Human Milk Microbiota Exhibits Taxonomic Divergence but Functional Stability

Wesolowska Aleksandra[1,10], Leigh Robert J.[2], Mariusz Radkiewicz[3], Oledzka Gabriela[1], Bzikowska-Jura Agnieszka[4], Molas Aleksandra[1], van den Akker Chris H. P.[5], Bertino Enrico[6], Drazkowska Izabela[7], Gandino Serena[8], Gawel Paulina[9], Gawronska Malgorzata[10], van Goudoever Johannes[5], Krolak-Olejnik Barbara[9], Sinkiewicz-Darol Elena[11], Sottemano Stefano[8], Tonetto Paola[8], Walsh Fiona[12], Mahon Bernard[12]

1 - Medical University of Warsaw, Laboratory of Human Milk and Lactation Reaserch, Department of Medical Biology, Warsaw, Poland
2 - School of Medicine, Trinity College Dublin, Dublin, Ireland
3 - Mass Spectrometry Laboraotry, Institute of Biochemistry and Biophysics PAS, Warsaw, Poland
4 - Medical University of Warsaw, Department of Clinical Dietetics, Warsaw, Poland
5 - University of Amsterdam, Vrije Universiteit Amsterdam, Department of Pediatrics-Neonatology, UMC - Emma Children's Hospital, Amsterdam, Netherlands
6 - City of Health and Science Hospital, Neonatal Care Unit of the University, Turin, Italy
7 - Medical University of Gdańsk, Division of Neonatology, Gdansk, Poland
8 - City of Health and Science Hospital, Neonatal Care Unit of the University, Turin, Italy
9 - Wroclaw Medical University,, Department of Neonatology, Wroclaw, Poland
10 - Human Milk Bank Foundation, Warsaw, Poland
11 - Kazimierz Wielki University in Bydgoszcz, Department of Physiology and Toxicology, Toum, Poland
12 - Maynooth University, Faculty of Science & Engineering, Co. Kildare, Ireland

Introduction

Human milk (HM) composition shapes the infant gut microbiota and intestinal barrier function. It may vary according to maternal diet, lactation stage, timing of expression and geographical origin. We established a multicentre international consortium including six human milk banks and collected over 180 HM samples from voluntary donors at three lactation time points. By combining metagenomics and mass spectrometry, we investigated temporal and geographical variation in HM microbiota and biochemical composition during the first six months of lactation.

Methods

HM samples were collected from six human milk banks in **Italy** (n=1), the **Netherlands** (n=1) and **Poland** (n=4) at 2–12 weeks (A), 13–16 weeks (B) and 17–24 weeks (C) postpartum. Nutritional composition of HM was assessed using tailored LC-MS/MS assays, including 12 B-vitamers, vitamin C, vitamin D, 9 fatty acids and 12 human milk oligosaccharides (HMOs). Microbial DNA was isolated using an optimised protocol and analysed by 16S rRNA gene sequencing. Statistical analyses were performed after harmonising maternal and neonatal covariates across datasets. Univariate models with robust HC3 covariance estimation assessed effects of time point, country and temporal trends. For multivariate analyses, features were residualised, winsorised and standardised before Principal Component Analysis and Euclidean distance-based PERMANOVA with 9,999 permutations, using a significance threshold of $p<0.005$.

Results

Among the 14 most abundant bacterial species identified in HM samples, five were short-chain fatty acid producers. Between timepoints A and C, thiamine (vitamin B1) increased (β=7.6, $p<0.001$), while thiamine monophosphate decreased (β=-76, $p<0.001$). Nine of 12 HMOs declined over time ($p<0.01$). Alpha diversity was comparable between countries, indicating similarly rich microbial communities. Caesarean section was associated with increased oleic acid (β=1250, $p<0.05$), while expressing milk by pump was associated with higher 3'-sialyllactose (β=26, $p<0.05$). Although species-level diversity differed significantly (F=5.0, p=0.0001), predicted metabolic pathways were more homogeneous (F=1.9, p=0.0089)

