The influence of maternal health on human breast milk composition with potential downstream effects on infant metabolism and gut colonization

MAINHEALTH

Research area: Infant Nutrition & Healthy Development

Main objective: To identify possible biological mechanisms or biological pathways activated by breast milk nutrients. We will integrate the fields of metabolomics, microbiology and medical science to study how evolution has shaped human breast milk and its effects on infants receiving it.

Project summary: The birth of a living human being is the result of an approximately nine-month pregnancy in which the developing foetus has taken exactly the building blocks necessary to grow and develop from its mother. However, growth and development continues in multiple dimensions at an increasing pace after birth. The nutrition in the first 1,000 days from conception to the child’s 2nd birthday plays a pivotal role in shaping the future health of the child. Yet, we know little of how breast milk components vary due to maternal factors or of the biological mechanisms behind the beneficial actions of many breast milk nutrients. We propose to overcome these obstacles by combining our specialties to give a more complete account of what breast milk is (major and minor milk constituents and microbiota), how it affects the infants directly or indirectly through breast milk-gut microbiome interactions and by which mechanisms. We will acquire longitudinal samples from 200 mother-infant dyads during the first year of life across three groups of pregestational maternal BMI; normal weight (BMI 18.5-24.99), overweight (BMI 25-30), and obese (BMI >30). The samples give a comprehensive record of what the infant has ingested (milk samples) and how the infant and infant gut microbiome responds to this milk (infant urine and feces). Maternal diet in pregnancy and at milk sample deliveries are recorded through a 24h online food recall and diary system. Maternal health attributes will, besides BMI, be analysed through clinical blood biochemistry parameters. Follow-up samples and infant dietary intake after three years allow investigating how early life diet shaped infant growth and gut colonization more long term. We have formed an experienced team of scientists within metabolomics, microbiology and medicine, holding leading positions within their respective fields in Denmark. Our novelty is the interdisciplinarity, unique study design and our emphasis to integrate a number of dynamic measurements thereby offering the ability to identify the factors in breast milk affecting infant metabolism and gut colonization. Knowing this enable the optimization of infant formula.

Project duration: 4 years, expected start April 2019, expected end March 2023

Project costs: Amount applied for from AFH: 5,000,000 DKK. Total project costs: 7,238,000 DKK

Applicant(s)/PI's:

- Ulrik K. Sundekilde (UKS), Assistant Professor, Department of Food Science, Aarhus University.
- Niels Uldbjerg (NU), Professor, consultant, DMSc, Department of Obstetrics and Gynaecology
- Dennis Sandris Nielsen (DSN), Professor MSO, University of Copenhagen

The project has been discussed with the following Arla Scientists:

- Henrik Jørgen Andersen and Anne Staudt Kvistgaard

Project manager: Ulrik K. Sundekilde, Assistant Professor, Department of Food Science, Aarhus University, Kirstinebjergvej 10, 5792 Årslev.
Project description

The questions we ask in this project are three-fold.

- First, we want to determine the variability of breast milk nutrients by application of metabolomics, proteomics and glycomics. The production of human breast milk has a high maternal metabolic cost. Thus, we hypothesise that maternal health attributes (metabolic dysfunction or obesity) influence which breast milk nutrients are made available to the infant.
- Second, we want to establish the microbiome of breast milk. Maternal obesity can lead to an apparent gut microbial ecology and increases the risk of obesity for the child. Thus, we hypothesise that maternal obesity confers distinct microorganisms to the infant.
- Third, we want to identify biological mechanisms for how breast milk nutrients are metabolised in the infants. Infants exclusively breast-feeding offer total compliance. Thus, we hypothesise that by deconstructing breast milk components and markers of infant metabolism through clever analysis of infant urine and feces, we can deduce the bioactivity of breast milk nutrients.

State-of-the-art

WHO recommends that infants should be exclusively breast-fed for the first six months of their life, after which partial breastfeeding should be performed up to 24 months of age or older.\(^1\) Despite these recommendations, only 37 percent of children under the age of 6 months are exclusively breastfeed in low- to middle-income countries, with the number being even lower in most high-income countries.\(^2\) Thus, there is a high demand for formula milk and despite the progress made in improving the composition of formula milk over the last 10-20 years, formula milk is still far from mimicking the content of bioactives and nutritious components found in breast milk. Yet, breast milk components and the specific activity of these components might have, has not been investigated in enough detail. Recent, preliminary data on peptidomics of human milk revealed almost 700 endogenous peptides from 30 different proteins\(^3\) and metabolomics identified different metabolite phenotypes depending on gestational age at premature birth.\(^4\) Now, our analytical platforms are sensitive, robust and high-throughput so we can begin to merge these data to deduce the bioactivity of these compounds, thereby warranting their inclusion into infant formula. Additionally, a skewed development of the intestinal microbiome during the first years of life has been found to influence future health of the infant.\(^5\) Birth mode influences early gut microbiome composition and mother-to-child transfer is important and e.g. linked to the transmission of obesity from mother to infant.\(^6\) Unfortunately, our understanding of the specific dietary components that shape the gut microbiome during the first year of life is still far from complete, and while it is known that breastfed infants differ from formula fed infants in their gut microbiome composition and function, very little is known on how inter-individual differences in breast milk composition influence the infant and its microbiome.\(^7\) It can be speculated that breastfeeding can assimilate a skewed infant microbiota.

