

Human Milk Components: Interaction with the Host

Josef Neu, MD
Professor of Pediatrics
University of Florida
neuj@peds.ufl.edu

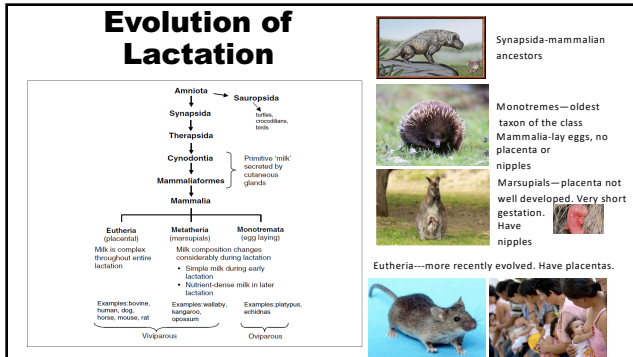
DISCLOSURE STATEMENT Speaker: Josef Neu

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.

Affiliation/Financial Interest	Organization
Infant Bacterial Therapeutics	Scientific Advisory Board Research Grant
Medela	Scientific Advisory Board Research grant
National Institutes of Health	Research Grant
Astarte	Scientific Advisory Board

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.



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.

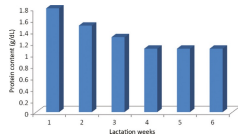


Macronutrients

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.

	Breast milk	Formula	Full-fat milk
Energy, kJ	270-290	280-290	270
Energy, kcal	65-70	67	65
Protein, g	0.9	1.2-1.8	3.4
Carbohydrates, g	6.7	7-8	4.4
Oligosaccharides, g	1.3	0	0
Fat, g	3.5	3.8	3.5
Calcium, mg	20-25	42	116
Phosphorus, mg	12-14	21	33
Sodium, mg	12-25	36	45
Potassium, mg	40-55	55	144
Iron, mg	0.03-0.09	0.4-0.7	0.09
Zinc, mg	0.1-0.3	0.4	0.42
Vitamin A, µg	30-60	50	29
Vitamin C, µg	10	7-9	1.2
Vitamin D, µg	0.03	1.0	0.1
Vitamin K, µg	0.2-0.5	2.8	1.6
Folic acid, µg	80-140	6.5	11

From Michaelsen et al. [2].

Calcium and Phosphorus in Human Milk, Term and Preterm Formulas



	Required per kg/day	Required per 100 kcal	Human Milk per 100kcal	Fortified human milk per 100kcal	Term Formula/100kcal	Preterm formula /100kcal
Ca, mg	184	170	45	156	75	170
P, mg	126	116	21	94	50	85

Ziegler, E. Nutritional Care of Preterm Infants, 2014

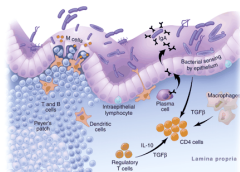
The Versatile Intestine: The intestine is not only a digestive-absorptive organ



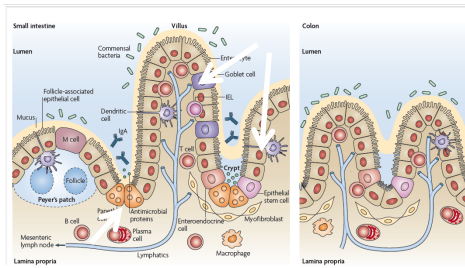
- largest immune organ of the body.
- harbors a huge microbial ecosystem.
- harbors the enteric nervous system

Intestinal Antigenic Load

- Gut content of microbes is $\sim 10^{13}$ 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.

