NEW INSIGHTS IN HUMAN MILK COMPOSITION AND BENEFITS FOR PRETERM INFANTS

Prof Ruurd van Elburg
Professor of Early Life Nutrition
Emma Children’s Hospital AMC Amsterdam
Chief Scientific Officer Nutricia Research Utrecht The Netherlands

4TH SIGNEC LONDON UK 27 SEP 2016
DISCLOSURE

Employee of Nutricia Research Utrecht, The Netherlands
NEW INSIGHTS IN HUMAN MILK COMPOSITION AND BENEFITS FOR PRETERM INFANTS

- Preterm infants and NEC
- Risk factors for NEC
- Human milk composition (quantity & quality)
- Role of storage and pasteurisation
- Conclusions
Lokku A, Miera L, Lee SK, Shah PS; Canadian Neonatal Network.
Am J Perinatol. 2016 Sep 20. [Epub ahead of print]
PMID: 27649293

Download CSV
PATHOPHYSIOLOGY NEC

**Figure 2. Pathophysiology of Necrotizing Enterocolitis.**
Factors conferring a predisposition to necrotizing enterocolitis include genetic factors and several immature characteristics of the fetal intestine, including altered microbiota, inadequate intestinal barrier function, and an excessive inflammatory response. These factors contribute to the severe necrosis of the small intestine that is characteristic of this disease. TLR denotes toll-like receptor.
Fig. 1. Inappropriate colonization or ‘dysbiosis’.
RISK FACTORS FOR NEC

• Preterm birth

• Altered intestinal microbiota development

• Prolonged (initial) antibiotics

• Feeding: human milk may be protective…
BENEFITS OF BREASTFEEDING

EXCLUSIVE BREASTFEEDING FOR FIRST 6 MONTHS
CONTINUED BREASTFEEDING INCL.
COMPLEMENTARY FEEDING UP TO 2 YEARS AND BEYOND

Term infants

- Protection against infection
- Oral tolerance
- Lower risk for allergies
- Lower risk for overweight
- Lower risk for diabetes
- Increased intelligence

Adapted from: Verhasselt, Mucosal Immunol, 2010
HUMAN MILK COMPOSITION
MILK EVOLUTION IN MAMMALS

First
Protective function

Second
Role in nutrition

Lactation may have its origin in the protection of young against microbial attacks by anti-infective secretions?

The egg-laying mammal platypus secretes milk from an incubation patch

Supplements of nutritive components during phylogeny gradually changed these secretions into a dual-purpose, highly protective/highly nutritive drink for the offspring: Human Milk.

MILK IS VARIABLE ACCORDING TO OFFSPRING NEEDS

Extreme example: Wallabies

Wallabies Produce Two Different Kinds of Milk Types at Once

- Milk for the Developing Joey Inside the Pouch
- Milk (e.g. higher fat) for the Developed Joey (Outside the Pouch)

Each Joey Suckles on a Different Teat in Order to Get the Right Milk!

Closer to Preterm

AN ORCHESTRA OF COMPLEX FUNCTIONS IN A COMPLEX MATRIX

- **HMOS**: Microbiota, Immunity
- **Bacteria**: Microbiota, Immunity, Digestion
- **Fat / LCPUFA**: Energy, Immunity, Brain, Growth
- **Hormons**: Growth, Mood
- **Vitamins**: Growth, Immunity
- **Minerals**: Growth, Bone & Teeth, Blood
- **Lactose**: Energy
- **Proteins**: Growth, Immunity, Signaling
- **Nucleotides**: Growth, Immunity, Brain
- **Lactose**: Energy
- **Living Cells**: Immunity, ...
MILK COMPOSITION: BUILDING COMPLEX STRUCTURES

Building Block: (“wagons”)

Complex Biomolecule: (“train”)

Monosaccharides

Fatty Acids

Amino Acids

Nutrition

Prebiotics

Lipids

Proteins

Synthesis & Digestion

Nutrition

Function
## Compounds with Immunological Properties in Human Milk

### Anti-microbial compounds
- Immunoglobulins: sIgA, sIgG, sIgM
- Lactoferrin, lactoferrin B and H
- Lysozyme
- Lactoperoxidase
- Nucleotide-hydrolizing
- Antibodies
- κ-casein and α-lactalbumin
- Haptocorrin
- Mucins
- Lactadherin
- Free secretory component
- Oligosaccharides and prebiotics
- Fatty acids
- Maternal leukocytes and
  - Cytokines
- sCD14
- Complement and complement receptors
- β-defensin-1
- Toll-like receptors
- Bifidus factor
- Tolerance/priming compounds
  - Cytokines: II10 and TGFβ
  - Anti-idiotypic antibodies

### Immune development compounds
- Macrophages
- Neutrophils
- Lymphocytes
- Cytokines
- Growth factors
- Hormones
- Milk peptides
- Long-chain polyunsaturated fatty acids
- Nucleotides
- Adhesion molecules

