Human Milk: Short Term Outcomes

- Infant Mortality
- Infectious Disease
  - Bacterial meningitis
  - Bacteremia
  - Diarrhea
  - Respiratory tract infection
  - Otitis media
  - Urinary tract infection

Human Milk: Long Term Infant Outcomes

- Cognitive Development
- Metabolic Syndrome
- Obesity
- Hypertension
- Diabetes Mellitus
- Lipid Profile
- Allergy and Asthma
- Celiac Disease
- Short Bowel Syndrome

Effect of Human Milk versus Formula on Cognitive Developmental Scores

- Meta-analysis of 20 studies (11 studies controlled for >5 covariates) concluded:
  - Unadjusted benefit of 5.2 (95% CI: 4.5-5.9)
  - Adjusted benefit of 3.2 (95% CI: 2.4-4.0)
  - Low birth weight infants showed the greatest benefit

Benefits of Human Milk to the Mother

Short Term
- Decreased postpartum bleeding
- More rapid uterine involution
- Decreased menstrual blood loss
- Increased child spacing
- Earlier return to pre-pregnancy weight

Long Term
- Decreased risk of breast cancer
- Decreased risk of ovarian cancer

References:

Benefits of Human Milk to Society

- Reduced health care costs
- Contributes to a more productive workforce: healthier mother and child
- Better for our environment: less trash and plastic waste

Incidence and Prevalence of Preterm Births

- Half a million preterm births in the United States

Worldwide Preterm Birth Rates

<table>
<thead>
<tr>
<th>Countries</th>
<th>Preterm Birth Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA</td>
<td>12.8</td>
</tr>
<tr>
<td>Africa</td>
<td>11.9</td>
</tr>
<tr>
<td>North America</td>
<td>10.6</td>
</tr>
<tr>
<td>Asia</td>
<td>9.1</td>
</tr>
<tr>
<td>Latin America &amp; Caribbean</td>
<td>8.1</td>
</tr>
<tr>
<td>Canada</td>
<td>7.0</td>
</tr>
<tr>
<td>Oceania (Australia/NZ)</td>
<td>6.4</td>
</tr>
<tr>
<td>Europe</td>
<td>6.2</td>
</tr>
</tbody>
</table>

Beck et al., 2009

Vulnerabilities of the preterm infant

- Poor neurocognitive outcomes ✔
- Increased risk of necrotizing enterocolitis ✔
- Increased risk for infection ✔
- Decreased immunoglobulin ✔
- Increased intestinal permeability ✔
- Poor intestinal motility ✔
- Abnormal gut colonization ✔
- Delayed gastric emptying ✔

These are human milk benefits to the preterm infant
Human milk and subsequent intellectual performance in preterm infants at 8 yr

<table>
<thead>
<tr>
<th>Factor</th>
<th>IQ Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breastmilk</td>
<td>+8.3</td>
</tr>
<tr>
<td>Social Class</td>
<td>-3.5/class</td>
</tr>
<tr>
<td>Mother’s Education</td>
<td>+2.0/group</td>
</tr>
<tr>
<td>Female Gender</td>
<td>+4.2</td>
</tr>
<tr>
<td>Mechanical Ventilation</td>
<td>-2.6/week</td>
</tr>
</tbody>
</table>


What is in human milk?

Human milk is a tissue

Human Milk

<table>
<thead>
<tr>
<th>CELLS</th>
<th>HORMONES</th>
<th>TRANSPORTERS</th>
<th>IMMUNE MODULATORS</th>
<th>GROWTH FACTORS</th>
<th>NUTRIENTS</th>
</tr>
</thead>
</table>

Blood

<table>
<thead>
<tr>
<th>ENZYMES</th>
<th>CYTOKINES</th>
<th>IMMUNE MODULATORS</th>
<th>NUTRIENTS</th>
</tr>
</thead>
</table>

