An Roinn Talmhaíochta,
Bia agus Mara
Department of Agriculture,
Food and the Marine

15F721 - The relation between the human milk microbiome, composition and infant nutrition

Final Report

This project was funded under the Department of Agriculture, Food and the Marine Competitive Funding Programme.

SUMMARY

The objective of the INFAMILK project was to undertake an interdisciplinary study of the impact of infant nutrition and the milk microbiome on the programming of the infant gut (through analysis of the ultimate faecal microbiome), with the overall goal of developing a holistic understanding of the factors that influence the gut development of the newborn infant.

A large cohort of almost 100 mothers being recruited through Cork University Maternity Hospital over the course of the study, one of the largest integrated national studies of this type. While the Covid-19 pandemic interfered with recruitment in the final year of the work (especially mothers of preterm infants) a very large bank of new knowledge was generated. The changes in both composition and microbial populations after birth (for up to 6 months) were mapped, and several new findings were generated.

In addition, a 'deep dive' was undertaken on several previously understudied aspects of human milk properties, such as creaming, and physical stability. Studies also addressed the physical and microbiological stabilisation of human milk in milk banks and hospitals by a range of novel techniques, bringing together the interdisciplinary strands of the project to deliver applied outcomes of direct practical relevance.

The bacterial communities in human milk over lactation were successfully characterised, and the evolution of the infant gut in both breastfed and exclusively formula fed infants over the first six months of life was documented. Milk microbiota composition significantly differed over lactation, and a gradual decrease in microbiota diversity was apparent, and lactation stage was the primary factor driving the composition changes in milk. Breast-fed infants had higher relative abundance of Bacteroides and Lactobacillus whereas formula-fed infants had increased relative abundances of Enterococcus and Blautia. Finally, milk is a source of potentially probiotic species which may enhance infant health and gut colonisation.

KEYWORDS

Human milk; nutrition; microbiome.

ACRONYM	INFAMILK
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Section 1 - Research Approach & Results

Start Date

01 December 2016

End Date

31 March 2021

Research Programme

Food Institutional Research Measure

TRL Scale

TRL 1: Basic Principles Observed

NRPE Priority area

Food for Health

Total DAFM Award

€600,308.00

Total Project Expenditure

€567,912.84

Rationale for undertaking the Research

Human breast milk (HM) is the Gold Standard feeding regime for newborn infants and represents a baseline for the functional performance of infant formulae. While many milk constituents – for example fatty acids, enzymes, and microbes – have been studied for decades, new analytical approaches, research findings, and paradigm shifts are rapidly providing new insights as to how they might impact both maternal and infant health. Moreover, interdisciplinary work is beginning to shed light on how these factors might be interacting in both the mammary gland and the infant's gastrointestinal tract and influencing the development of the gut microbiota. Establishment of the intestinal microbiota commences at birth and it plays a major role in maturation of the immune system, protection against pathogens, and the long-term metabolic welfare of the infant. In terms of infant health, it is imperative to understand how early infant nutrition influences the development of a healthy gut microbiota. Thus, the objective of the INFAMILK project was to undertake a systematic holistic study of the nutritional and microbiological aspects of human milk, to greatly extend our knowledge of how these influence early infant development and health.

Methodology

The INFAMILK Study was a dual-centre study performed at University College Cork (UCC) and Teagasc. Term and preterm human milk (HM) samples were collected from University Maternity Hospital (CUMH) using a standardised sample collection procedure. The application of long experience in methodologies in the area of dairy chemistry to the understanding of these aspects of bovine milk to the topic of human milk was a key benefit of the project. The exact point at which the sample for analysis was taken was specified and recorded. Then at UCC, key compositional attributes of the collected bank of HM samples were measured, including gross composition, protein profile, enzyme activities, somatic cells, and fatty acid profiles. HM were also sent from UCC to Aarhus university for studying the changes of metabolites during lactation by NMR methods. In cases where there was sufficient sample volume available, analysis of structural features of HM caseins was undertaken and physical stability of such samples (such as physical changes including creaming, little measured

previously for human milk) were monitored. Moreover, novel methods as alternatives to pasteurization to preserve the nutritional value of human milk were evaluated, including high-pressure treatment, filtration and use of bacteriocins. At Teagasc, microbiota composition of breast milk was characterized and correlated with the gut microbiota composition of infants, over the first six months of life. DNA was extracted from breast milk collected over the first 24 weeks of lactation. A unique bank of HM microbes was developed and suitability of microbes as probiotics for infants was characterized.

