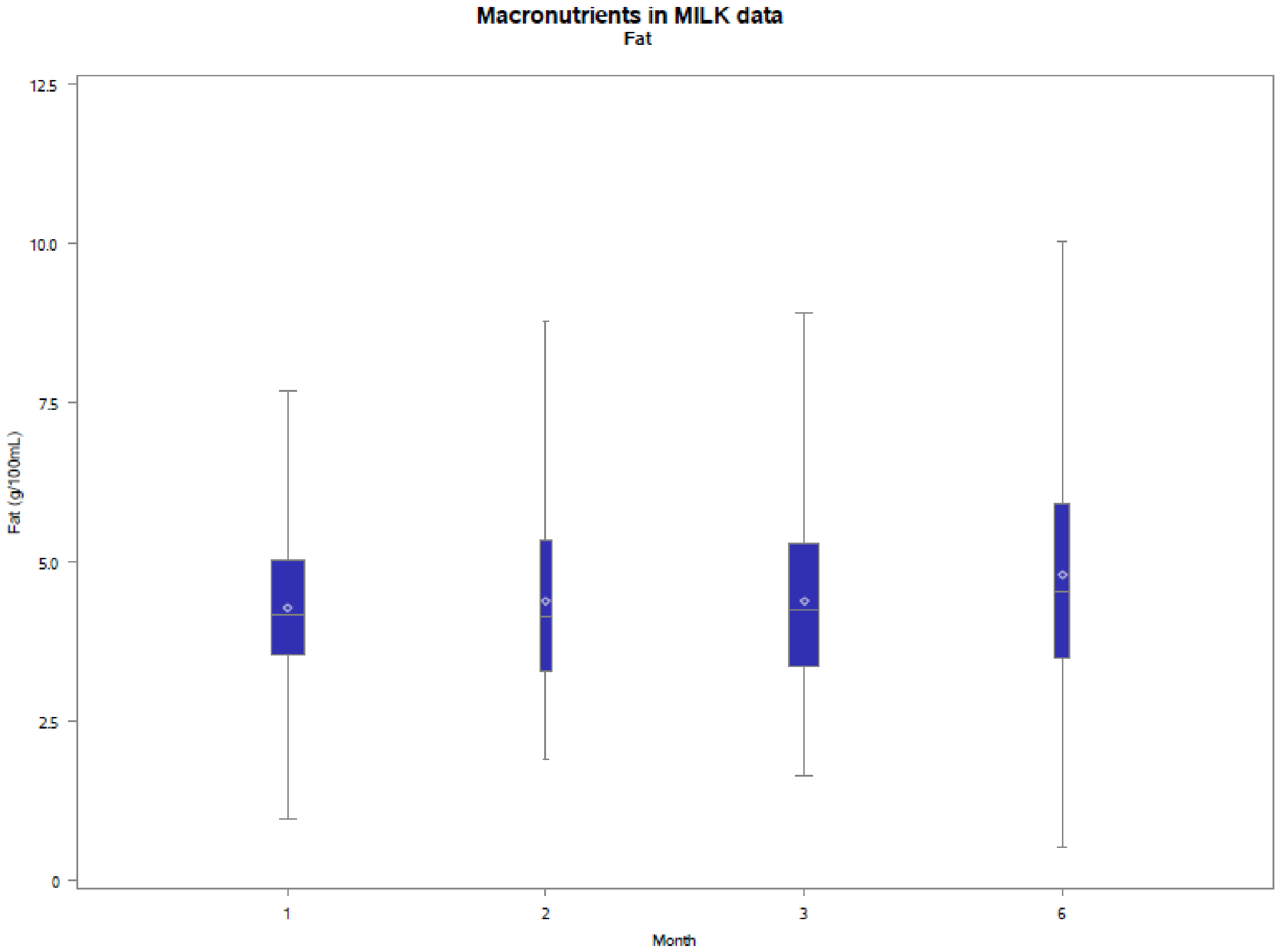
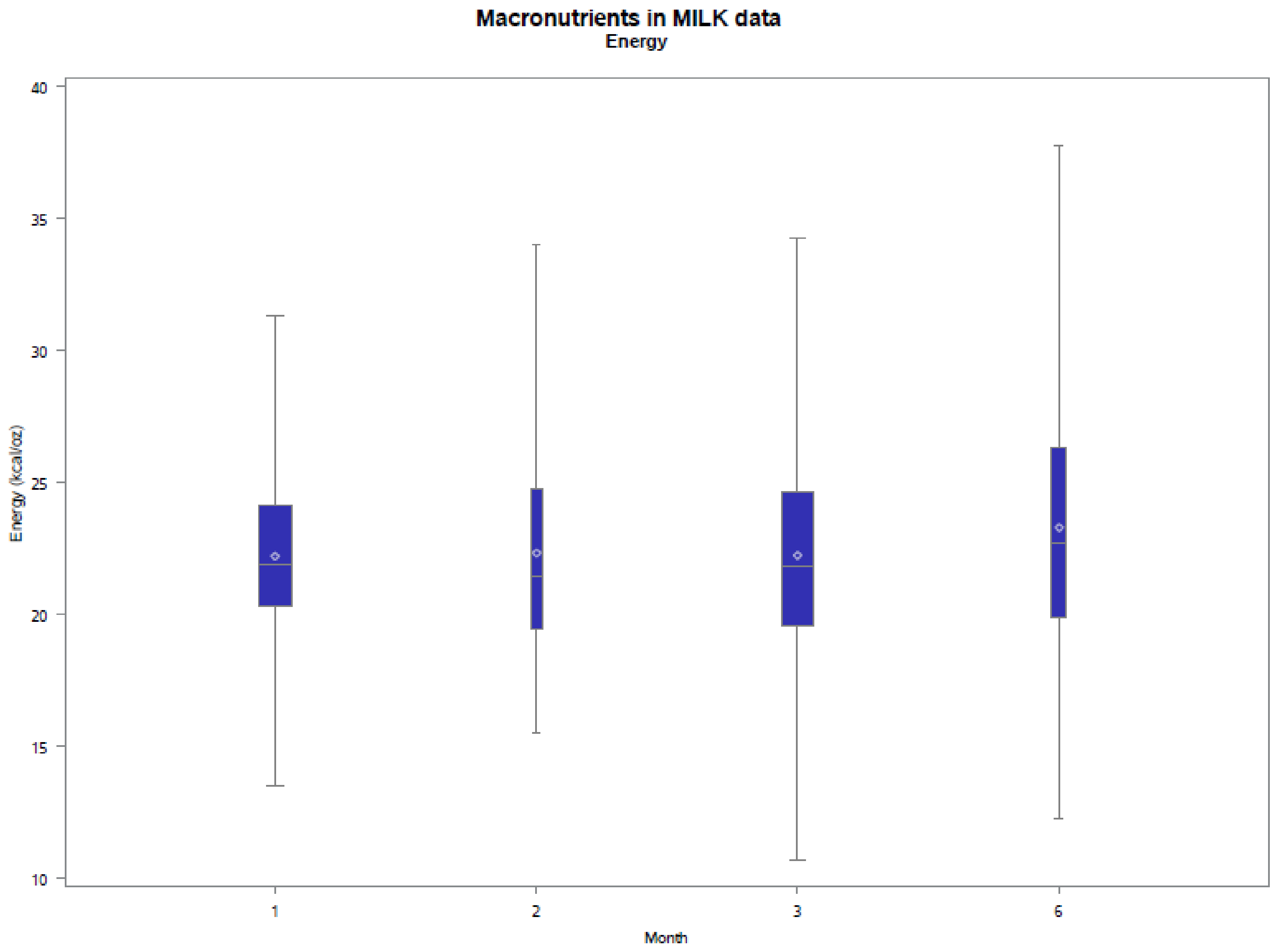


Figure 5 Fat content distribution of preterm and term breast milk by postnatal age over the first 12 weeks of lactation, weighted mean and 95% reference interval. Preterm milk ···· Term milk —— : mean +/- 2 standard deviations.

Fat concentrations at 1, 2, 3, and 6 months (MILK Study)



Energy concentrations at 1, 2, 3, and 6 months (MILK Study)



Somewhat richer milk with exclusive breastfeeding

- Study of 614 dyads at 4-8 weeks postpartum in England
- Gold standard laboratory assessments of macronutrients
- Milk of exclusively breastfeeding mothers compared to mixed feeding mothers contained:
  - ~3 kcal/100 ml higher calories and 0.3 g/100 ml higher fat
  - Slightly lower protein and carbohydrate

Prentice et al Acta Pædiatrica 2016 105, pp. 641–647