Nutritional Components of Human Milk

MACRONUTRIENTS

Proteins
- Mucins
- Caseins
- Whey
- Amino acids

Carbohydrates
- Lactose
- Glucose
- Galactose
- Oligosaccharides (neutral and acidic, ~1%, >200 identified)

Lipids
- Triglycerides (167 identified)
- Phospholipids
- Sphingolipids
- Sterols
- Fatty acids

Enteral Administration of Fat

- How does fat get digested?
  - Different between human milk and formula
- How much should we give?
  - Formula – not highly variable
  - Human milk – most variable macronutrient
  - ~50% of total calories

Enteral Lipids

Human Milk Composition
- 3-4.5% fat in human milk
- Mostly triacylglycerols 98.99%
- Unique fat globules
Human Milk Fat Globules

Digesting Fat
- Step 1: Salivary lipase
- Step 2: Human milk lipase and gastric lipase
  - attack the core of human milk globule
- Step 3: Bile salts and formation of micelles
- Step 4: Pancreatic lipase/collaps
  - generation of monoacylglycerols and FFA

Fat Comparison between Human Milk Versus Formula

<table>
<thead>
<tr>
<th>Nutritional Component</th>
<th>Human Milk</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat globules</td>
<td>&lt;50%</td>
<td>40-50%</td>
</tr>
<tr>
<td>Medium-Chain Triglycerides</td>
<td>8-10% of total fatty acids</td>
<td>40-50% of total fatty acids</td>
</tr>
<tr>
<td>Long-Chain Fatty Acids</td>
<td>palm, oleic</td>
<td>high oleic, safflower, soy, corn oil</td>
</tr>
<tr>
<td>Trans-fatty acids</td>
<td>&lt;5%</td>
<td>trace</td>
</tr>
<tr>
<td>Free fatty acids</td>
<td>&lt;2%</td>
<td>trace</td>
</tr>
</tbody>
</table>

Digestion of Milk

Vitamin D levels in human milk
- Vit D levels in human milk reflect maternal vitamin D status
- Reduced sun exposure has markedly increased vitamin D insufficiency in mother's milk
- Supplementation with 4,000 to 6,000 Units/day of vitamin D can restore adequate levels of vitamin D during lactation

Nutritional Components of Human Milk

Vitamins
- A
- C
- D
- E
- K
- B1
- B2
- B3
- B6
- B12
- Biotin
- Choline
- Folate
- Niacin
- Pantothenic acid
- Riboflavin
- Thiamin
- Vitamin B6
- Vitamin B12

Minerals
- Calcium
- Copper
- Iron
- Magnesium
- Manganese
- Phosphorus
- Potassium
- Sodium
- Zinc

MICRONUTRIENTS
Vitamins

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td></td>
</tr>
<tr>
<td>K</td>
<td></td>
</tr>
<tr>
<td>B1</td>
<td></td>
</tr>
<tr>
<td>B2</td>
<td></td>
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<tr>
<td>B3</td>
<td></td>
</tr>
<tr>
<td>B6</td>
<td></td>
</tr>
<tr>
<td>B12</td>
<td></td>
</tr>
<tr>
<td>Biotin</td>
<td></td>
</tr>
<tr>
<td>Choline</td>
<td></td>
</tr>
<tr>
<td>Folate</td>
<td></td>
</tr>
<tr>
<td>Niacin</td>
<td></td>
</tr>
<tr>
<td>Pantothenic acid</td>
<td></td>
</tr>
<tr>
<td>Riboflavin</td>
<td></td>
</tr>
<tr>
<td>Thiamin</td>
<td></td>
</tr>
<tr>
<td>Vitamin B6</td>
<td></td>
</tr>
<tr>
<td>Vitamin B12</td>
<td></td>
</tr>
</tbody>
</table>