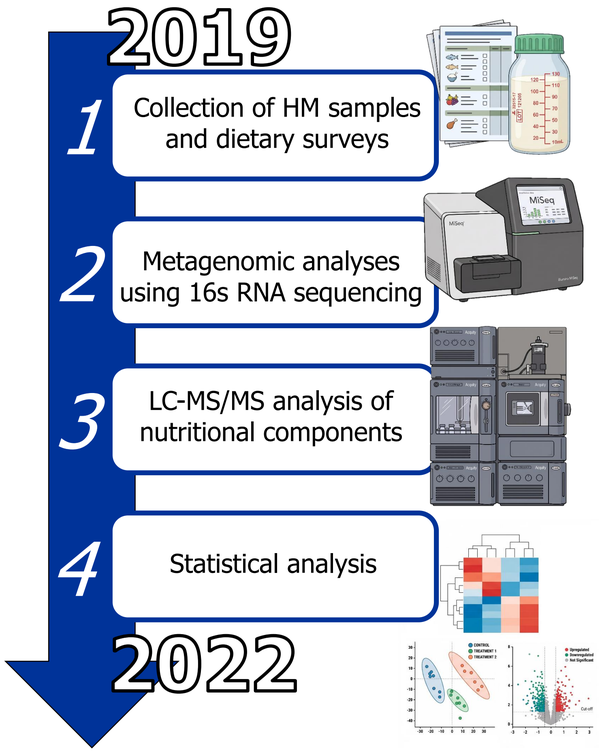
Dietary profiling revealed distinct nutritional patterns across the three cohorts. PL was characterized by significantly higher intakes of dietary cholesterol (median 323.9 vs. 181.0 mg in IT, p<0.001), animal protein, and ruminant-derived odd-chain fatty acids like FA 15:0 (240 vs. 110 mg in IT, p<0.001). NE exhibited the highest total caloric intake (2077 vs. 1736 kcal in IT, p< 0.001) alongside elevated consumption of fiber (24.2 vs. 15.8 g in IT, p<0.001) and PUFAs (14.0 vs. 9.4 g in IT, p<0.001). IT demonstrated the lowest overall intake of calories, saturated fats, and retinol (239.8 vs. 425.8µg in NE, p<0.001) compared to the NE and PL groups.

Discussion

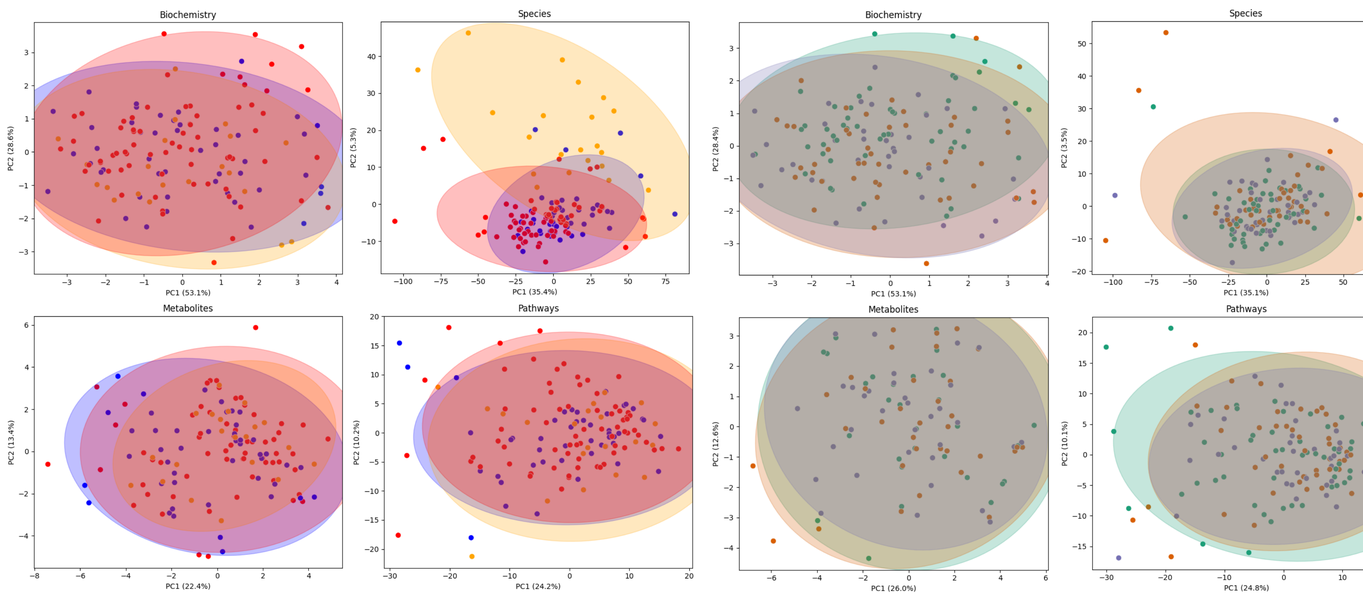
To our knowledge, this is the first study combining HM microbiota profiling with in-depth metabolic analysis to demonstrate a balanced bacterial metabolite profile naturally optimized for infant health.
HM multivariate structure varied more by geographical location than by lactation stage, with PCA showing clear country-level separation at the bacterial species level. However, after adjustment for covariates, metabolic and biochemical parameters were generally comparable across regions.