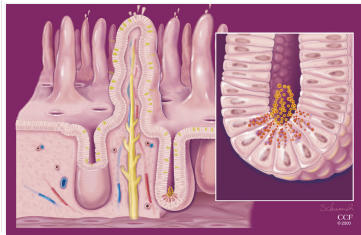


The Intestinal Barrier: Cells



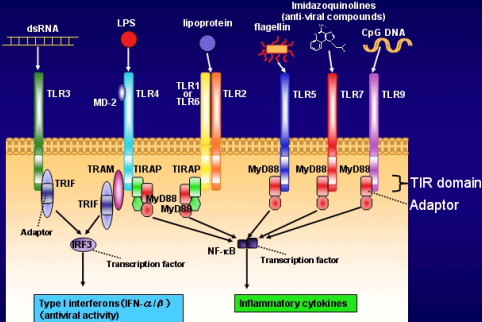
Abreu, M. Nature Immunology Feb, 2010

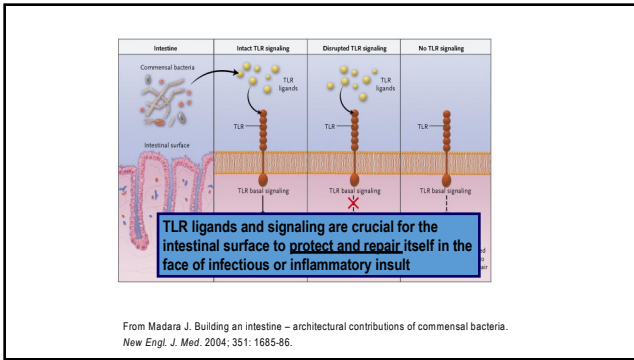
Paneth Cells

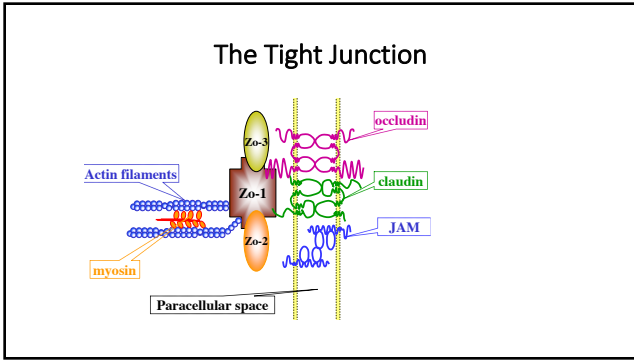


Blikslager AT, et al. Physiol Review 87:545-564,2007


Toll-like receptors: ligands and signaling pathways







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.

A formal photograph of Dr. Widdowson sitting at her desk, which incidentally is untypically tidy. (Photograph taken by Mr David Reed for the National Portrait Gallery, reproduced courtesy of Mr David Reed and Dr Eva Crane.)

Ashwell M
Nature **406**, 844 (24 August 2000)

Morbidities: Early vs. Late Feeding

Table 3. Univariate Analysis of Neonatal Morbidities by Group.

Outcomes (%)	Early (n = 79)	Late (n = 51)
NEC	6.3	10.0
ROP	16.7	52.1**
CLD	21.5	69.4**
PVL	0.0	6.0*
IVH	24.1	24.0
Comorbidities	8.0	25.0**

* Early vs. Late p<0.05;

** Early vs. Late p<0.0001

Neurotizing Enterocolitis (NEC); Retinopathy of Prematurity (ROP); Chronic Lung Disease (CLD); Periventricular Leukomalacia (PVL); Intraventricular Hemorrhage (IVH); Comorbidities = The presence of 2 or more neonatal outcomes.

Konnikova, et al. PLOS One 2015

THE NEW ENGLAND JOURNAL OF MEDICINE

ORIGINAL ARTICLE

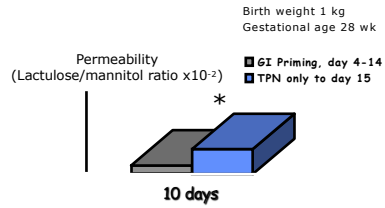
Controlled Trial of Two Incremental Milk-Feeding Rates in Preterm Infants

Jon Dorling, M.D., Jane Abbott, B.A., Janet Berrington, M.D., Beth Bosiak, M.Sc., Ursula Bowler, Elaine Boyle, Ph.D., Nicholas Embleton, M.D., Oliver Hewer, M.A., Samantha Johnson, Ph.D., Edmund Juszczak, M.Sc., Alison Leaf, M.D., Louise Linsell, D.Phil., Kenny McCormick, M.D., William McGuire, M.D., Omar Omar, M.Sc., Christopher Partlett, Ph.D., Mehali Patel, B.Sc., Tracy Roberts, Ph.D., Ben Stenson, M.D., and John Townend, Ph.D., for the SIFT Investigators Group^a

