Impact of Human Milk on Gut Colonization and Subsequent Health Outcomes

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Rush University Medical Center, Chicago
Within the NICU, the RUSH Mothers’ Milk Club is a state-of-the-art lactation and human milk feeding program

Translational Research within a Clinical Program of Lactation Care

- Practice: Families receive state-of-the-art, evidence-based lactation care by the entire NICU staff
- Research: Families and infants serve as subjects in the team’s internally and externally funded research projects

RUSH MOTHERS’ MILK CLUB

Rush Research Team 2017

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Clinical Staff that Provide Direct Lactation Care and Optimize Recruitment and Retention for Research

Breastfeeding Peer Counselor Team*

*All are parents of former Rush NICU infants

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• The first 1000 days is a critical window in human development that is heavily influenced by early nutrition
  • 270 days of gestation
  • 365 days = 1st year post-birth
  • 365 days for 2nd year post-birth

  – Organs, immunomodulatory and enzymatic pathways develop and are influenced by early diet
  – Early diet and growth trajectories influence childhood and adult health by multiple mechanisms

Outcomes for the Term Infant

• Acute otitis media
• Gastroenteritis
• Lower respiratory tract infections
• SIDS
• Atopic dermatitis
• Asthma
• Diabetes (Types I and II)
• Obesity (Childhood and Adult)
• Childhood leukemia
• Neurocognitive outcomes
• Post-neonatal death
• Lower costs for family and society

Ip et al., Breastfeeding and maternal and infant health outcomes in developed countries. AHRQ Evidence report #153; www.ahrq.gov; Oddy et al.; Pediatrics, 2011; Chen & Rogan, Pediatrics, 2004; Bartick & Raimbault, Pediatrics, 2010
• Dose-dependent reduction in the risk/incidence/severity of:
  – NEC
  – Late Onset Sepsis
  – Bronchopulmonary Dysplasia
  – Retinopathy of Prematurity
  – Neurodevelopmental problems at 20 months CA
  – Rehospitalization after NICU discharge

• Reduced risk of breast and ovarian cancers
• Reduced risk of Type 2 diabetes
• Reduced risk of obesity (retaining pregnancy weight gain)
• Reduced risk of hypertension
• Reduced risk of Cardiovascular Disease
• Reduced risk of myocardial infarction
  in mid-late adulthood
• Increased costs = $17.4 billion

A Single Layer of Epithelial Cells Lines the GIT

- Barrier between lumen of the gut and underlying mucosa
- Digestion and absorption of nutrients
- Establish molecular relationships with the myriad of bacteria in the gut through multiple mechanisms

Intestinal Lumen

Enterocytes

- Open Paracellular Pathways permit entry of large-molecular-weight antibodies from colostrum, then close to prevent translocation of pathogenic organisms as the gut undergoes rapid colonization with bacteria following birth

Intestinal Lumen

Enterocytes

Intestinal Tract has High Intestinal Permeability During Early Post-Birth Period

- Commensals:
  - Anti-inflammatory
  - Protect gut epithelial border
- Pathogens:
  - Upregulate inflammation
  - Predispose to gut dysbiosis

Intestinal Lumen

Enterocyte

- Intestinal Tract Becomes Home to More Bacteria than there are Cells in the Human Body
Intestinal Lumen

Enterocyte

Pathogens and their toxins perpetuate open paracellular pathways (high intestinal permeability) permitting translocation of pathogens into underlying mucosa

Under healthy circumstances the epithelial border is a barrier, but dendritic cells sample bacteria in a manner that induces tolerance to antigens

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**Microbiota, Microbiome, Metabolome**

**The Gut Microbiota Engage in Bacterial-Enterocyte Cross-Talk, Shaping Life-Long Health**

Yu, LC et al. *World J Gastrointestinal Pathophysiology, 2012*
The composition of the gut microbiota throughout life, with an emphasis on early life.

Juan Miguel Rodríguez1, Kiera Murphy2, Catherine Stollman2,9, R. Paul Ross3,4, Olivia I. Kocab4, Nathalie Juge4, Ekatrina Anversa5, Knut Rudi5, Asger Norby5, María C. Jemmenn6,7, Johan R. Marchesi7,8 and Maria Carmen Collado7,9

Antenatal/Perinatal Maternal Factors that Influence Infant Gut Microbiota

- Maternal overweight and obesity
- Maternal diabetes
- Preterm birth, antibiotics
- Chorioamnionitis
- Cesarean Delivery

Rautava et al., Gastroenterology & Hepatology, 2012.
- Dominguez-Bello et al. Proc Nat Acad Sci USA, 2010
- Jealissang et al. Gut, 2014

Maternal Overweight and Obesity

Distinct composition of gut microbiota during pregnancy in overweight and normal-weight women1,2

Maria Carmen Collado, Brita Eulernaut, Karin Laftinen, and Seppo Salmela

Effect of mother’s weight on infant’s microbiota acquisition, composition, and activity during early infancy: a prospective follow-up study initiated in early pregnancy1

Maria Carmen Collado, Brita Eulernaut, Karin Laftinen, and Seppo Salmela

Maternal weight and excessive weight gain during pregnancy modify the immunomodulatory potential of breast milk.

