Human Milk Components: Interaction with the Host

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DISCLOSURE STATEMENT
Speaker: Josef Neu
Dr. Neu has disclosed the following relevant financial relationships. Any real or apparent conflicts of interest related to the content of this presentation have been resolved.

<table>
<thead>
<tr>
<th>Organization/Interest</th>
<th>Relationship</th>
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<td>Infant Bacterial Therapeutics</td>
<td>Scientific Advisory Board</td>
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<td>Medela</td>
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<td>National Institutes of Health</td>
<td>Research Grant</td>
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<td>Scientific Advisory Board</td>
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Agenda

• Brief History and Evolutionary aspects.
• Critical components of human milk beneficial for preterm.
• Intestinal mucosal immunity and milk.
• Mother’s own milk versus donor milk: Compositional advantages?
• How can we make donor milk more like own mother’s milk?
  • Transfaunation
  • Improving “pasteurization” techniques.
Evolution of Lactation

Monotremes — oldest taxon of the class Mammalia, lay eggs, no placenta or nipples
Marsupials — placenta not well developed, very short gestation, have nipples
Eutheria — more recently evolved, have placentas

Reason for being fussy in evaluating composition of breast milk

Considered the “gold standard” Components of human milk are being viewed as prophylactic or therapeutic agents.

Evaluating Breast Milk Composition General Concepts

- Dynamic composition: varies within a feeding, over lactation and between mothers and populations.
- Studies of human milk composition should include:
  - sample over 24 hours
  - collect on multiple occasions over time (not easy to do)
  - standardize at specific time of day,
  - avoid collection from a breast that was used for nursing within the past 2-3 hours
  - record other conditions such as whether milk was pumped, freezing, thawing, duration of storage, the “gold standard”, components of human milk are being viewed as prophylactic or therapeutic agents.
Colostrum

- Produced in low quantities in first days after parturition.
- Rich in immunologic components: secretory IgA, lactoferrin, white blood cells, epidermal growth factor and other growth factors.
- Tight junction closure occurs in mammary epithelium and sodium to potassium ratio declines and lactose concentration increases with onset of transitional milk.
- Transitional milk—after 2-3 days: markers include sodium concentration, citrate and lactose.

Macronutrients

Vary between mothers and across lactation but is conserved across populations despite variations in maternal nutritional status

Term Milk Macronutrient composition

- Protein: 0.9-1.2 gram per dL
- Fat: 3.2-3.6 gram per dL
- Carbohydrate: 6.7-7.8 gram per dL for fat
- Energy: 67-70 kcal/dL

Micronutrients (Vitamins and Minerals)

- Vitamin K is extremely low and injection is needed to prevent hemorrhagic disease of the newborn.
- Vitamin D also low in human milk—supplementation suggested.
- Vitamins may vary in diet of mother and multi-vitamins to mothers is recommended.
Calcium and Phosphorus in Human Milk, Term and Preterm Formulas

<table>
<thead>
<tr>
<th></th>
<th>Required per Kg/day</th>
<th>Required per 100 kcal</th>
<th>Human Milk per 100 kcal</th>
<th>Fortified Human Milk per 100 kcal</th>
<th>Term Formula/100 kcal</th>
<th>Preterm Formula/100 kcal</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ca, mg</strong></td>
<td>184</td>
<td>170</td>
<td>45</td>
<td>34</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td><strong>P, mg</strong></td>
<td>126</td>
<td>116</td>
<td>21</td>
<td>8</td>
<td>50</td>
<td>85</td>
</tr>
</tbody>
</table>

Ziegler, E. Nutritional Care of Preterm Infants, 2014

The Versatile Intestine: The intestine is not only a digestive-absorptive organ

- largest immune organ of the body.
- harbors a huge microbial ecosystem.
- harbors the enteric nervous system

Intestinal Antigenic Load

- Gut content of microbes is ~10^{11} cells.
- An individual consumes at least 2,500 kg. Food antigen during a lifetime.
- A single layer of epithelial cells separates the luminal contents from effector immune cells in the lamina propria.
TLR ligands and signaling are crucial for the intestinal surface to protect and repair itself in the face of infectious or inflammatory insult.

The Tight Junction

What about enteral feeding?

