Composition of major proteins in breast milk: high-throughput techniques for quantitative protein analysis

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Nestlé R&D

39 centers
200 partnerships
1 innovation Hub

in 2015
Nestlé Research Centre Locations

Staff of ~600 including...
- over 250 PhD Scientists from over 50 nationalities

Lausanne (CH)
- Food Safety & Integrity
- First 1'000 Days & Healthy Kids
- Healthy Ageing
- Healthy Pleasure
- Sustainable Nutrition

Asia (Beijing & Singapore)
- Brain Development
- Ageing
- Metabolic Health
- Cardiovascular Health
- Traditional Chinese Medicine

St. Louis (US)
- Pet care research

Scientific publications

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Patent applications

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2010 2011 2012 2013 2014 2015
Scientific publications
Patent applications

29/09/2016 M. Affolter - NRC
Why breast milk research?

- Comprehensive data on human milk composition
  - Infant factors:
    - Term vs. Preterm; SGA vs. AGA vs. LGA
    - Male vs. female
  - Mother factors:
    - Pre-pregnancy BMI
    - Mode of Delivery (vaginal vs. Caesarean)
    - Stages of lactation
    - Geographical/Socio-Economic status
- Association milk composition: infant growth
- Scientific concepts for novel products and used for regulatory & communication
Breast Milk Research at NRC

Objectives
To better understand breast milk in relation to:

1. Nutritional needs of infants (term, special needs)
2. Infant growth and health outcomes; and
3. Nutritional requirements of breastfeeding mothers

...using state-of-the-art validated analytical methodologies
Breast Milk Research Activities

Collaboration with 50+ Investigators/co-investigators

Europe: term infants; 13 centres

Europe: pre-term infants; 2 centres

Europe: allergic mothers/infants

China: term infants; 3 centres

India: SGA vs. AGA; 5 centres

Singapore: term infants, 1 centre

Singapore: intervention micronutrients, 1 centre
Human milk is dynamic

Factors that impact quality and quantity of human milk:

**Low to Minimal Impact**
1. Infant suckling duration
2. Maternal moderate physical activity
3. Social factors
4. Maternal stress

**Factors that have impact and must be **RECORDED:**
1. Stage of lactation
2. Maternal diet
3. Maternal anthropometry
4. Maternal lifestyle (e.g. severe physical activity)
5. Maternal obstetric history (e.g. parity, mode of delivery, etc.)
6. Maternal demographics
7. Infant birth weight
8. Infant gender

**Factors that have impact and must be **STANDARDIZED:**
1. Time of milk sampling (Circadian Rhythm)
2. Type of milk sampling (single full breast milk sampling)
3. Treatment of samples post collection
4. Length and temperature of milk storage until analyses
5. Freeze-thaw cycles (limit to zero if possible)
6. Choice of analytical procedures
# Human milk is complex

A non-exhaustive list of the components of human milk includes:

### Water

### Carbohydrate
- Lactose
- HMOs...

### Proteins
- Whey
- Casein
- MFGM ...
- Peptides

### Non-protein nitrogen
- Creatine
- Urea ...

### Fats
- Triglycerides
- Fatty Acids
- Phospholipids
- Sphingolipids
- Gangliosides
- Sterols
- Cholesterol

### Vitamins
- A, B, C, D, E, K, carotenoids ...

### Minerals
- Ca, Na, K, Fe, Zn, Cl, P, Mg, Cu, Mn, I, Se, Cr, Co, ...

### Growth Factors
- IL ...
- GF ...

### Hormones
- Insulin
- Leptin
- Ghrelin
- TSH, TRH, ....

### Enzymes
- protease, amylase
- BSSL ...

### Anti-proteases
- Antimicrobial factors
- slgA, IgG, IgM, lysozymes ...

### Cytokines + anti-inflammatory

### Transporters
- lactoferrin

### DNA
- RNA (miRNA)
Nutrients in breast milk (selection for analyses with foresight)

Neurodevelopment
- Gangliosides
- Phospholipids
- Carbohydrates
- Energy
- Adiponectin
- Insulin
- Leptin
- Proteins
- Insulinogenic amino acids

Immune Development
- LC-PUFA
- Folate
- IGF1,2; IgA, IgG, IgM
- Vitamins D, B6
- Minerals
- Lipids
- Cholesterol
- HMOs
- Lactoferrin
- Microbiota

Physical Growth
- Triacylglycerol structure profiling

Metabolic Health Programming
- Gangliosides
- Phospholipids
- Carbohydrates
- Energy
- Proteins
- Insulinogenic amino acids

Gut development and prevention of gut disorders
- Vitamins D, B6
- Minerals
- Lipids
- Cholesterol
- HMOs
- Lactoferrin
- Microbiota
Breast milk protein analysis