Results from the present project could offer an attractive route to optimise formula milk composition through inspiration from nature’s design of human breast milk. We thus propose to study milk composition in combination with the study of infant metabolism and gut microbiome to further advance the fields of infant nutrition.

Project content

There is a pressing need for a project that bridges the gap between breast milk nutrients and the infant’s metabolism stimulating healthy growth; a biological investigation of the mechanisms by which nutrients and possibly microbes from the milk glands are included in breast milk and how the infant or its microbiome utilise these selected nutrients. We need to combine the biological (protein,
peptide, metabolite, and glycan) levels in breast milk, with the effects on infants and their gut microbiome to reveal how nature’s system of personalised infant nutrition performs. If successful, we know to which extend and by which means, we need to adapt infant formula to the growing infant to improve its nutrition, which will be of immense value for formula milk producers. The project is divided into three major phases covering seven work packages (Figure 1). The first phase is the establishment of a biobank with samples and diet diaries from 200 mother-infant dyads followed from birth until the infant’s first birthday (with a possibility to extend this period until the 5th birthday). The second phase is the biological OMICS-data knowledge generation, where metabolomics, proteomics and peptidomics, microbiomics and glycomics are deconstructing the breast milk, infant microbiome, maternal microbiome, milk microbiome and infant metabolism. The third and last phase is the systems biological integration of all data from the multiple biological levels possibly resulting in elucidations of biological mechanisms behind several milk components’ effect on infant metabolism or gut microbiota.

**Project Outcome**

The predominant outcome of the first phase in the project is the establishment a unique set of coordinated samples; initial samples from the birth and samples synchronized in time with breast milk offered to the infant and biofluids from the infant, showing how the infant utilized the breast milk (metabolic wastes through urine and feces) at three time points. Research based on this sampling approach has not been described before. Phase two demonstrate the high-level of detail each breast milk is deconstructed into. Such a high level of detail coupled with the unique study design will be first of its kind and be pivotal for identifying nutrients or components of nutritional importance in breast milk. This identification will be important for the dairy industry as these components may be identified in dairy streams, thereby offering a new product opportunity. This will also be of value to the individuals, and the society in general, by facilitating the development of better infant nutrition or better advice to mothers of breast-fed infants.

**Risk Assessment**

The segments of mothers we target to include in our study generally have low rates of breast-feeding. If we are unable to recruit enough mothers to our observational study, we will lengthen the
recruitment period or optionally offer compensation for participating in our studies, to ensure that the participants are completing the study. In the Aarhus region there is yearly 5,000 births.

Summary of experimental tasks and work packages
The first phase: WP1: Establishment of a cohort and biobank
The study is a longitudinal, non-intervention study to create a cohort of 200 mothers-infant dyads, including a biobank of milk samples, infant biofluids (Figure 1) and meta-data. Two hundred women will be recruited during pregnancy (~ 28 weeks of gestation) until the infant reaches its 1st birthday. Immediately after signed consent, the participants will give a blood sample for clinical biochemistry, which together with BMI will be used to score the participants’ health status.

At week 30 the women will fill out two 24h food recall questionnaires, using the validated MyFood24 system to assess maternal dietary patterns. Mothers are divided into three segments according to their pregestational BMI of equal sizes (n~66; a total of 200 mothers). A control group (normal weight, BMI 18.5-24.99), overweight (BMI 25-29.99), and obese (BMI >30). We will collect synchronised samples and dietary assessments from the dyad at regular intervals (Figure 1).

The second phase: WP2-5: OMICS-data generation.
In these work packages we will comprehensively determine the individual variability of breast milk metabolites, glycans, proteins, peptides and microbiota as well as establish the infant metabolome, the metabolome of infant gut microbiota and infant gut microbiota itself.

WP2: Metabolomics
The aim of this WP is to determine the metabolome of breast milk, infant urine and feces. We will use a combination of NMR, LC-MS and GC-MS-based metabolomics. Breast milk metabolite profile include bioactive molecules such as phospholipids, amino acids, energy molecules, all thought to have an effect on the infant metabolism. The urinary metabolites offers a snapshot of infant’s metabolism and which dietary compounds are utilized. Whereas, the fecal metabolome offers a measure of microbial activity. PI (UKS) is an expert in metabolomics.

WP3: Prote- and peptidomics
The aim of this WP is to determine the protein and peptide profiles of breast milk. Peptidomics expertise is achieved through collaboration with Assistant Professor Dave Dallas, Oregon State University, USA, a forerunner in human milk peptidomics (Stay abroad for PhD2). Additionally, collaboration with proteomics-expert Professor Fuquan Yang at University of Chinese Academy of Sciences, China is in place (stay abroad for PhD1).

