



Early Insights into Human Milk Analysis

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USLCA Saturday, 9/24 at 10:10am

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I have no relevant financial relationship(s) with ineligible companies to disclose for this presentation.

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Objectives

- Identify the nutritional components of human milk
- Describe normal physiologic variance
- Analyze case studies

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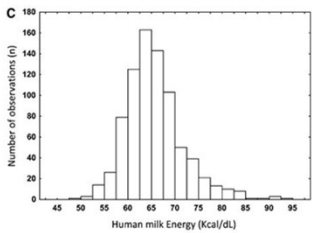
Species Specific Milk

- Brain grows rapidly
- Body grows slowly
- Dynamic “changing” milk
 - Minutes
 - Hours
 - Weeks
 - Environment

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Energy Content


- Assume 20 kcal/oz (67 kcal/dL)
- Often 17-22 kcal/oz (50-85 kcal/dL)
- Can be 12-32 kcal/oz (40-108 kcal/dL)



McLeod et al, 2021
De Halleux and Rigo, 2013

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Energy Yielding Components



- 1-2 g/dL Protein
- 3-4 g/dL Fat
- 5-6 g/dL Lactose (sugar) + Oligos (fiber) =
- 7-8 g/dL Carbohydrate

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Protein

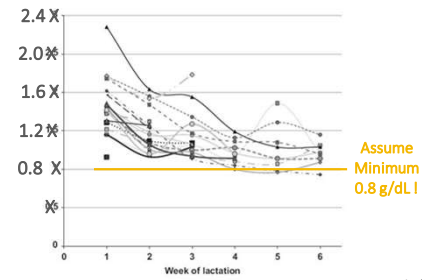
- **Total Protein**
 - Also called “Gross” or “Crude” Protein
 - Represents all nitrogen including bioactive factors
- **Nutritional Protein**
 - Also called “True” Protein
 - Assumed to be 80% of Total (variable, as low as 54-70%)
- **Colostrum ~2 g/dL**
- **“Preterm” ~1.3-1.5 g/dL**
- **Normalizes to ~1 g/dL (0.8 g/dL)**

Fenton and Elmrayed, 2021
McCauley et al, 2023
Fenton and McCauley, 2020

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Protein in Preterm Milk

Protein
~1-2 g/dL
(0.8 g/dL)



Arnold et al, 2017

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Fat

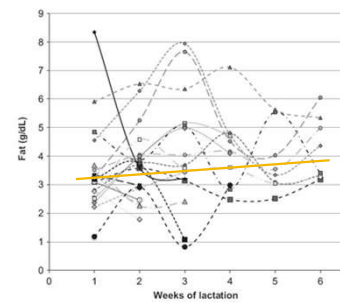
- **Energy**
- **Building block for the brain**
 - Unique triglyceride form
 - Fat globule size increases with time
 - Variety enhanced by diet
- **Colostrum ~2-3 g/dL**
- **Normalizes to ~4 g/dL**
(Most Variable ~18 g/dL)

Kim and Froh, 2012

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Fat in Preterm Milk

Fat
~3-4 g/dL
(varies)



Arnold et al, 2017

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Foremilk/Hindmilk: Yes

✓

Foremilk	Hindmilk	Average
~1 g/dL 13 kcal/oz	~9 g/dL 33 kcal/oz	~23 kcal/oz



Prior Full
Expression

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Foremilk/Hindmilk: No

X

X

Foremilk	Hindmilk	Average
~7 g/dL 30 kcal/oz	~6 g/dL 27 kcal/oz	~28 kcal/oz
~10 g/dL 34 kcal/oz	~6 g/dL 23 kcal/oz	~29 kcal/oz



Prior
Incomplete
Expression

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Carbohydrates

Lactose (sugar)

- Important energy source
- Averages 80% Lactose (variable, 70-85%)
- Colostrum ~5 g/dL → Normalizes ~6.5 g/dL

+ Oligos (fiber)

- Important for gut biome (>200 unique)
- Averages 20% Oligosaccharides (variable, 15-30%)
- Colostrum ~2 g/dL → Normalizes ~1.5 g/dL