### Anti-inflammatory compounds
- Cytokines: II-10 and TGFβ
- II-1 receptor antagonist
- TNFα and II-6 receptors
- sCD14
- Adhesion molecules
- Long-chain polyunsaturated fatty acids
- Hormones and growth factors
- Osteoprotegerin
- Long-chain polyunsaturated fatty acids
- Hormones and growth factors

---

*Field J Nutr 2005*
MEASUREMENT OF HUMAN MILK COMPONENTS

**BREASTMILK**

<table>
<thead>
<tr>
<th>WATER</th>
<th>CARBOHYDRATES (energy source)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactose</td>
<td>Oligosaccharides (see below)</td>
</tr>
</tbody>
</table>

**CARBOXYLIC ACID**

| Alpha-hydroxy acid | Lactic acid |

**PROTEINS**

<table>
<thead>
<tr>
<th>Whey protein</th>
<th>Alpha-Lactalbumin</th>
</tr>
</thead>
<tbody>
<tr>
<td>HANKEL [human alpha-lactalbumin]</td>
<td>Made lethal to tumor cells</td>
</tr>
</tbody>
</table>

**FATS**

<table>
<thead>
<tr>
<th>Triacylglycerols</th>
<th>Long-chain polyunsaturated fatty acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Docosahexaenoic acid (DHA)</td>
<td>(important for brain development)</td>
</tr>
<tr>
<td>Arachidonic acid (ARA)</td>
<td>(important for brain development)</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>Alpha-Linolenic acid (ALA)</td>
</tr>
<tr>
<td>Eicosapentaenoic acid (EPA)</td>
<td>Conjugated linoleic acid (Ruminic acid)</td>
</tr>
<tr>
<td>Free Fatty Acids</td>
<td>Monounsaturated fatty acids</td>
</tr>
<tr>
<td>Olean</td>
<td>Palmitic acid</td>
</tr>
<tr>
<td>Steric</td>
<td>Heptadecenic acid</td>
</tr>
<tr>
<td>Saturated fatty acids</td>
<td>Stearic acid</td>
</tr>
<tr>
<td>Palmitic acid</td>
<td></td>
</tr>
</tbody>
</table>

**VITAMINS**

<table>
<thead>
<tr>
<th>Vitamin A</th>
<th>Biotin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin B6 (pyridoxal)</td>
<td>Vitamin B12</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>Vitamin E</td>
</tr>
<tr>
<td>Thiamine</td>
<td>Riboflavin</td>
</tr>
<tr>
<td>Niacin</td>
<td>Pantothenic acid</td>
</tr>
</tbody>
</table>

**PEPTIDES**

<table>
<thead>
<tr>
<th>(combinations of amino acids)</th>
<th>(in human growth factor)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HGK</td>
<td>HGK II</td>
</tr>
<tr>
<td>HGK III</td>
<td>Cholecystokinin (CCK)</td>
</tr>
<tr>
<td>Glucagon</td>
<td>Parathyroid hormone (PTH)</td>
</tr>
<tr>
<td>Parathyroid hormone-related peptide (PTHrP)</td>
<td>1-29-BP</td>
</tr>
<tr>
<td>25-Hydroxyvitamin D3</td>
<td>Calcitriol</td>
</tr>
<tr>
<td>Calcitonin</td>
<td>Gastrin</td>
</tr>
<tr>
<td>Motilin</td>
<td>Bombesin (gastric releasing peptide, also known as neuropeptide B)</td>
</tr>
<tr>
<td>Neuropeptide B</td>
<td>Somatostatin</td>
</tr>
</tbody>
</table>

**ANTIPROTEASES**

<table>
<thead>
<tr>
<th>(thought to bind themselves to macromolecules such as enzymes and as a result prevent allergic and inflammatory reactions)</th>
<th>α-1-proteinase inhibitor</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-1-antichymotrypsin</td>
<td></td>
</tr>
</tbody>
</table>

**ANTIMICROBIAL FACTORS**

<table>
<thead>
<tr>
<th>(are used by the immune system to identify and neutralize foreign objects, such as bacteria and viruses)</th>
<th>Leukocytes (white blood cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-1-proteinase inhibitor</td>
<td>Phagocytes</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>Lymphocytes</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>Macrophages</td>
</tr>
<tr>
<td>Mast cells</td>
<td>Lactoferrin</td>
</tr>
<tr>
<td>Cytokines (also known as cytokines)</td>
<td>Leukocytes (also known as C cells)</td>
</tr>
<tr>
<td>Gammaglobulin</td>
<td>Erythropoietin</td>
</tr>
<tr>
<td>Erythropoietin</td>
<td>Interleukin-1 (IL-1)</td>
</tr>
</tbody>
</table>

