

WP2 RESEARCH INFRASTRUCTURES

Task 2.3 Unlocking the collaborative potential of EDUC Research Infrastructures

Advancing healthcare using the Research Infrastructure
CeSAR of UniCa: Nutrition during the first two years of
life

Cagliari, 23th and 24th November 2023



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from the European Union's Horizon
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No 101017526

Use of NMR facilities in the clinic and study of infant milk

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Presentation structure

PART 1 – NMR

- ✓ Probes
- ✓ Sample preparation
- ✓ NMR spectrum

PART 2 – HUMAN MILK



NMR advantages

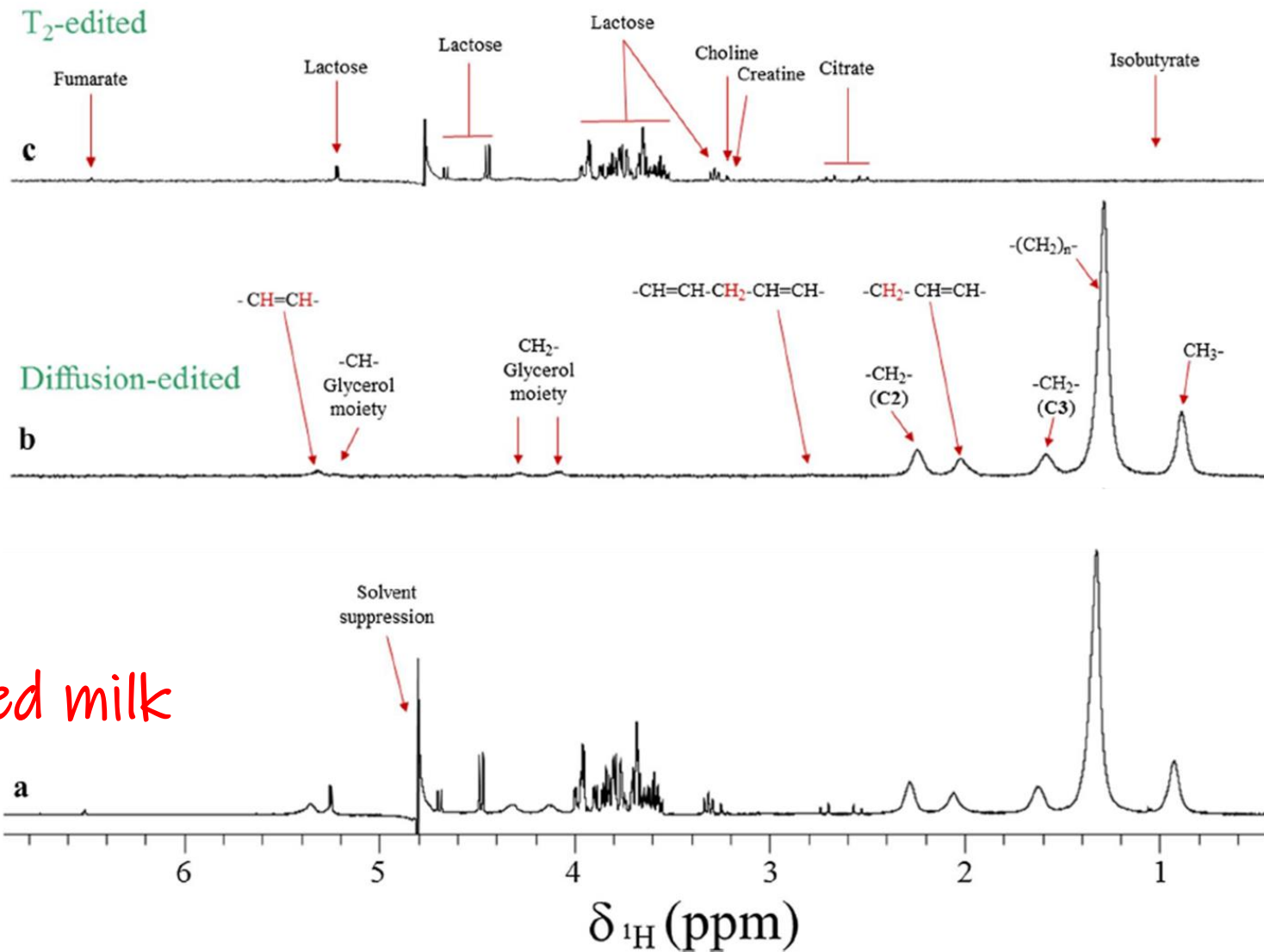
High
reproducibility

Minimal sample
preparation

All metabolites at a detectable
concentration level
can be observed
in one measurement