= Total Carbohydrate

Colostrum ~7 g/dL → Normalizes ~8 g/dL

Fenton and Elmsayed, 2021
Fenton and McLeod, 2020
Mish, 2017
Kim and Froh, 2012

Lactose (Sugar)

Preterm Milk	0-2 Weeks	2-4 Weeks	≥4 Weeks
Median	6.5 g/dL	6.6 g/dL	6.5 g/dL
±1 SD	6.0-7.0 g/dL	6.3-6.9 g/dL	6.3-6.7 g/dL
Range	5.1-7.9 g/dL	6.4-7.5 g/dL	5.9-7.1 g/dL

Lactose
~5-6 g/dL

Radmacher et al, 2013

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Oligos (Fiber)

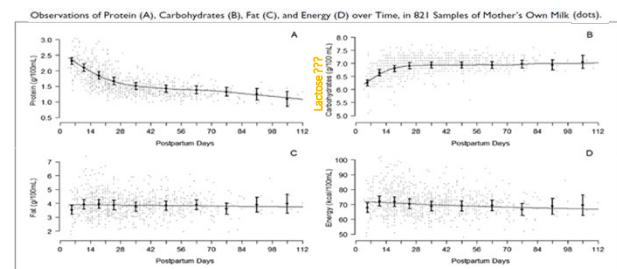
Median	1-2 Weeks	3-4 Weeks
Preterm Milk	2.1 g/dL	1.7 g/dL
Term Milk	1.9 g/dL	1.6 g/dL

Lactose + Oligos =
~7-8 g/dL Carbohydrate

Gidrewicz and Fenton 2014

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Nutritional Components Over Time (Preterm Milk)



Sjöström et al, 2014

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Energy Yielding Components

- 1  • 1-2 g/dL Protein (0.8 g/dL)
- 4 • 3-4 g/dL Fat
- 8 • 5-6 g/dL Lactose + Oligos =
- 7-8 g/dL Carbohydrate

Metabolizable Energy

Protein ✕ 4.4 kcal/g ✕ 4 kcal/g

- Total Nitrogen
- Nutritional Protein

Fat ✕ 9.25 kcal/g ✕ 9 kcal/g

Carbohydrate ✕ 4 kcal/g

- Lactose ✕ 4 kcal/g
- Oligosaccharides ✕ 4 kcal/g ✕ 2 kcal/g

***Current methods
may overestimate
by 5-10%**

Fenton and Elmsayed 2021
Mish, 2017

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Sampling Errors

+ Protein	+ Fat	- Fat	Sample Selection
Hemoglobin	Vancomycin	Citalopram	
		Sertraline	
		Pseudoephedrine	
		Ampicillin	
		Clindamycin	
		Cephalexin	

Miris, 2017

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Human Milk Analyzers



Miris, 2017

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Protein: 1-2 g/dL (0.8 g/dL)

Should we use?	28 Weeker	24 Weeker	Should we use?
	Week #1: 1.5 g/dL	Week #1: 2.2 g/dL	
	Week #2: 1.3 g/dL	Week #2: 2.0 g/dL	
	Week #3: 1.2 g/dL	Week #3: 1.7 g/dL	
	Week #6: 0.8 g/dL	Week #6: 1.2 g/dL	
		Week #12: 0.9 g/dL	

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Protein: 1-2 g/dL (0.8 g/dL)

Term Newborn	Preterm Newborn + Tandem Nursing Toddler
Week #1: 1.4 g/dL	Week #1: 1.4 g/dL
Preterm and Term Mature Milk	What do you expect?
5 Months: 0.8 g/dL	

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Fat: 3-4 g/dL (variable)

4 Bottles in 1 Session

1.7 g/dL Fat = 14 kcal/oz

2.1 g/dL Fat = 15 kcal/oz

6.0 g/dL Fat = 26 kcal/oz

6.6 g/dL Fat = 27 kcal/oz

What would cause this variation?

Average ~21 kcal/oz

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Fat: 3-4 g/dL (variable)

Foremilk

2.9 g/dL Fat = 18 kcal/oz

3.3 g/dL Fat = 19 kcal/oz

Hindmilk

7.9 g/dL Fat = 31 kcal/oz

10.0 g/dL Fat = 37 kcal/oz

How does this fractionated milk compare?