Project Results

From 2017 to 2021, INFAMILK project collected breast milk, infants' stool, and mothers' skin swab from 117 participants. The INFAMILK project took full advantages of these samples to study the impact of infant nutrition (including the lipidome and metabolome) and the milk microbiome on the programming of the infant gut. Much of the research has been published (or is about to be published) in international journals and has significantly improved our knowledge on the composition and properties of human milk, which is of major benefit to food scientists, microbiologists, medical staff, neonatologists and of course to parents and infants. Very briefly, some study conclusions include:

1. There was a significant influence of lactation stage on levels of protein and energy contents of milk, but not on levels of fat and carbohydrate in term milk. Levels of protein in milk at 4 weeks after birth were higher in milk from mothers with high pre-pregnancy body mass index (BMI) compared to mothers with low BMI values.
2. There were changes in some important fatty acids in human milk over lactation. For example, levels of caproic acid and α-linolenic acid increased significantly whereas arachidonic acid and docosahexaenoic acid decreased, during six months after birth. Significant impacts of maternal pre-pregnancy BMI and infant gender on fatty acids profiles were also found. These may be related to physiological processes such as neural development.
3. There were significant changes in milk microbiota composition over time. A significant decrease in milk microbiota diversity was observed throughout the first 6 months of lactation, with the greatest difference seen between week 8 and week 24. Nine genera predominated in milk over lactation from week 1 to week 24, comprising of Staphylococcus, Streptococcus, Pseudomonas, Acinetobacter, Bifidobacterium, Mesorhizobium, Brevundimonas, Flavobacterium, and Rhodococcus; however, fluctuations in these core genera were apparent over time. A decrease in milk microbiota diversity throughout lactation was also observed.
4. Significant changes in milk metabolites were seen during lactation. For example, levels of human milk oligosaccharides and citric acid decreased during lactation, whereas that of glutamate, an essential amino acid in protein metabolism, increased during lactation.
5. Mode of delivery of a baby (e.g., by caesarean section) was not a primary factor in driving the compositional changes, compared to lactation stage, which resulted in significant changes over time.
6. The infant feeding regime also significantly impacts the infant gut microbiota from birth to six months of age.
7. High-pressure treatment (cold-pressing) of human milk provides optimal retention of the nutritional properties of milk and is suggested as a possible future alternative compared to heat pasteurisation.

Section 2 - Research Outputs

Summary of Project Findings

The project findings offer benefits on a number of levels:

1. Scientific Community: the carefully conducted trials in this study add significantly to the scientific literature relating to human milk, including the dynamic changes of human milk macronutrients, microbiome, and metabolome during the first six month of lactation.
2. Public: The IFAMILK study provides strong evidence to support health benefits of human milk. The findings help mothers understanding the dynamics of human milk, as well as offering recommendations for the storage of human milk, and lay summaries of the outcomes of the project for participating mothers have been prepared. The current findings provide evidence of effect of freezing on human milk macronutrients and microbiota composition, which may help improve the good practice for handling of human milk.
3. Industry: The study helps infant formula manufacturers understanding the compositional changes of human milk and therefore their products can be further optimised based on compositionally mimicking human milk. Moreover, the milk samples were screened, and a biobank of human milk derived strains were generated for their characterization and potential use in the infant health and nutrition market. The project provided a sophisticated biological 'readout' by which any formula/ingredient can be assessed and hence could lead to a new generation of ingredients which actively influence the composition of the infant gut microbiota in a healthy (breast-fed) direction.