Minerals

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td></td>
</tr>
<tr>
<td>Copper</td>
<td></td>
</tr>
<tr>
<td>Iron</td>
<td></td>
</tr>
<tr>
<td>Magnesium</td>
<td></td>
</tr>
<tr>
<td>Manganese</td>
<td></td>
</tr>
<tr>
<td>Phosphorus</td>
<td></td>
</tr>
<tr>
<td>Potassium</td>
<td></td>
</tr>
<tr>
<td>Sodium</td>
<td></td>
</tr>
<tr>
<td>Zinc</td>
<td></td>
</tr>
</tbody>
</table>
Non-Nutritional Components of Human Milk (>250 compounds)

Antimicrobial factors
- Secretory IgA, IgM, IgG
- Lactoferrin
- Lysozyme
- Complement C3
- Leukocytes
- Bifidus factor
- Lipids and fatty acids
- Antiviral mucins, GAGs
- Oligosaccharides

Cytokines and anti-inflammatory factors
- Tumour necrosis factor
- Interleukins
- Interferon-gamma
- Prostaglandins
- A1-antichymotrypsin
- A1-antitrypsin
- Platelet-activating factor: acetyl hydrolase

Hormones
- Feedback inhibitor of lactation (FIL)
- Insulin
- Prolactin
- Thyroid hormones (T2, T3, Reverse T3)
- Corticosteroids, ACTH
- Oxytocin
- Calcitonin
- Parathyroid hormone
- Erythropoietin
- Progesterone
- Estrogen

Growth factors and GI regulatory peptides
- Epidermal (EGF)
- Nerve (NGF)
- Insulin
- Insulin-like (IGF)
- Transforming (TGF)
- Taurine
- Polyamines
- Gastrin
- Gastric inhibitory peptide (GIP)
- Gastric regulatory peptide (GRP)
- Neuropeptide
- Histidine

Peptide YY (PYY)

Digestive enzymes
- Amylase
- Bile acid-stimulating esterase
- Bile acid-stimulating lipases
- Lipoprotein lipase
- Proteases

Transporters
- Lactoferrin
- Xanthine oxidase
- Glutathione peroxidase
- Alkaline phosphatase
- Folate binder
- Cobalamin binder
- IgF binder
- Thyroxine binder
- Corticosteroid binder

BIOLOGICS
- 1. Nucleotides (adenosine 5’-monophosphate, cytidine 5’-monophosphate, disodium guanosine 5’-monophosphate, disodium uridine 5’-monophosphate)
- 2. Prebiotics
- 3. Probiotics

CAN WE IMITATE HUMAN MILK?

- Gross nutrient compositional identity close but structural profiles still different
- No bioactive factors – very expensive
- Cellular components – very, very difficult
- Main goal is to try to match major functional effects
- THEREFORE, NOT IN OUR LIFETIME

Human Milk Contains Bioactive Factors That Stimulate the GI Tract

| TABLE 1 | Gastrointestinal target for selected bioactive factors in human milk |
| --- | --- | --- |
| Agent in HM | Epithlum | Immune system | Nervous system |
| IGF-1 | - | - | - |
| IGF-2 | 1 | - | - |
| EGF | 2 | 3 | - |
| A1-antitrypsin | - | - | - |
| Cortisol | - | - | - |
| Thyroxine | - | - | - |
| Enzymes | - | - | - |
| Peptides | - | - | - |

NEC is the most common life-threatening gastrointestinal emergency in the newborn period
- NEC is marked by intense inflammation and acute intestinal necrosis
- Most common sites include terminal ileum, cecum and ascending colon

Ingredient Listing of Term Formula

MACRONUTRIENTS
- Water
- Nonfat milk
- Lactose
- High oleic safflower oil, soy oil, coconut oil, C. cohnii oil (DHA), M. alpina oil (ARA)

MINERALS
- Potassium citrate, calcium carbonate, potassium chloride, magnesium chloride, sodium chloride, ferrous sulfate, choline chloride

TRACE ELEMENTS
- Zinc sulfate, cupric sulfate, manganese sulfate, sodium selenate