The consistency of predicted functional pathways suggests that different bacterial taxa may provide functional redundancy within a shared HM metagenomic profile.

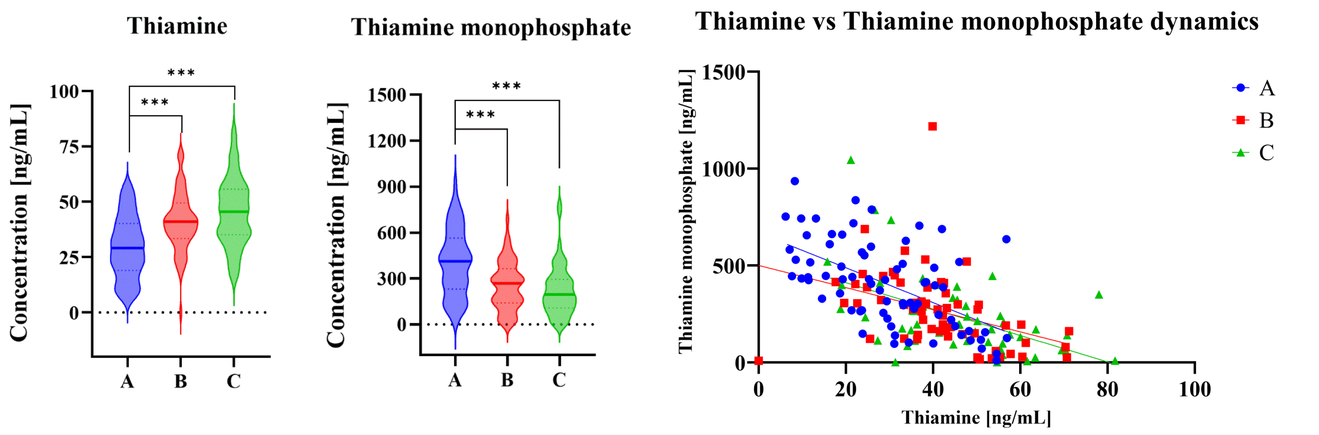




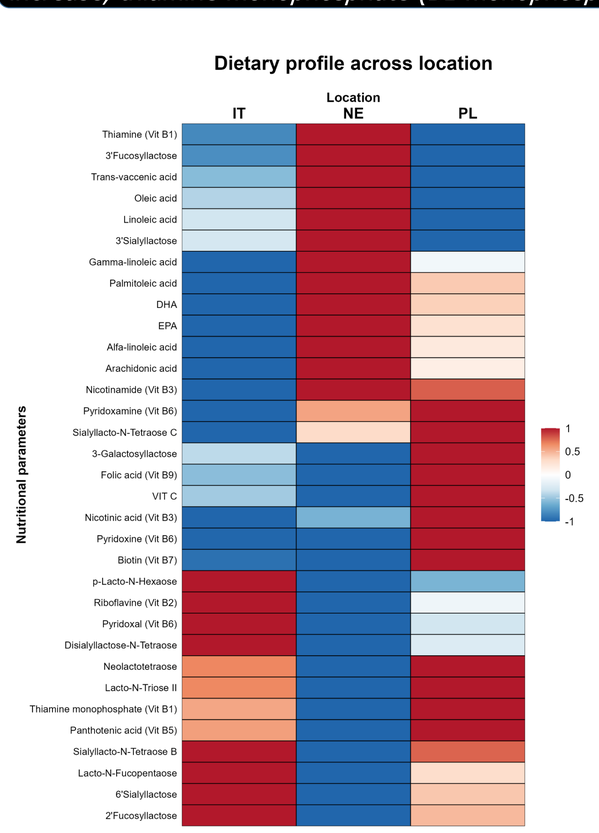
The map shows locations from where HM samples were collected.The diagram on the right shows the project's workflow consisting of sample and dietary surveys collection, as well as rRNA sequencing and LC-MS analyses.

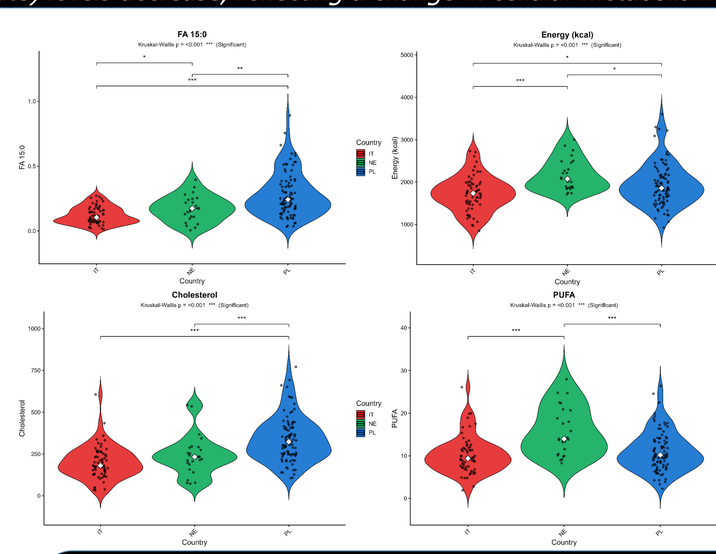


The PCA score plots illustrate interspecies differences while demonstrating overall metabolic and physiological similarities across both sampling locations (left) and time points (right). The different colours refer to NE, IT and PL



The graphs demonstrate significant codependent alteration in thiamines excretion. While thiamine (B1 vitamin) levels increase, thiamine monophosphate (B1 monophosphate) levels decrease, reflecting a change in cellular metabolism.





Heatmap (left) pictures the relative differences in metabolic landscape of HM samples from IT, NE and PL. NE cohort demonstrates highest levels of health promoting nutrients. Violin plots (right) show the significant differences in cholesterol, PUFA, FA15:0 and total caloric intake between cohorts.

Project „Health promoting importance of human milk donation" financed under the grant of Polish National Agency for Academic Exchange for International Academic Partnerships

POSTER PRESENATION COFINANCED BY THE NATIONAL INSTITUTE OF FREEDOM – CENTER FOR CIVIL SOCIETY DEVELOPMENT AS PART OF THE GOVERNMENT PROGRAMME FOR CIVIL SOCIETY ORGANISATIONS DEVELOPMENT FOR 2018–2030 CSODev