Summary of Staff Outputs

Research Output	Male	Female	Total Number
PhD Students	0	2	2
Other / Temporary	0	2	2
Post Doctorates	0	1	1

Summary of Academic Outputs

Research Outputs	Total Number	Details
Publications in Peer Reviewed Scientific Journals	6	1. Lyons, K., O'Shea, C.-A., Grimaud, G. M., Ryan, A. C., Dempsey, E., Kelly, A., . . .Stanton, C. (2022). The human milk microbiome aligns with lactation stage and not birth mode. Scientific Reports, 12. 2. Lyons, K., Fouhy, F., O'Shea, C.-A., Ryan, A. C., Dempsey, E., Ross, R., & Stanton, C. (2021). Effect of storage, temperature, and extraction kit on the phylogenetic composition detected in the human milk microbiota. Microbiology Open, 10. 3. Lyons, K., Ryan, A. C., Dempsey, E., Ross, R., & Stanton, C. (2020). Breast Milk, a Source of Beneficial Microbes and Associated Benefits for Infant Health. Nutrients, 12, 1039. 4. Meng, F., Uniacke-Lowe, T., Lyons, K., Murphy, K., O'Mahony, J., Stanton, C., &Kelly, A. (2021). Human Milk. In. Meng, F., Uniacke-Lowe, T., Ryan, A. C., & Kelly, A. L.

		(2021). The composition and physico-chemical properties of human milk: A review. Trends in Food Science & Technology, 112, 608-621. 5. Meng, F., Uniacke-Lowe, T., Lanfranchi, E., Meehan, G., O'Shea, C. A., Fox, P. F.,. . . Kelly, A. L. (2020). Factors affecting the creaming of human milk. International Dairy Journal, 108, 104726. 6. Overgaard Poulsen, K., Meng, F., Lanfranchi, E., Yong, J. F., Kelly, A. L., & Sundekilde, U. K. (2022). Dynamic changes in the human milk metabolome over 25 weeks of lactation. Fronteirs in Nutrition. Jult 14; 9. 917659.
PhD Theses	2	1. Katriona Lyons (UCC, 2021): The relationship between the human milk microbiome and infant gut microbiota composition over the first six months of life. 2. Fanyu Meng (UCC, 2021): Biochemistry, physicochemical properties, and processing of human milk and comparison to bovine milk.

Intellectual Property

There are no current plans to protect outcomes of the study.

Potential Impact related to Policy, Practice and Other Impacts

Impact	Details
Industry	The study provides instructions for hospital and human milk banks in terms of good practice of handling of human milk.

Dissemination Activities

Activity	Details
Other	Seminars: 1. Meng, F., Uniacke-Lowe, T., Lanfranchi, E., Meehan, G., O'Shea, C. A., Fox, P., Huppertz, T., Ryan, C.A., & Kelly, A. L. (2019). Factors Affecting the creaming of human milk. Poster session presented at 48th Annual Food Science and Technology Conference. 2. Meng, F., Uniacke-Lowe, T., Lanfranchi, E., Meehan, G., O'Shea, C. A., Ryan, C. A., & Kelly, A. L. Effect of milk storage processes on physicochemical properties of breast milk. Poster presented at 47th Annual Food Science and Technology Conference; 2018 Dec 6-7; Cork, Ireland.

	3. Meng, F., Uniacke-Lowe, T., O'Shea, C. A., Fox, P. F., Ryan, C. A., & Kelly, A. L. Physical stability of human milk to processing and storage. Poster presented at Irish Paediatric Association Annual Meeting; 2017 Dec 8-9; Kilkenny, Ireland. 4. Poulsen, K. O., Meng, F., Lanfranchi, E., Kelly, A., & Sundekilde, U. K. (2019). Human breast milk metabolomes over the course of 6 months lactation. Poster session presented at 16th Symposium on Milk Genomics and Human Health, Aarhus, Denmark. 5. The effects of storage method, temperatures and extraction kits on the human milk microbiota. A poster was presented at the 9th Europaediatrics Conference in Dublin, June 13th -15th, 2019 and APC Symposium: Challenges for Microbiome Science, University College Cork, October 11th, 2019. • Alan Kelly presented at 11th NIZO Dairy Conference in Papendal, The Netherlands, October 8th to 11th, 2019. The title of the presentation is ''Physicochemical and enzymatic stability of human milk'' • Catherine Stanton gave presentations on the INFAMILK work programme in Brussels JPI meeting, London conference, Human Nutrition conference in Mexico City, and a conference at Wuxi & Fuzhuo.