Dr. Elsie Widdowson (1906-2000)

The suckled pig’s duodenum gains 42% of its weight in the first 24 hours after birth.
MORBIDITIES: EARLY VS. LATE FEEDING

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Early (n = 78)</th>
<th>Late (n = 91)</th>
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<tbody>
<tr>
<td>NEC</td>
<td>6.3%</td>
<td>10.0%</td>
</tr>
<tr>
<td>ROP</td>
<td>19.7%</td>
<td>30.0%</td>
</tr>
<tr>
<td>CLD</td>
<td>21.5%</td>
<td>68.8%**</td>
</tr>
<tr>
<td>PTL</td>
<td>0.6%</td>
<td>0.6%</td>
</tr>
<tr>
<td>HMI</td>
<td>24.1%</td>
<td>24.8%</td>
</tr>
<tr>
<td>Extremities</td>
<td>8.6%</td>
<td>25.4%</td>
</tr>
</tbody>
</table>

*Early vs. Late p<0.05.
**CLD vs. Late p<0.001.
Intestinal Obstruction (NEC), Retinopathy of Prematurity (ROP), Chronic Lung Disease (CLD), Pulmonary Volutic Lesion (PTL), Intraventricular Hemorrhage (HMI), Extremities = The presence of 2 or more non-relate outcomes.

Konekova, et al. PLOS One 2015

THE NEW ENGLAND JOURNAL OF MEDICINE

Controlled Trial of Two Incremental Milk-Feeding Rates in Preterm Infants


THE INTESTINAL BARRIER, FEEDING AND LATE ONSET SEPSIS

![Image of a baby with a feeding tube]

7
Effect of GI Priming on Intestinal Permeability

- Birth weight 1 kg
- Gestational age 28 wk
- Permeability (Lactulose/mannitol ratio x10⁻²)
- GI Priming, day 4-14
- TPN only to day 15
- 10 days


Immunologic and Growth Factors

- Human Milk Fat Globule
- Innate Immunologic factors
- Oligosaccharides
- Microbes
- Growth Factors
- MicroRNAs

Quiz: What's this?
Human Milk Fat Globule

• Antimicrobial defense:
  Releases free fatty acids and monoglycerides via the digestive process, which disrupt cell membranes of microbes.
• Components have important functions in brain and gut.


Quiz: What are we seeing? Why is this important?
IgA and IgM

SARS-CoV-2 specific IgA in breastmilk of lactating healthcare workers pre and post COVID-19 vaccination (1:3 dilution)

Donor human milk after Holder Pasteurization

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-pasteurization</th>
<th>Pasteurized</th>
</tr>
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<tbody>
<tr>
<td>Bactericidal activity</td>
<td>94%</td>
<td>96%</td>
</tr>
<tr>
<td>Virus neutralizing activity</td>
<td>2.34</td>
<td>2.34</td>
</tr>
<tr>
<td>Antibody titer</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Heat stability</td>
<td>25°C, 24 hours</td>
<td>25°C, 24 hours</td>
</tr>
<tr>
<td>pH stability</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Freezing-thawing stability</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Shelf life</td>
<td>2.5 years</td>
<td>2.5 years</td>
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Courtesy of Peter Hartman, et al.
Implications of Abolishing BSSL

- Lower pancreatic lipases in preterms.
- Combined with gastric lipase, milk BSSL facilitates lipid hydrolysis and prepares intraluminal lipid for further cleavage by pancreatic enzymes.
- BSSL is higher in milk of mothers delivering pre-term than in those delivering at term.

Underlying Mechanisms of Alkaline Phosphatase

- IAP dephosphorylates various pro-inflammatory microbial components including LPS, thus making them unrecognizable by Toll Like receptors.
- IAP can directly inhibit NFκappaB pathway components.
Effects of Alkaline Phosphatase on Experimental Sepsis in Mice

Microbiome: Why all the fuss?

Over 86,000 publications cited in Pub Med as of today

The Mother-infant “Dyad” is actually a Triad!

- New concept: Placenta, amniotic fluid, and milk contain microbes.
- Not only are the nuclear and mitochondrial genomes transmitted from the mother to the infant, so are the microbial genes—a second genome! About 35% of the metabolites in mammalian blood have a bacterial origin.
- The pregnant mammal is herself a symbiotic community, a “holobiont”.