Pediatric Research, 72: 77-85, 2012

Of the bugs that shape us: maternal obesity, the gut microbiome, and long-term disease risk.

Pediatric Research, 77: 196-204, 2015

Rodriguez et al., 2015
Dominguez-Belloa et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. PNAS. 2010;107(26):11971-5

Cesarean Delivery has long-lasting impact on infant gut microbiota

Original Article
Decreased gut microbiota diversity, delayed Bacteroides colonisation and reduced Th1 responses in infants delivered by Caesarean section


Intestinal Colonization (Band Patterns) Becomes Similar Among Premature Infants Over the NICU Stay Compared to Breast-Fed Term Infants


The composition of the gut microbiota throughout life, with an emphasis on early life

Juan Manuel Rodriguez1, Kiera Murphy2, Catherine Stanton1,2, R. Paul Ross3,4, Olivia I. Koby5, Nathalie Juge6, Ekaterina Avrorina2,3, Knut Rudi7, Anja Kiel5,8, Maria C. Jerums9, Julian R. Marchesi10,11 and Maria Carmen Gollad10,11

Several lines of evidence suggest that artificial milks exert a separate, detrimental effect on the growth, maturation and integrity of the gut epithelium

Rodriguez et al., 2015

Clinical Outcomes

Experimental Mechanisms

Ohta et al., J Parenter Enter Nutr 34: 271-279, 2010
Sanghvi, Curr Opin Pediatr 2015 doi:10.1097/MOP.0000000000000237
Thompson et al., J Mda Nursing 1015, 2008
Thompson et al., Sc Med J 1113-1116, 1994
Infant Formula: Separate Detrimental Impact

- Increases Intestinal Permeability: Undigested casein attracts neutrophils which separate the tight junctions, allowing entry of bacteria and toxins into the mucosa
- Direct Cytotoxicity of Epithelial Cells

Components in digested and undigested formula are directly cytotoxic to intestinal cells in animal studies and incubated intestinal cell lines

Pathogens and their toxins perpetuate open paracellular pathways (high intestinal permeability) permitting translocation of pathogens into underlying mucosa

Mannitol

Lactulose

- Mannitol is “small”, and diffuses easily through the small intestinal cell membrane
- Lactulose is “large”, and can be absorbed only via the paracellular pathways

Lactulose-to-Mannitol (L/M) ratios are a measure of intestinal permeability

Urine samples measure L/M ratios

High L/M ratio = High intestinal permeability

Human Milk Decreases Intestinal Permeability in a Dose-Response Manner in Premature Infants

Mannitol

Lactulose

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Infants who received >75% of feedings as human milk had significantly lower intestinal permeability, when compared to infants with <25% (or no) human milk feedings at postnatal days 7, 14, and 30.

- 3.8-fold lower permeability (L/M ratio) when these 2 dosage groups were compared as composite over 1st 30 days


Infants receiving 100% formula had significantly higher intestinal permeability, when compared to infants receiving ANY human milk at days 7 and 14.

- 2.8-fold higher permeability (L/M ratio) in 100% formula group when compared to composite over 1st 30 days

- Volume of formula positively associated with high permeability on day 7, suggesting “toxic dose”


Human milk is a source of probiotic bacteria: The milk microbiome


Martin et al. Human milk is a source of lactic acid bacteria for the infant gut. J Pediatr 2003; 143: 754-8

Cabrero-Rubio et al. The human milk microbiome changes over lactation and is shaped by maternal weight and mode of delivery. AJCUL2012, 36 (2): S64-S65.


The milk microbiome is exceptionally mother-infant specific, and is accompanied by an array of prebiotic oligosaccharides that serve as "food" for the probiotic bacteria.
Growth Factors Target Enterocytes to Markedly Increase Surface Area and Stimulate Epithelial Cell Migration and Turnover in the GIT

Tapper et al., 1979; Walker, 2010

Other Human Milk Components work Synergistically with the Gut Microbiota

Immunologic programming by breast milk creates an anti-inflammatory cytokine milieu in breast-fed infants compared to formula-fed infants.