Knowledge Transfer Activities

Identify knowledge outputs generated during this project.	The knowledge outputs generated target a number of different groups: Scientific Community: the carefully conducted trials in this study add significantly to the scientific literature related to human milk, including the dynamic changes of human milk macronutrients, microbiome, and metabolome during the first six month of lactation. Public: The IFAMILK study provides strong evidence to support health benefits of human milk. The findings help mothers understanding the dynamics of human milk as well as offer the recommendation of the storage of human milk. Industry: The study helps infant formula manufacturers understanding the compositional changes of human milk and therefore their products will be developed based on compositionally mimicking human milk. Moreover, the milk samples were screened, and a biobank of human milk derived strains were generated for their characterization and potential use in the infant health and nutrition market. The project provided a sophisticated biological 'readout' by which any formula/ingredient can be assessed and hence will lead to a new generation of ingredients which actively influence the composition of the infant gut microbiota in a healthy (breast-fed) direction.
Identify any knowledge transfer activities executed within the project.	• Meetings with hospitals and industry to discuss the results and potential use of our results. • A newsletter to all participants on outcomes of the studies. This ensured that the outcomes of project were clearly disseminated to the public and in particular to those who took part as participants in the research. • Presentation at conferences - a number of talks were delivered throughout the project highlighting the work to the scientific community.

	• Theses Submissions - 2 theses submitted to CORA, the UCC online thesis repository.
List any impacts resulting from the knowledge transferred during the project.	• Training – obtained at PhD and Post Doc level. Significant training of the next generation of scientists took place throughout the INFAMILK project. • Evidence for hospitals and human milk banks: The current findings show the effect of processing and storage on physical stability and compositional changes in human milk. Therefore, the results could help hospitals and human milk banks improve their human milk handling methods.

Section 3 – Leveraging, future Strategies & Reference

Leveraging Metrics

Type of Funding Resource	Funding €	Summary
Exchequer National Funding	€96,000.00	PhD student funded through Alimentary Pharmabiotic Centre.

Future Strategies

The project team are committed to exploring future studies in the area of human milk research to build on the outcomes of this project. In the short term, some follow-on studies were undertaken during Summer 2022 to add replicates for some of the processing studies where Covid-19 disruptions resulted in fewer repeat trials being performed than would have been preferred. In addition, an additional PhD student (funded through the Alimentary Pharmabiotic Centre) has started on work which parallels and extends some of the processing studies.

Project Publications

Project publications

1. Lyons, K., O'Shea, C.-A., Grimaud, G. M., Ryan, A. C., Dempsey, E., Kelly, A., . . . Stanton, C. (2022). The human milk microbiome aligns with lactation stage and not birth mode. Scientific Reports, 12.
2. Lyons, K., Fouhy, F., O'Shea, C.-A., Ryan, A. C., Dempsey, E., Ross, R., & Stanton, C. (2021). Effect of storage, temperature, and extraction kit on the phylogenetic composition detected in the human milk microbiota. Microbiology Open, 10.
3. Lyons, K., Ryan, A. C., Dempsey, E., Ross, R., & Stanton, C. (2020). Breast Milk, a Source of Beneficial Microbes and Associated Benefits for Infant Health. Nutrients, 12, 1039.
4. Meng, F., Uniacke-Lowe, T., Lyons, K., Murphy, K., O'Mahony, J., Stanton, C., & Kelly, A. (2021). Human Milk. In. Meng, F., Uniacke-Lowe, T., Ryan, A. C., & Kelly, A. L. (2021). The composition and physico-chemical properties of human milk: A review. Trends in Food Science & Technology, 112, 608-621.
5. Meng, F., Uniacke-Lowe, T., Lanfranchi, E., Meehan, G., O'Shea, C. A., Fox, P. F., . . . Kelly, A. L. (2020). Factors affecting the creaming of human milk. International Dairy Journal, 108, 104726.
6. Overgaard Poulsen, K., Meng, F., Lanfranchi, E., Yong, J. F., Kelly, A. L., & Sundekilde, U. K. (2022). Dynamic changes in the human milk metabolome over 25 weeks of lactation. Frontiers in Nutrition. In print.