*Adapted from Gilbert, SF. Frontiers in Genetics. Aug. 2014
Culture versus Non Culture

400 species  2000 species

Major Microbiome Sequencing Technologies

Who's There?

What's there and what are they doing?

Adapted from: https://www.neb.com/tools-and-resources/addressing-challenges-in-microbiome-dna-analysis

The Versatile Intestine

- Largest immune organ of the body
- Houses the enteric nervous system
- Houses a huge microbial ecosystem
Regional Differences

Metabolomic (Bioreactor) Role

How do Microbial Genomics Affect Phenotype?

- Nutrition
- Drugs
- Other environmental factors

Gut bacteria and obesity
“Holy shit!”
Nov 12th 2009
From The Economist print edition
A new way of finding out how diet affects gut microbes

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Human Milk Microbes: Are they Pathogens, Commensals or just Inert Bystanders?

Breast milk microbes Over time

Jeurink, PV, Beneficial Microbes, March 2013:17-30

The Maternal GI Tract as a Source of Breast Milk Microbes

Jeurink, PV, Beneficial Microbes, March 2013:17-30
Bacterial Load over Lactational Stages: FusA Gene PCR

Microbial Dose from Human Milk

- Assume intake of 800 ml/day
- Assume $10^{5-6}$ bacterial cells/ml
- This will provide $10^{7-8}$ bacterial cells (personalized?) daily, close to the dose in most probiotic studies.

Human Milk Personalization and Dynamic Interactions: Enteromammary Immune System
The Future: Questions

- Should we be focusing on use of Fresh mother’s milk rather than banked donor milk?
- How can we improve on banked donor milk or formula?
- How can we promote mother’s own fresh milk use?
Transfaunation Summary

- Each mother has a unique milk microbiota and the live microbiome in DBM can be restored with these unique bacteria using small amounts of MOM.
- This is a novel approach to possibly improving the bioactivity of DBM by adding specific MOM microbes in small quantities to personalize her own infant’s milk.
- The agreement between the results obtained from the viable bacterial counts and the microbiome analyses indicate that DBM incubated with 10 percent of the MOM for 4 h is a reasonable restoration strategy.
- Future studies should include larger samples sizes, activity of the microbes in RM in comparison to DBM and MOM samples, and clinical evaluation of the safety and efficacy.

Fecal Microbiota after CS vs. Vaginal Delivery: Can Human Milk Restore the Microbiome?

Azad, et al. CMAD, March 19, 2013: 185(S)

Ultraviolet-C Irradiation: A Novel Pasteurization Method for Donor Human Milk

Abstract

Background: Ultraviolet-C irradiation (UVC) is one of the standard treatments for donor human milk. Although the effects of UVC on human milk bioactivity and other characteristics are well-documented, there is a marked difference in the efficacy of the standard method for providing the required pasteurization. The UV-C irradiation is an essential element in the treatment of milk products.

Methods: The pasteurization method was implemented in the standard method. The study was conducted using a 20% and 30% concentration of the milk samples. The irradiation dose was measured using a UV light chamber (300 W/cm²). The total number of live bacteria was determined using the colony-forming unit (CFU) method. The live bacteria were calculated by multiplying the number of colonies on the plated milk samples by the dilution factor. The total number of live bacteria was calculated using the colony-forming unit (CFU) method. The total number of live bacteria was calculated using the colony-forming unit (CFU) method. The total number of live bacteria was calculated using the colony-forming unit (CFU) method.
Oligosaccharides Human Milk and Optimization of Microbiota

- At least 200 Human Milk Oligosaccharides: Some of these are bioactive in the promotion of microbial growth, primarily Bifidobacteria species.
- Fut 2 secretor status (associated with greater Bifidobacteria in the stools) initially thought to be protective against adverse neonatal outcomes, but validation studies suggest otherwise.
- This is a controversial area and studies are ongoing.

Breast Milk microRNAs

Take Home Messages

- Evidence is accumulating that the microbial environment of the fetus and infant have a major effect on subsequent health and disease.
- Numerous environmental influences (antibiotics, mode of delivery, composition of diet) can alter the human "holobiont".
- These effects are especially important in early development and may actually play a role in evolutionary processes that occur more rapidly than previously recognized.
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