The Intestinal Barrier, Feeding and Late Onset Sepsis



Effect of GI Priming on Intestinal Permeability

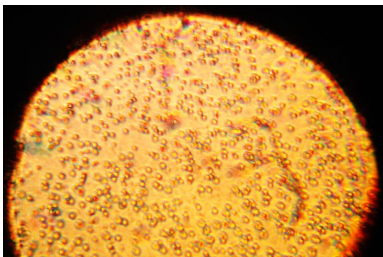


Shulman et al, Pediatr Res 1998;44:519

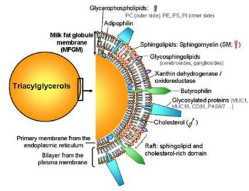
Immunologic and Growth Factors

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

Quiz: What's this?

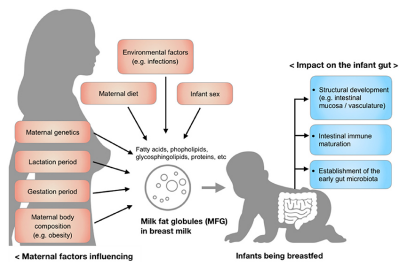


Human Milk Fat Globule



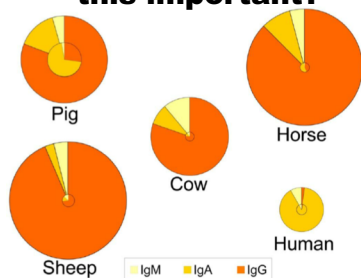
- Antimicrobial defense: Releases free fatty acids and monoglycerides via the digestive process, which disrupt cell membranes of microbes.
- Components have important functions in brain and gut.

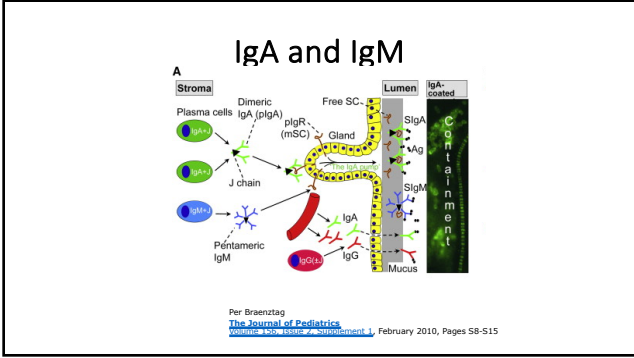
Human Milk Fat Globule

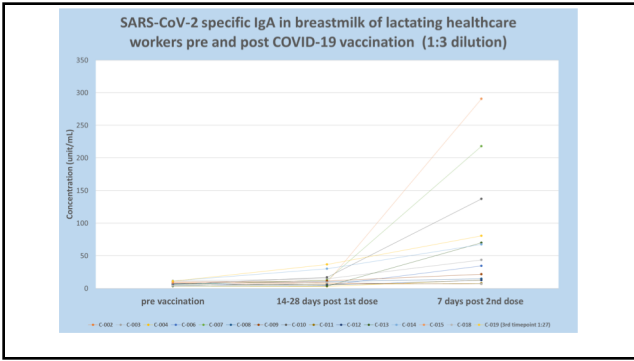


From Lee, et al. Front. Pediatr. 24 October 2018

Quiz: What are we seeing? Why is this important?







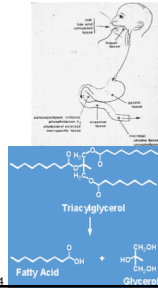
Donor human milk after Holder Pasteurization

Total Lipid	NA or ↓ 3.5-5.5%
Free fatty acids	↑ 83%
Lactose and oligosaccharides	NA
Total protein	NA or 4% reduction
Alkaline phosphatase (ALP)	↓ 99%
Dile salt-stimulated lipase (BSL)	Abolished
Lactoferrin (LF)	NA or ↓ 44-91%
LF-iron-binding capacity	↓ 71%
IgA	NA or ↓ 20-60%
IgM	Abolished
IgG	↓ 34%
Lactoperoxidase (LP)	↓ 82%
LP activity	↓ 88%
Lysozyme	↓ 24-60%
Lysozyme activity	NA or ↓ 65-85%
TGF-β	NA
IGF-I	↓ 39%
IGF-II	↓ 31%
IGFBPs	↓ 7-19%
EGF	NA

3/7/21
Courtesy of Peter Hartman, et al.
30

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.