**Total milk protein:**
- Analysis of macronutrient content (including total protein) by human milk analyzer system (MIRIS AB, Sweden)
  - The MIRIS Human Milk Analyzer is based on approved Mid-IR-technology (Infrared transmission spectroscopy)

**Major milk proteins:**
- α-lactalbumin (~30% in human milk), caseins (α-, β- and κ-casein, total ~40%), lactoferrin (~20%) and albumin (~5%), absolute quantification
- LabChip G XII microfluidic chip technology (Caliper/Perkin Elmer) for high-throughput protein analysis
Breast milk protein analysis

Details on the analysis of major human milk proteins:

- 25-50 μL human milk required for analysis
- Normal protein concentration in human milk ca. 9-12 g/L
- Pure human milk proteins (α-lactalbumin, lactoferrin and serum albumin, caseins not available thus bovine homologues) used as standards to measure individual calibration curves for precise quantification (fully validated method)
- Protein sizing on the LabChip GXII achieved by integrating features of one-dimensional SDS-PAGE such as separation, staining, destaining, and detection steps on the chip
- Chip format (96 well format) dramatically reduces separation time (maximum of 600 milk samples in two days) and provides automated sizing and quantitation information in a digital format (allowed triplicate measurements)
### Subject recruitment

- Subjects assessed for eligibility (n=421)
- Excluded (n=281) - did not meet criteria (n=85) - declined to participate (n=196)
- Subjects enrolled (n=140)
- Human milk samples (n=140)
- Colostrum samples (n=90)
- Analyzed protein samples (n=456)

### Subject demographics & anthropometry

#### Study population

<table>
<thead>
<tr>
<th></th>
<th>5-11 d (n=90)</th>
<th>12-30 d (n=90)</th>
<th>1-2 m (n=90)</th>
<th>2-4 m (n=90)</th>
<th>4-8 m (n=90)</th>
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</thead>
<tbody>
<tr>
<td><strong>Mother</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years), Mean (SD)</td>
<td>27 (4)</td>
<td>27 (3)</td>
<td>28 (4)</td>
<td>27 (4)</td>
<td>26 (4)</td>
</tr>
<tr>
<td>Height (cm), Mean (SD)</td>
<td>160 (4)</td>
<td>160 (5)</td>
<td>161 (5)</td>
<td>161 (5)</td>
<td>159 (5)</td>
</tr>
<tr>
<td>Weight (kg), Mean (SD)</td>
<td>60.7 (8.7)</td>
<td>60.8 (7.9)</td>
<td>61.9 (8.9)</td>
<td>58.4 (8.3)</td>
<td>56.2 (8.1)</td>
</tr>
<tr>
<td>BMI (kg/m²), Mean (SD)</td>
<td>23.7 (3.3)</td>
<td>23.7 (2.8)</td>
<td>23.9 (3.1)</td>
<td>22.5 (2.9)</td>
<td>22.2 (3.1)</td>
</tr>
<tr>
<td>Gestational weight gain (kg), Mean (SD)</td>
<td>16.7 (7.4)</td>
<td>16.2 (6.0)</td>
<td>15.9 (5.7)</td>
<td>15.9 (5.9)</td>
<td>14.9 (7.6)</td>
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<tr>
<td>Postpartum weight loss (kg), Mean (SD)</td>
<td>9.1 (6.1)</td>
<td>8.6 (5.3)</td>
<td>9.8 (4.0)</td>
<td>10.0 (6.2)</td>
<td>10.6 (5.9)</td>
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<tr>
<td>Caesarean delivery, N (%)</td>
<td>39 (42)</td>
<td>43 (48)</td>
<td>53 (59)</td>
<td>35 (39)</td>
<td>35 (38)</td>
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</table>

#### Infant

<p>| | | | | | |</p>
<table>
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<tr>
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<tbody>
<tr>
<td>Males, N (%)</td>
<td>51 (57)</td>
<td>48 (53)</td>
<td>48 (53)</td>
<td>54 (60)</td>
<td>43 (48)</td>
</tr>
<tr>
<td>Gestational age at birth (weeks), Mean (SD)</td>
<td>39.3 (1.2)</td>
<td>39.2 (1.3)</td>
<td>39.2 (1.6)</td>
<td>39.4 (1.3)</td>
<td>39.5 (1.5)</td>
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</table>
MINING study - macronutrient composition

<table>
<thead>
<tr>
<th>Protein Concentration (g/100 mL)</th>
<th>Lactose Concentration (g/100 mL)</th>
<th>Fat Concentration (g/100 mL)</th>
<th>Energy Content (kcal/100 mL)</th>
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<tbody>
<tr>
<td>1.6</td>
<td>0.9</td>
<td>0.8</td>
<td>0.7</td>
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<tr>
<td>2.9</td>
<td>3.5</td>
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<td>4.9</td>
</tr>
<tr>
<td>6.3</td>
<td>6.7</td>
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</table>