VITAMINS
- D-alpha-tocopheryl acetate, ascorbic acid, niacinamide, riboflavin, calcium pantothenate, vitamin A palmitate, thiamine chloride hydrochloride, pyridoxine hydrochloride, biotin, cyanocobalamin, vitamin D3

EMULSIFIERS
- Mono- and diglycerides, soy lecithin, carrageenan, choline bitartrate, taurine, m-inositol, L-carnitine

BIOLOGICS
- 1. Nucleotides
- 2. Prebiotics (term formula only)
- 3. Probiotics (term formula only)
Pathogenesis of NEC

- Loss of barrier function
- Intrinsic factors
- Acute injury
- Infectious agents
- Mucosal disruption
- Toxins

Epidemiology of NEC

- NEC comprises 1-5% of all NICU admissions
- NEC affects more than 10% of infants born less than 1000 grams
- No sex differences but males have greater chance of death
- ~30-50% go to surgery
- Case fatality rate with surgical intervention as high as 50%
- Stricture formation occurs in 25% to 35% of survivors after either medical or surgical therapy

Onset of NEC is inversely correlated with gestational age

Death from NEC

- Time periods
  - 1960-70: 24-65%
  - 1990’s: 9-28%
  - 2000+: 0-10%
- Concept of Zero NEC possible

Economic costs of NEC

- Medical NEC (22 days): US $73,700 (1992-94)
- Surgical NEC (2 months): US $186,200
- Increased length of stay, hospital costs
- US $ 7.2 million/year on unit and US $238,333 per survivors (not included doctor’s fee + TPN + antibiotics)

- Costs to health care system
  - $5 billion annually (assume 10%, incidence of NEC and 30% mortality)
  - Constitutes 19% of the total costs for initial care for all newborns in the US

Reduction of NEC with Human Milk

- NICHD, CNN

- Reduction in NEC with human milk

NICHD=National Institute Child Health and Human Development
CNN=Canadian Neonatal Network

Fractional Human Milk Feeding Reduces Chances of NEC

**Fraction of Human Milk Feeding**

<table>
<thead>
<tr>
<th>Age in Days</th>
<th>0.00</th>
<th>0.10</th>
<th>0.20</th>
<th>0.30</th>
<th>0.40</th>
<th>0.50</th>
<th>0.60</th>
<th>0.70</th>
<th>0.80</th>
<th>0.90</th>
<th>1.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fraction</td>
<td>1.00</td>
<td>0.90</td>
<td>0.80</td>
<td>0.70</td>
<td>0.60</td>
<td>0.50</td>
<td>0.40</td>
<td>0.30</td>
<td>0.20</td>
<td>0.10</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Meinzen-Derr et al. NICHD Network, J Perinatol 2008;1-6

**Human Milk Fortification**

- **Bovine HMF**
- **Human Milk**
- **Human HMF**
- **Exclusively Human**

Standard

**Hypothesis**

- Short-term health benefits (decreased duration of parenteral nutrition, late-onset sepsis and NEC incidence) of an exclusively human milk-based diet would exceed those of a diet containing bovine-based products, without detrimental effects on growth.

**Methods**

- Multi-centered randomized trial
- 12 NICUs, 2 countries
- Birth weight: 500 - 1250 g
- Intention to provide own mother’s milk
- Enteral feeding before 21 days of life
- Parenteral nutrition within 48 hours of birth
- Excluded: major congenital malformations
- Study endpoint = earliest of the following milestones:
  - 92 days of age, discharge from hospital, or attainment of 50% oral feedings

**Study Design**

- Enrolled subjects randomized to:
  - Human Milk-Based Human Milk Fortifier at 100 mL/kg/day
  - Donor Milk if no own mother’s milk
  - Bovine Milk-Based Human Milk Fortifier at 100 mL/kg/day
  - Preterm Formula if no own mother’s milk
  - HUM100
  - HUM40
  - BOV

Exclusive human milk reduces NEC and NEC surgery

Results

- No difference in durations of parenteral nutrition or hospitalization, rates of late-onset sepsis or growth between infants fed exclusively human milk-based vs bovine milk-based diet.