Hamosh, *Biol Neonate* 1987;52 (Suppl. 1):50-64

OPEN

rhBSSL Improves Growth and LCPUFA Absorption in Preterm Infants Fed Formula or Pasteurized Breast Milk

¹Charlotte Casper, ¹Virgilio P. Carnielli, ¹Jean-Michel Hascoet, ³Alexandre Lapillonne, ¹Luca Maggio, ⁴Kristina Tindahl, ⁵Birgitta Olsson, ⁶Mårten Vägerö, and ⁶Olle Hernell

ABSTRACT

Objectives: Preterm infants often experience suboptimal growth, which can affect organ development. The aim of this study was to improve growth by treatment with bile salt-stimulated lipase (BSSL) naturally present in breast milk, but lost after pasteurization, and absent in formula.

Methods: Two clinical trials were performed with a predefined analysis of combined data to investigate the effects of recombinant human BSSL (rhBSSL) treatment on growth velocity and fat absorption in preterm infants. The studies were randomized and double-blinded comparing 7-day treatment with rhBSSL and placebo, administered in pasteurized breast milk or formula, using a crossover design.

Results: Sixty-three infants were evaluated for safety. At randomization, the mean standard deviation weight was 1467 (151) g and mean postmenstrual age was 32.6 (0.5) weeks. Sixty and 46 infants were evaluated for growth velocity and fat absorption, respectively. rhBSSL treatment significantly improved mean growth velocity by 2.95 g · kg⁻¹ · day⁻¹ (P < 0.001)

compared with placebo (mean 16.86 vs 13.91 g · kg⁻¹ · day⁻¹) and significantly decreased the risk of suboptimal growth (<15 g · kg⁻¹ · day⁻¹) (20% vs 32%, P = 0.004). rhBSSL significantly increased absorption of the long-chain polyunsaturated fatty acids, docosahexaenoic acid, and arachidonic acid by 5.76% (P = 0.013) and 8.55% (P = 0.001), respectively, but had no significant effect on total fat absorption. The adverse event profile was similar to placebo.

Conclusions: In preterm infants fed pasteurized breast milk or formula, 1 week of treatment with rhBSSL was well tolerated and significantly improved growth and long-chain polyunsaturated fatty acid absorption compared to placebo. This publication presents the first data regarding the use of rhBSSL in preterm and the results have led to further clinical studies.

Key Words: Clinical study, fat absorption, growth velocity, preterm infant, recombinant human bile salt-stimulated lipase

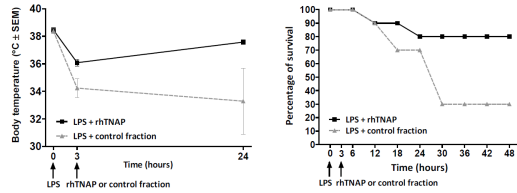
Received October 16, 2013; accepted February 28, 2014.
From the ¹Unit of Neonatology, Children's Hospital, Paul Sabatier

(PFGN 2014;59: 61–69)

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 NFkappaB pathway components.

Effects of Alkaline Phosphatase on Experimental Sepsis in Mice



Bender B, et al. Physiol. Res. 64:731-738,2015

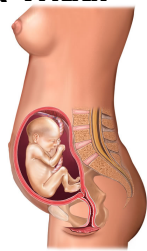
Microbiome: Why all the fuss?



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

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

- New concept: Placenta, amniotic fluid meconium 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 has a bacterial origin.
- The pregnant mammal is herself a symbiotic community, a “holobiont”.