Introduction: Emerging Roles of Bioactive Components in Pediatric Nutrition

J Pediatrics, 2016

The Role of Lactoferrin in Gastrointestinal and Immune Development and Function: A Proximal Perspective

J Pediatrics, 2016

Human milk provides peptides highly stimulating the growth of bifidobacteria

European J Biochemistry, 2002

American J Respiratory and Critical Care Medicine, 190: 298-308, 2014.
Growth, Maturation and Protection of the GIT with Colostrum and Human Milk

Walker, J Pediatrics, 2010

• Prebiotic
• Anti-adhesive
• Anti-microbial
• Decoy function
• Immune cell modulators
• Epithelial cell modulators

• Found in urine, feces and systemic circulation in recipient infants
• Highly individualized for mother-infant dyad

Grahming KC et al. PLOS ONE 9, 2014.

Soluble CD14 mediates bacterial-enterocyte cross-talk in the immature intestine

• Soluble CD-14 is a pattern recognition receptor serving as co-receptor for TLR-II and TLR-IV
  – Mediates bacterial-enterocyte “cross-talk”
• Present in serum, amniotic fluid, breast milk, and other fluids, but not in infant formulas


Soluble CD14

In μg/ml; *n= 22 (0-71 days post-birth)
**n = 10 (≤ 6 days post-birth)
The gut has a major role in brain development via the gut-brain axis. Emphasizes:
- The role of the enteric nervous system in inflammation
- The indirect impact of intestinal inflammation on brain development even in the absence of sepsis
- The role of nutrition in down-regulation of intestinal inflammation with consequent neuroprotection

Recommends:
- Human milk that is not frozen or pasteurized
- Colostrum singled out and fed early
- Re-thinking timing of bovine fortification (references oxidative stress, citing Friel, 2011)
- Avoidance of formula (due to epithelial cell cytotoxicity, citing Penn, 2012)
Rethinking the Role of Nutrition in Brain Development for Premature Infants

- Appropriate growth of the brain is only one aspect of optimal nutrition.
- In the last several years, the research standard for evaluation of brain development is magnetic resonance that reveals separate brain structures.
- In premature infants, the developing white matter is vulnerable to inflammation and oxidative stress.
- White matter mass and integrity is related to neurodevelopmental outcome in term and premature infants.
- Nutrition now seen as having potential for neuroprotection as well as neuromodulation during active infection.
- Many "neuroprotective" nutritional supplements now being studied are present in high concentrations in human milk, and are highest in the early milk of mothers who deliver prematurely. *Patra et al., in review.*

Selective Brain Growth and Development Most Vulnerable with Preterm Birth

- Serial brain imaging to determine the relationship between the growth of cortical surface area relative to cerebral volume
- Critical window of selective brain growth
  - Cortical surface area grows more rapidly cortical volume
  - Pattern is defined by specific algorithm
- Susceptible to extrauterine events that interrupt this unique pattern of development

*Kapellou et al., PLOS Medicine 3: 2006; e265*

| NeuroImage, 2013, 77-86 |

Breastfeeding and early white matter development: A cross-sectional study

- 133 Term Infants followed with neuroimaging and neurodevelopmental testing from 10 months-4 years of age

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<th>Relationship between duration of breastfeeding, white matter microstructure, and neurodevelopmental testing</th>
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<td>Duration of breastfeeding (mo)</td>
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*NeuroImage, 2013, 77-86*
• 104 VPT (≤ 32 weeks gestation), 107 term infants: 1998-2000)
• Structural MRI scan at term equivalent for all VPT infants
• Ages 4 and 6: comprehensive ND assessment (general intellectual ability, language, executive functioning)
• Findings:
  • VPT children without white matter abnormalities at term were equivalent to term infants
  • VPT children with mild and moderate-severe white matter abnormalities had performance deficits across all time points and measures
  • Associations persisted after adjustment for gender, neonatal medical risk factors and family social risk

More recent studies confirm the concept of differential areas of growth and vulnerability in the premature brain

Infection-induced inflammation and cerebral injury in preterm infants

• Exceptionally thorough review paper with tables of previous studies
• Emphasizes:
  • Antibiotics alone are ineffective in preventing infection-related inflammation in developing white matter
  • Injury to developing white matter can occur without bacterial entry into the CSF due to inflammation that targets the myelin sheath
• Concludes:
  • Strategies to reduce inflammation secondary to activated immune cells—among these is lactoferrin
• Nutrition can be neuroprotective of rapidly developing grey and white matter processes for preterm infants
• Emphasizes that nutrition strategies should:
  • Target selective growth of vulnerable structures (not just cerebral volume growth)
  • Provide neuroprotection of vulnerable structures (e.g., downregulation of inflammation)
• Recommends:
  • Probiotics
  • Prebiotic oligosaccharides (focus on gut colonization and gut-brain axis)
  • Neuroprotective supplements: glutamine, Vit E, lactoferrin