MING study - protein gender differences

Protein concentration (in g/100 mL)

**MING study - major milk protein composition**

**α-lactalbumin**

<table>
<thead>
<tr>
<th>Stage</th>
<th>5-11 days</th>
<th>12-20 days</th>
<th>1-2 months</th>
<th>2-4 months</th>
<th>4-6 months</th>
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<tbody>
<tr>
<td>p</td>
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<td>0.000</td>
<td>0.002</td>
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**Lactoferrin**

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<th>Stage</th>
<th>5-11 days</th>
<th>12-20 days</th>
<th>1-2 months</th>
<th>2-4 months</th>
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<tbody>
<tr>
<td>p</td>
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<td>0.000</td>
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**Serum albumin**

<table>
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<th>Stage</th>
<th>5-11 days</th>
<th>12-20 days</th>
<th>1-2 months</th>
<th>2-4 months</th>
<th>4-6 months</th>
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<tbody>
<tr>
<td>p</td>
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<td>0.02</td>
<td>0.000</td>
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**Total casein**

<table>
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<tr>
<th>Stage</th>
<th>5-11 days</th>
<th>12-20 days</th>
<th>1-2 months</th>
<th>2-4 months</th>
<th>4-6 months</th>
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<tbody>
<tr>
<td>p</td>
<td>0.002</td>
<td>0.000</td>
<td>0.002</td>
<td>0.002</td>
<td>0.02</td>
</tr>
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</table>


MING study - alpha-lac gender differences

**α-lactalbumin concentration (in g/L)**

- **5-11 days**
- **12-30 days**
- **1-2 months**
- **2-4 months**
- **4-8 months**

- **Female**
- **Male**

*p = 0.000*  
*p = 0.000*  
*p = 0.002*  
*p = 0.01*  
*p = 0.01*

**29/09/2016**  
**M. Affolter - NRC**
MING study - lactoferrin gender differences

M. Affolter - NRC
Temporal Changes of Protein Composition in Breast Milk of Chinese Urban Mothers and Impact of Caesarean Section Delivery

Michael Affolter 1,2, Clara L. Garcia-Rodenas 1,2, Gerard Vinyes-Pares 1,2, Rosemarie Jenni 1,2, Iris Roggero 1,2, Ornella Avanti-Nglo 1,2, Carlos Antonio de Castro 1,2, Al Zhao 1,2, Yumei Zhang 1,2, Peiyu Wang 1,2, Sagar K. Thakkar 1,2 and Laurent Favre 1,2

Amino Acid Composition of Breast Milk from Urban Chinese Mothers

Clara L. Garcia-Rodenas 1,2, Michael Affolter 1,2, Gerard Vinyes-Pares 1,2, Carlos A. De Castro 1,2, Leonidas G. Karagounis 1,2, Yumei Zhang 1,2, Peiyu Wang 1,2 and Sagar K. Thakkar 1,2

Temporal Change of the Content of 10 Oligosaccharides in the Milk of Chinese Urban Mothers

Sean Austin 1,2, Carlos A. De Castro 1,2, Thierry Bénet 1,2, Yangfeng Hou 2,2, Hengen Sun 2,2, Sagar K. Thakkar 1,2, Gerard Vinyes-Pares 1,2, Yumei Zhang 1,2 and Peiyu Wang 1,2

Breast milk macronutrient composition and the associated factors in urban Chinese mothers

Yang Titi, Zhang Yumei, Ning Yibing, You Lili, Ma Defu, Zheng Yingdong, Yang Xiaoguang, Li Wenjun, Wang Junkun and Wang Peiyun

Microbiota in Breast Milk of Chinese Lactating Mothers

Olga Sakwinska 1,2, Déborah Moine 3, Michèle Delley 1, Séverine Combremont 1, Enea Rezzonico 1, Patrick Descombes 1, Gerard Vinyes-Pares 3, Yumei Zhang 1, Peiyu Wang 1, Sagar K. Thakkar 1
MING study - whey to casein ratio

Whey %

5-11 d 12-30 d 1-2 m 2-4 m 4-8 m

62:38 50:50 49:51 45:55 44:56


Summary

- Broad geographical footprint of human milk studies
- Validated quantitative methods for nutrient analyses
- High-throughput method for major milk protein analysis
- Protein results comparable with previous studies carried out in other parts of the world
- Integration of all breast milk study results into one unified data analysis and visualization platform
What’s next?

• Robust, state-of-the-art validated methods (⇒ publications)
• Protein analytics:
  • Increased resolution ⇒ quantification of individual caseins
  • More proteins quantified ⇒ UPLC-MRM-MS/MS approach
  • Explore modifications ⇒ intact protein LC-MS/MS
  • Target protein functionalities ⇒ measure protein functionalities (in vitro/in vivo)
Teamwork

Acknowledgements