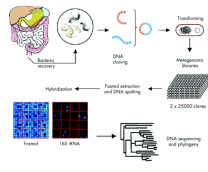


*Adapted from Gilbert, SF, Frontiers in Genetics, Aug. 2014

Culture versus Non Culture

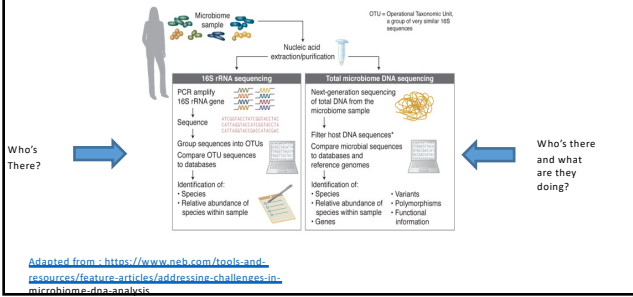


400 species



2000 species

Major Microbiome Sequencing Technologies



THE HUMAN MICROBIOME

Over 100 trillion and diverse microbial organisms live in the folds of the skin, the mucosal surfaces of the mouth, nose, throat, gut, and vagina, and in the large intestine. They are essential for the normal development and function of the immune system, and play a role in the prevention of disease. The human microbiome is estimated to contain 100 trillion organisms that live on the body.

25 SPECIES in the mouth include:

- Streptococcus
- Staphylococcus

500-1,000 SPECIES in the skin include:

- Staphylococcus
- Streptococcus
- Corynebacterium
- Propionibacterium

600+ SPECIES in the gut, oral cavity and respiratory system include:

- Streptococcus
- Bacteroides
- Clostridium
- Escherichia coli

1,000 SPECIES in the gut include:

- Streptococcus
- Bacteroides
- Clostridium
- Escherichia coli

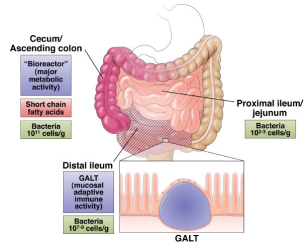
60 SPECIES in the vagina include:

- Lactobacillus
- Streptococcus
- Clostridium

The Versatile Intestine

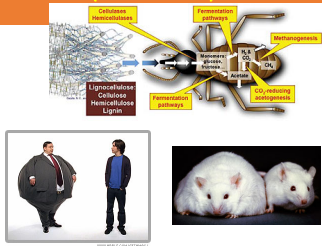
- largest immune organ of the body.
- harbors the enteric nervous system
- harbors a huge microbial ecosystem

Regional Differences



Neish, A. Gastroenterology vol. 136, No. 1, 2009

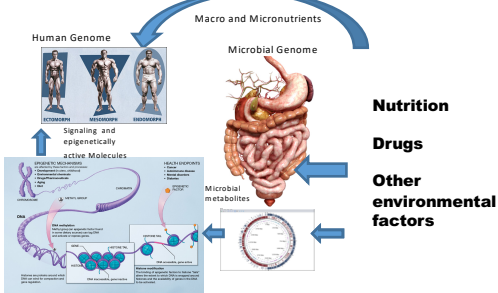
Metabolomic (Bioreactor) Role



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

Roy C. Nutr. Clin Practice 21:351:2006
 Turnbaugh, PJ Cell Host Microbe Volume 3, Issue 4, 17 April 2008, Pages 213-223

How do Microbial Genomics Affect Phenotype?



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

Breast milk microbes
Over time



Hunt, et al. PlosOne
2011

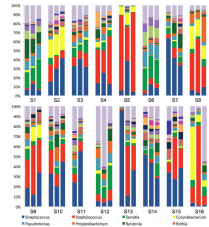
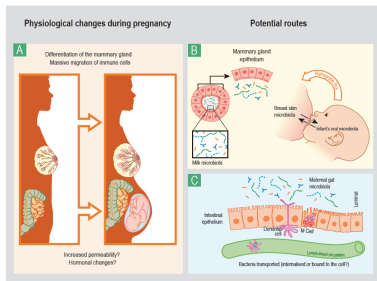


Figure 1. The community composition of the 16S rRNA-sequenced bacterial genera in a panel of 168 samples from 161 women over 24 weeks postpartum. The community composition is shown as stacked bar charts for each time point. The color key is as follows: Bacteroidetes (blue), Firmicutes (red), Proteobacteria (green), Actinobacteria (purple), Fusobacterium (orange), Veillonellaceae (yellow), Streptococcus (pink), and Clostridiaceae (grey).