- For extremely preterm infants, an exclusively human milk-based diet is associated with protection from NEC and surgical NEC.

Gut Colonization in Newborns

Human Milk
- Bifidobacteria
- Lactobacilli

Formula Milk
- Clostridia
- Enterococci
- Enterobacter
- Bacteroides

Human Milk Contains Probiotics

- Probiotics are defined as live microorganisms that may beneficially affect the host upon ingestion by improving the balance of the intestinal microflora

Risk of the Preterm Infant

- Often not delivered from birth canal
- Frequent use of broad spectrum antibiotics
- Delay in enteral feedings
- Sterilization of infant formulas
- Nosocomial bacterial colonization

Impaired Colonization of the Preterm Gut

- Delay in establishment of gut flora
- Reduction in the number of bacteria
- Reduction in the diversity of bacteria


### NEC Pathogenesis

- Reduced Probiotic Diversity
- Decreased Permselectivity
- Altered Colonic Membrane & Tight Junctions
- Altered Host Proteins
- Increased Epithelial Permeability
- Invasion of Bacteria

*Hunter et al., 2008* *Pediatr Res* 63:2:117

### Probiotic Therapy and NEC

<table>
<thead>
<tr>
<th>Strain</th>
<th>Probiotic Agent</th>
<th>Dosage</th>
<th>Outcome</th>
</tr>
</thead>
</table>
| Bifidobacterium infantis | 10 ng/kg | Improved 
| E. coli | 100 mcg/kg | Improved |
| L. reuteri | 10 mcg/kg | Improved |

*Deshpande et al., 2007* *Lancet*

### Effect of Probiotics on NEC Stage 2 or Greater

- Non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improve host health.

- Human milk contains numerous different prebiotics primarily in the form of oligosaccharides.

### Donor Milk Banking History

- The first human milk bank opened in Vienna in 1909.
  (Happy centennial!)
- Milk banking in North America began in 1910 in Boston.
- In 1974, Canada has its first milk bank in Vancouver.
- In the 1980’s banks closed because of the fears of transmission of HIV (North America: 14 → 8)

### Human Milk Contains Prebiotics

- Non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improve host health.

- Human milk contains numerous different prebiotics primarily in the form of oligosaccharides.

### How is Donor Human Milk Different from Mother’s Own Breastmilk?

- Donor milk undergoes freezing for long-term storage and Holder pasteurization (62.5°C x 30 min)
- Most nutrients and components found in breast milk are retained after donor milk is pasteurized.
- All cells including WBC are destroyed
- Most bacteria, viruses and other pathogens are destroyed
- Many immunoglobulins and other infection fighting factors are unchanged or minimally decreased
Donor Milk Banking in North America

- HMBANA (Human Milk Banking of North America)
- 1.4 million ounces of milk annually

Routine Human Milk Analysis in the NICU

- Human milk is best, but:
  - Varies in composition by
    - mothers
    - time of day, week, month
    - timing of pumping
  - delivery system
  - Current fortification methods are BLIND!
  - Error margin at least 50%.

Ideal Features of a Milk Analyzer

- Point-of-care
- Accurate
- Measures Protein, Fat, and Carbohydrates
- Uses only a small volume of milk
- Affordable
- Fast
- Small footprint

Changes in Human Milk Protein and Energy with Time

Variation in Human Milk Between Individuals
NIR Milk Analysis

- 17 NICU mothers donated breast milk samples
- Near Infrared Analysis compared to standard Mid Infrared Analysis
- Now compared by standard primary chemical methods (data not shown)


Summary

- Human milk is best for human babies!
- Human milk is best for preterm babies!
- Human milk contains a multitude of bioactive factors that confer optimum health to the preterm infant
- Human milk has highly variable composition between mothers and over time