Average ~26 kcal/oz

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Carbohydrate: 7-8 g/dL

Term Newborn with Hypoglycemia

Day #4: 8.1 g/dL Carb

34 Weeker with Prolonged Hypoglycemia

Day #7: 7.5 g/dL Carb

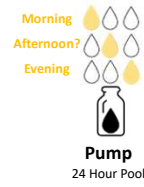
Day #9: 7.4 g/dL Carb

Day #13: 8.2 g/dL Carb

**Not the milk –
must look at the
bigger picture!**

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Sampling Technique



Fenton and Elmhurst, 2021

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Term Newborn

Would like to stop artificial supplement
Breastfeeding + Supplement + Pump
Noticing more milk (storing)

What samples would you request?

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Term Newborn

Sample	Protein ~1-2 g/dL	Fat ~3-4 g/dL	Carb ~7-8 g/dL	Energy ~20 kcal/oz
Morning	1.5 g/dL	~6 g/dL	~8 g/dL	= 28 kcal/oz
Afternoon	1.5 g/dL	~4 g/dL	~8 g/dL	= 23 kcal/oz
Evening	1.4 g/dL	~5 g/dL	~8 g/dL	= 25 kcal/oz

What would you recommend?

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Photo Credit: Medela

5 Months (Former Preterm)

Slow growth and feeding intolerance
Exclusively pumping 18oz x 3 per day

What questions do you have?

What samples would you request?

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5 Months Old

Time	Protein ~1 g/dL	Fat ~4 g/dL	Carb ~8 g/dL	Energy ~20 kcal/oz
Morning #1	0.8 g/dL	4.0 g/dL	8.5 g/dL	= 23 kcal/oz
Morning #2	0.9 g/dL	5.3 g/dL	8.5 g/dL	= 26 kcal/oz
Afternoon #1	0.9 g/dL	5.1 g/dL	8.5 g/dL	= 25 kcal/oz
Afternoon #2	0.8 g/dL	6.8 g/dL	8.5 g/dL	= 30 kcal/oz
Evening #1	0.8 g/dL	6.4 g/dL	8.5 g/dL	= 25 kcal/oz
Evening #2	0.7 g/dL	4.1 g/dL	8.3 g/dL	= 23 kcal/oz

What would you recommend?

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Photo Credit: Medela

Term 5 Months

Poor growth

Exclusive breastfeeding

- What questions do you have for mom?
- What samples would you request?

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5 Months Old

Time	Protein ~1 g/dL	Pre-BF Fat ~4 g/dL = 30 kcal/oz	Post-BF Fat ~4 g/dL = 27 kcal/oz	Carb ~8 g/dL	Average
Morning #1	~0.8 g/dL	~7 g/dL = 30 kcal/oz	~6 g/dL = 27 kcal/oz	~8.5 g/dL	= 28 kcal/oz
Morning #2 *Engorged	~0.8 g/dL	~3 g/dL = 17 kcal/oz	~9 g/dL = 34 kcal/oz	~8 g/dL	= 26 kcal/oz
Afternoon #2	~0.8 g/dL	~10 g/dL = 34 kcal/oz	~6 g/dL = 23 kcal/oz	~8 g/dL	= 29 kcal/oz
Evening #2	~0.8 g/dL	~5 g/dL = 21 kcal/oz	~9 g/dL = 34 kcal/oz	~8 g/dL	= 28 kcal/oz

What would you recommend?

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When Pumping is Necessary



Pump Frequently: 8-12 x day



If volumes are high...
Freezer can overflow quickly...

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First In First Out (FIFO)



- **Colostrum** in order, even if frozen
 - Considered first 3-4 days
- Then ~50% **oldest frozen milk** + ~50% **fresh**
 - "FIFO" highest protein, early bioactive factors

Especially for preterm infants, suggest using up first 3-4 weeks frozen milk (at 50%) before switching to all fresh

Meier et al, 2010

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Choose appropriate bottle size for full expression "1 Breast per Bottle"



Can combine after pumping (mix well before pouring).
Allow room for the milk to expand with freezing!

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What's happening here?
Mix well before pouring.

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Conclusions

- Human milk varies, usually of little concern
- Analysis gives us a few data points, trends
- Must look at bigger picture, mom and baby supporting long term breastfeeding

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Questions?

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