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

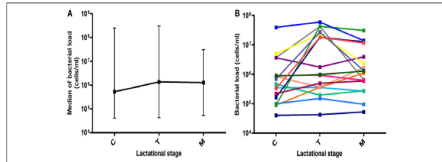


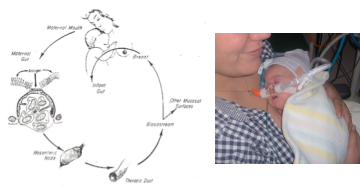
FIGURE 1 | Bacterial load over lactational stages. (A) Data show the median with ranges (maximum and minimum values for each group) of bacterial load at the three time points. C, colostrum samples (n = 19); T, transition milk samples (n = 20); M, mature milk samples (n = 17). (B) Lines show individual bacterial load for each mother at the three time points (n = 17).

Boix-Amaros, A. *Frontiers in Microbiology*, 20 April, 2016

Microbial Dose from Human Milk

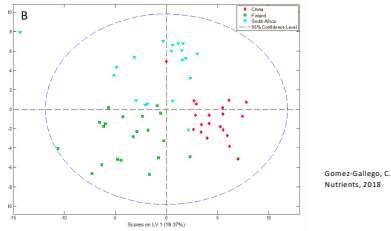
- Assume intake of 800 ml/day
- Assume 10⁵⁻⁶ bacterial cells/ml
- This will provide 10⁷⁻⁸ bacterial cells (personalized?) daily, close to the dose in most probiotic studies.

Human Milk Personalization and Dynamic Interactions: Enteromammary Immune System



Kleinman, RE and Walker, WA. *Dig Dis Sci.*, 1979

Metabolome of Human Milk Different Geographic Regions



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?

Personalization of the Microbiota of Donor Human Milk with Mother's Own Milk

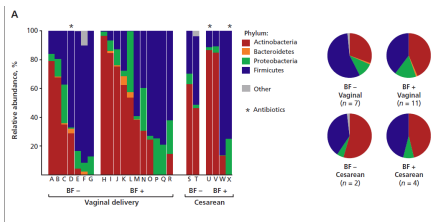
The American Academy of Pediatrics recommends that exclusively breastfed infants receive mother's own milk (MOM) until 6 months of age. For infants who are not breastfed, the American Academy of Pediatrics recommends that they be fed donor human milk (DHM) until 12 months of age. The goal of this study was to determine whether DHM could be personalized with MOM-like microbes to promote infant health. DHM was personalized with MOM-like microbes and a probiotic formulation in milk. The personalized DHM, MOM, and the probiotic formulation were tested in a murine model to evaluate the microbiota in the small intestine and feces. The results of this study are available at <https://doi.org/10.3389/fmicb.2017.01811>.

Frontiers in Microbiology, August 2017

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(5)

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

Lukas Christen^{1,2*}, Ching Tat Lai¹, Ben Hartmann^{3,4}, Peter E. Hartmann¹, Donna T. Geddes¹

1 School of Chemistry and Biochemistry, Faculty of Science, The University of Western Australia, Crawley, Western Australia, Australia, **2** Dorig AG, Basle, Switzerland, **3** Person Rotary Express Milk Bank, King Edward Memorial Hospital, Subiaco, Western Australia, Australia, **4** Centre for Neonatal Research and Education, The University of Western Australia, Crawley, Western Australia, Australia

Abstract

Background: Holder pasteurization (milk held at 62.5°C for 30 minutes) is the standard treatment method for donor human milk. Although this method of pasteurization is able to inactivate most bacteria, it also inactivates important bioactive components. Therefore, the objective of this study was to investigate ultraviolet irradiation as an alternative treatment method for donor human milk.