**WP4: Microbiomics**

The aim of this WP is to define the extent of bacterial vertical transmission from mother to infant. Total DNA from breast milk, maternal vaginal and rectal fluid, infant saliva and infant feces will be extracted and the entire 16S-ITS-23S rRNA operon (4200 bp) amplified and sequenced using the Oxford Nanopore Promethion platform. This will compared to conventional 16S rRNA gene amplicon sequencing (up to 450 bp fragments) normally carried out using the Illumina MiSeq platform allow much improved resolution ensuring species/subspecies level relative quantification. PI (DSN) is an expert in analysing complex microbial societies including the gut and linking this information with other omics-data.

**WP5: Glycomics**

The aim of this WP is to establish the influence of maternal health on HMOs. HMOs are profiled using a nanochip-LC-MS glycan assay run in multiplex in collaboration with Assoc. professor Daniela Barile at University of California, Davis, USA or alternatively through NMR analysis (Stay abroad for PI).

The third phase is the data integration of the different biological levels measured. Here we will use available data to construct mode of action of specific milk nutrients or bacteria.

**WP6: Maternal and Infant metabolism and WP7: Breast milk gut microbiome interactions**

The aims of these WPs is to elucidate the effect maternal health or diet on breast milk nutrients and a subsequent possible effect on infant metabolism and on microbial metabolism. We use a systems biology based approach determine the biological pathways used as the infant or microorganisms processes the breast milk nutrients by combining the biological data from WP1-5. These WPs are carried out in collaboration between KU-FOOD and AU-FOOD.

**The cohort**

The Ethical Approval for the study will is granted for five initial years of the study. After these five years, the sample material is transferred to a biobank. Follow-up/additional studies on this material can then be granted by reapplying the Ethics committee.

The main sampling in the current project, will be in the first year of the infant. After this, at each infant birthday (2, 3, 4 and 5 years of age), the mothers will be sent a questionnaire to give information about child anthropometrics and diet. Additionally, the mothers are asked to collect a fecal sample from their child and mail by post.

**The research teams**

Eight individuals will be conducting research on MAINHEALTH, including the three PIs. Two PhD-students will be working on relatively self-contained sub-questions. They will be supervised by UKS, but also draw on expertise from the other PIs, postdoc, and collaborators.

**PhD1 (Katrine O. Poulsen):** Katrine is already funded (Sino-Danish Center and Aarhus University) and started 1 Aug 2018 on a 4-year PhD. She is responsible for the human trial. Project title: Human milkOMICS - a study of effects of milk components on infant metabolism and gut microbial colonization. Located at AU-FOOD.

**PhD2 (NN):** Project title: Mechanistic effects of breast milk components on infant metabolism and gut microbiota. Located at AU-FOOD.
PhD3 (NN): WP4 and WP7 require specialist knowledge within microbiology. A postdoc with a background in microbiology is necessary to bring their time, experience and expertise to ensure the success of the work packages with microbiology-content. Located at KU-FOOD. Associate Professors Lukasz Krych (WP4), expert on microbiome characterisation, and Morten Arendt Rasmussen, expert on data-fusion (WP6 and WP7), both KU-FOOD, will contribute as co-PIs (CVs enclosed).
Postdoc (NN): 3-month to assist in interpreting diet data collected from both mothers and infants. As PIs, we will oversee the project, provide leadership to all members, participate in relevant work packages, and ensure that MAINHEALTH delivers on its objectives.

Contribution to education
The project will strongly align with research competences and educational activities of the three PIs. Three PhDs and one 3-month postdoc will be associated and trained within the project. PhD1 (Katrine O. Poulsen) has already started on a 4-year scholarship (1. August 2018) at AU-FOOD under UKS supervision funded by Sino-Danish Center and Aarhus University. PhD2 will be based at AU-FOOD (UKS), whereas the PhD3 will be based at KU-FOOD (DSN), which will ensure interdisciplinarity.

Plan for publication
At least six publications are planned submitted to highest ranked peer-reviewed journals within our fields; 1) on longitudinal variability of human breast milk nutrients, 2) on the bioactivity of specified breast milk nutrients, 3) on the vertical transmission of bacteria in infant gut colonization, 4) on the utilization of specific breast milk nutrients in selected gut bacteria, 5) on the effect of breast milk nutrients on infant metabolism, and 6) on the combined effects of gut microbiota and utilization of breast milk nutrients on infants.

Dissemination plan
The proposed research is anticipated to generate a very significant body of knowledge of interest to a wide range of stakeholders, from the clinical (paediatricians and other health professionals) to the research community in the area and industrial producers of both infant milk formula and ingredients for such products. There is also likely to be significant public interest in the results. Hence, a multi-layered dissemination strategy will be followed. The international scientific community will be informed via peer-reviewed publications and presentations at scientific conferences, whereas the industry will be informed at regular meetings and reports.
Reference list


List of appendices

1. CVs (Ulrik K. Sundekilde, Niels Uldbjerg, Dennis S. Nielsen, Lukasz Krych, Morten A. Rasmussen)
2. Letters of commitment from all participants
3. Budget
4. Reference list