**MINERALS**

<table>
<thead>
<tr>
<th>Copper</th>
<th>Iron</th>
</tr>
</thead>
<tbody>
<tr>
<td>Managanese</td>
<td>Zinc</td>
</tr>
<tr>
<td>Manganese</td>
<td>Zink</td>
</tr>
<tr>
<td>Manganese</td>
<td>Manganese</td>
</tr>
<tr>
<td>Manganese</td>
<td>Manganese</td>
</tr>
</tbody>
</table>

**SUGARS**

<table>
<thead>
<tr>
<th>Galactose</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fructose</td>
<td>Lactose</td>
</tr>
</tbody>
</table>

**FIBRONECTIN**

<table>
<thead>
<tr>
<th>(makes phagocytes more aggressive, minimizes inflammation, and repairs damage caused by inflammation)</th>
<th>Collagen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibronectin</td>
<td>Collagen</td>
</tr>
</tbody>
</table>

**LACTOFERRIN**

<table>
<thead>
<tr>
<th>(binds to iron to grow)</th>
<th>(binds to iron to grow)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactoferrin</td>
<td>Lactoferrin</td>
</tr>
</tbody>
</table>

**ENZYMES**

<table>
<thead>
<tr>
<th>(catalysts that support chemical reactions in the body)</th>
<th>(catalysts that support chemical reactions in the body)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amylase</td>
<td>Proteoglycan</td>
</tr>
<tr>
<td>Protease</td>
<td>Catalase</td>
</tr>
<tr>
<td>Lipase</td>
<td>Catalase</td>
</tr>
<tr>
<td>Peptidase</td>
<td>Catalase</td>
</tr>
</tbody>
</table>

**MIMOSAL Tv**

<table>
<thead>
<tr>
<th>Intestinal brush border enzymes</th>
<th>(also known as somatostatin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(also known as somatostatin)</td>
<td>(also known as somatostatin)</td>
</tr>
<tr>
<td>(also known as somatostatin)</td>
<td>(also known as somatostatin)</td>
</tr>
</tbody>
</table>

**MINERALS**

<table>
<thead>
<tr>
<th>Calcium</th>
<th>Iron</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnesium</td>
<td>Zinc</td>
</tr>
</tbody>
</table>

**FIBRONECTIN**

<table>
<thead>
<tr>
<th>(binds to iron to grow)</th>
<th>(binds to iron to grow)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactoferrin</td>
<td>Lactoferrin</td>
</tr>
</tbody>
</table>

**ENZYMES**

<table>
<thead>
<tr>
<th>(catalysts that support chemical reactions in the body)</th>
<th>(catalysts that support chemical reactions in the body)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amylase</td>
<td>Protease</td>
</tr>
<tr>
<td>Protease</td>
<td>Catalase</td>
</tr>
</tbody>
</table>

**MIMOSAL Tv**

<table>
<thead>
<tr>
<th>Intestinal brush border enzymes</th>
<th>(also known as somatostatin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(also known as somatostatin)</td>
<td>(also known as somatostatin)</td>
</tr>
</tbody>
</table>
HUMAN MILK COMPOSITION

QUANTITY
HUMAN MILK FOR PRETERM INFANTS

1. All preterm infants with BW <1800 grams should be fed fortified HM (protein, vitamins & minerals to enable appropriate growth)
2. Start with standard fortification
3. If growth is not appropriate, individualized fortification is advisable:
   - targeted fortification (based on HM analysis)
   - adjustable fortification (based on BUN measurements)
Both are advisable depending on the NICU experience and facilities.

Translating the recommendation of Ziegler EE, Carlson SJ. J Matern Fetal Neonat Med 2009, 22(3):191-197
PROTEIN CONCENTRATION IN HUMAN MILK

Preterm vs. Term

DOSE RESPONSE OF BREAST FEEDING TERM INFANTS

INFECTIONS (IMMUNITY)

OVERWEIGHT OBESITY


Von Kries et al. 1999 BMJ (319): 147-150
EARLY NUTRITION STUDY

MULTICENTER STUDY IN PRETERM INFANTS

- RCT First 10 days donor milk vs preterm formula if own mother’s milk was not (sufficiently) available
- Primary outcome parameter: cumulative incidence of LOS/Meningitis, NEC or death in first 60 days
- No difference in primary outcome
- However 89 vs 84,5% received own mother’s milk in first 10 days!
- >50% own mother’s milk associated with lower risk in primary outcome compared to <50% own mother’s milk

Corpeleijn et al JAMA Pediatr 2016;170:654-61
HUMAN MILK COMPOSITION

QUALITY
HUMAN MILK QUALITY

Impact of Pasteurization on Growth of Preterm Infants

• 17% higher fat absorption and more weight gain and linear growth in infants fed mother’s own raw milk compared to mother’s own pasteurized milk (Andersson, 2007). Probable reason: deactivation of BSSL by pasteurization.