Methods: Human milk samples were inoculated with five species of bacteria and then UV-C irradiated. Untreated and treated samples were analysed for bacterial content, bile salt stimulated lipase (BSL) activity, alkaline phosphatase (ALP) activity, and fatty acid profile.

Results: All five species of bacteria reacted similarly to UV-C irradiation, with higher dosages being required with increasing concentrations of total solids in the human milk sample. The decimal reduction dosage was 289±17 and 945±164 J/l for total solids of 107 and 146 g/l, respectively. No significant changes in the fatty acid profile, BSL activity or ALP activity were observed up to the dosage required for a 5-log₁₀ reduction of the five species of bacteria.

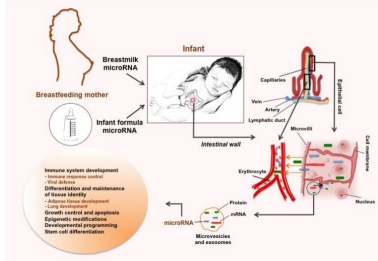
Conclusion: UV-C irradiation is capable of reducing vegetative bacteria in human milk to the requirements of milk bank guidelines with no loss of BSL and ALP activity and no change of FA.

Citation: Christen L, Lai CT, Hartmann B, Hartmann PE, Geddes DT (2013) Ultraviolet C Irradiation: A Novel Pasteurization Method for Donor Human Milk. PLOS ONE 8(6): e68120. doi:10.1371/journal.pone.0068120

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



Alsaweed, M. et al. Int. J. Environ., Res. Public Health 2015

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

Acknowledgments

University of Florida

- Ron Li
- Eric Tamm
- Leslie Foster
- Elizabeth Lanza
- Mark Robinson
- Ben Coker
- Chae Wansu/Chae
- Cindy Miller
- Kelly Cury
- Eric Wang
- Christina Jahn
- Melissa Kucharski
- Valeriya
- Benjamin Jones
- Jonathan Mueller
- Matt Leach
- Christine Wood
- Melissa Miller Wood
- Andrew Greg
- Joseph Lamb
- Catherine Kucenic
- Melissa Lomon (Nursing Nurse)
- Ana Carolina -Cepeda
- Elizabeth
- Elizabeth
- Nicholas Curtis
- Mark Smith (Dermatologist)
- Alexanna (Dermatologist)

Special thank you to Nursing Staff,
Families and Babies at UF Health NICU

Recent Fellows

- Anand (China) (currently faculty at UF)
- Shantanu (in Out) (currently faculty at UF)
- Chuanxin (Graduate)
- Sarah Egner
- Wang (currently JG-CG)
- Doreen Wu (Newest) (PhD in Otolaryngology)
- Liqiang
- Jun (former graduate student—microbiology and cell sciences)

Visiting Scholars

- Missa Mohitpour (Texas, Georgia)
- Wu Yan (Bao Hospital, Shenzhen, China)
- Yanyan (Guangdong University, Hainan, China)
- Yuan Zhong (Japan, China)
- Wang (Department of Pediatrics, Fudan University, Shanghai, China)
- Ji Kim (Chairman of Korean Society of Neonatology, Seoul, Korea)
- Jungho (Seoul National University)
- Li (Liqiang) (investigator) (currently here from Changqing, China)

Non-UF Faculty, Fellows and Grad Students

- Richard (Paris) (Columbia University)
- Sharon (Dorset) (J. of Bristol—Nutrition)
- Bill (Daphne) (Texas A and M, not neonatologist)
- Dominique (Darmstadt, France)
- Jean-Christophe (Rouen, France)
- M Carmen (Catalonia, Valencia, Spain—human milk studies)
- Anthony (Foster) (J. North Carolina, Charlotte—neonematology)
- Anthony (Wright) (Graduate Student, U. North Carolina, Charlotte)
- W. Allen (Walker) (Purdue)
- Nathan (Farrow) (Baylor)
- Mark Knight (Bioinformatics) (UCSD)
- Paolo (Melo) (Baylor, MD)
- Mike (Carter) (Ohio)
- Bill (Barnes) (Baylor)

RECENT FUNDING SOURCES

NIH (R01, R03, CSR), CIRM, Weizmann, Infant Microbiota Therapeutics