• Improved weight gain and a decreased risk for NEC was found in premature infants fed with mother’s own raw milk compared with pasteurized donor milk (Montjaux-Régis, 2011).
MILK QUALITY – FUNCTIONAL MILK PROTEINS

Frozen Human Milk

Host Defense Proteins
Activity lowered up to 70%

Pathogens* applied to HM
grow up to 3.6 fold in 6h

* S aureus, P aeruginosa, E coli

Pasteurised Human Milk

Host Defense Proteins
Activity lowered up to 90%

Pathogens* applied to HM
grow up to 4.6 fold in 6 h

Akinbi et al JPGN 2010
• Holder pasteurisation is currently the most used method.

• But affects several of the active ingredients.

• Data about safety for microbiological control are still scarce for most of the novel technologies.

• Consensus on processing conditions is necessary for non-thermal technologies, before any conclusions on the qualitative and nutritional advantages of these techniques can be drawn.

*Peila C, Emmerik NE, et al, JPGN 2016 (accepted for publication)*
HUMAN MILK COMPOSITION

Maternal factors:
- Diet & health condition of the mother
HUMAN MILK COMPOSITION

OTHER NEW INSIGHTS
DIFFERENT HUMAN MILK FOR SONS & DAUGHTERS

Energy content of breast milk for boys is higher

- HM for boys had 25% greater caloric content than HM for girls

Boys grow faster than girls, thus, have an increased energy demand.

Breastfed boys consume more milk

How gender-specific milk is physiological regulated is yet unknown.

- Powe CE et al. (2010): American Journal of Human Biology 22, 50–54
HUMAN MILK IS VARIABLE ⇔ CONSISTENCY IN BENEFITS

- Mums Diet and Local Environment, Lifestyle
- Mums & Babies Health Conditions
- Genetic Background
- Compositional Changes during Lactation
- Human milk composition in different regions of the world
Maternal vitamin D status by season and site, $p < 0.001$ by ANOVA,

Widespread seasonal gene expression in the immune system

23% of the genome (5,136 unique genes out of 22,822 genes tested) show significant seasonal differences in gene expression

---

**Giuseppe Arcimboldo**
(* 1526; † 11. July 1593 in Milano)

---

HM REFLECTS HEALTH STATUS OF THE INFANT

- During active infection in the infant, total number of white blood cells, specially number of macrophages, and TNFα levels in breast milk increase.

- Dynamic immunological connection between lactating mothers and their infants

- Baseline level of leukocytes (0-2%) in mature milk unless the mother and/or her infant became infected, when leukocyte numbers significantly increased up to 94% leukocytes out of total cells (P < 0.001).
Cells
- Macrophages
- Neutrophils
- T cells
- DC
- Epithelial cells

Milk fat globules
- Origin: breast epithelial cells
- Plasma membrane enveloped lipid droplet
- Lipid trilayer enclosed
- Main fat storage units in milk
- 0.2 – 15 µm in size

Extracellular vesicles
- Origin: various cell types
- Lipid bilayer enclosed
- Mediators of intercellular communication
- <0.2 µm in size

Casein micelles
- a-casein, b-casein, k-casein
- Non-globular proteins with a micellar structure
- Human milk micelles ~43 nm
EV FORMATION

Raposo et al. JCB, 2013
EXTRACELLULAR VESICLES (EV)

**EV CARGO**

**Lipids**
- Ceramide
- Cholesterol
- Phosphatidylserine

**Proteins**
- MHC class I/II
- Co-stimulatory molecules
- Cytokines (e.g., IL-1β, TGF-β)
- Heat shock proteins

**Genetic material**
- miRNA
- mRNA
- Small non-coding RNA

Adapted from EVpedia
BIOLOGICAL ROLE OF EV

Adapted from: Robbins et al. Nat Rev Imm, 2014
THE COMPOSITION & BENEFITS OF HUMAN MILK

...there are different orchestra...

...and even one orchestra can play very different music!

No WW reference Value for HM composition
NEC AND HUMAN MILK

NEC AND PROBIOTICS

Pubmed search 2016:

- NEC and human milk: 219 hits
- NEC and Probiotics: 181 hits
- First publication 2002
- One of the first publications by Kate Costeloe (2003)
NEW INSIGHTS IN HUMAN MILK COMPOSITION AND BENEFITS FOR PRETERM INFANTS

CONCLUSIONS

• No worldwide standard for human milk composition
• Quantitative and qualitative aspects of human milk are important for the benefit of preterm infants
• Freezing and processing have major impact on bioavailability of ingredients of human milk
• Human milk & supplements (eg prebiotics, probiotics, synbiotics, lactoferrin) may have protective effect for the development of NEC in preterm infants
THANK YOU