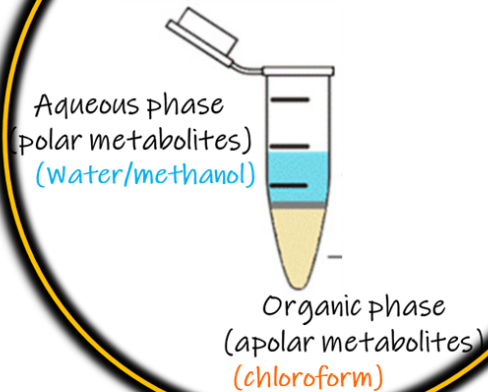
Nondestructive

^1H spectrum of milk

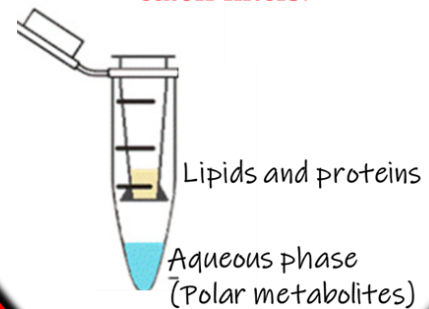


Milk sample preparation for NMR

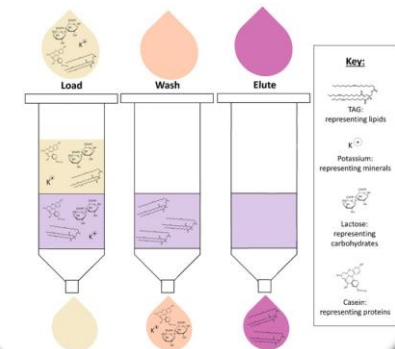
Liquid-liquid extraction (Folch method)



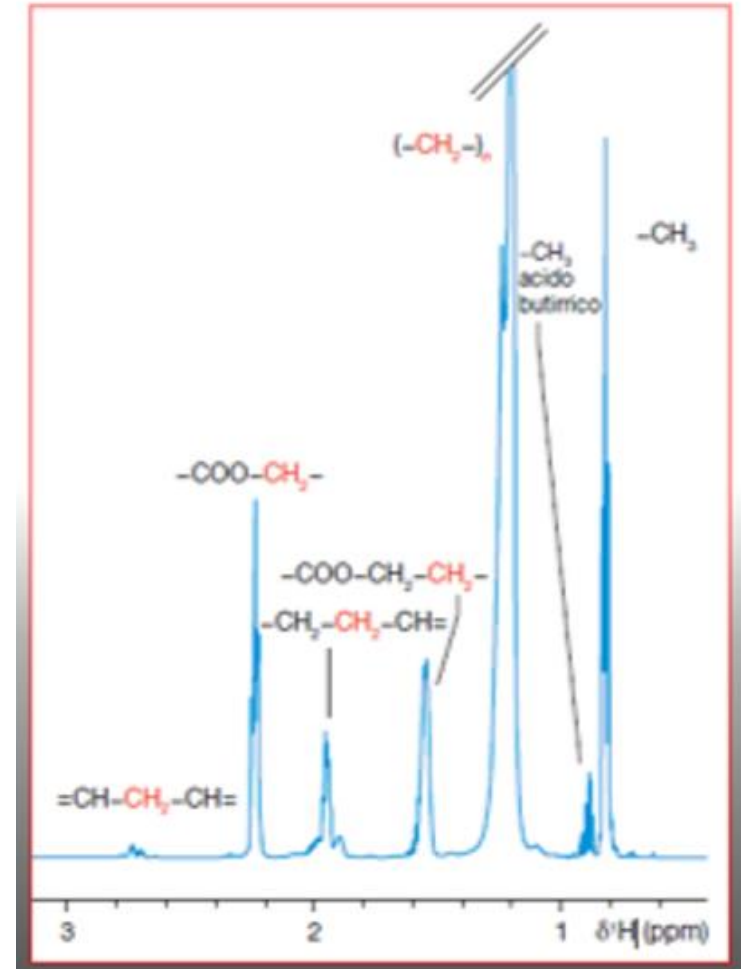
Ultrafiltration centrifugal molecular weight cutoff filters.



SPE Solid phase extraction

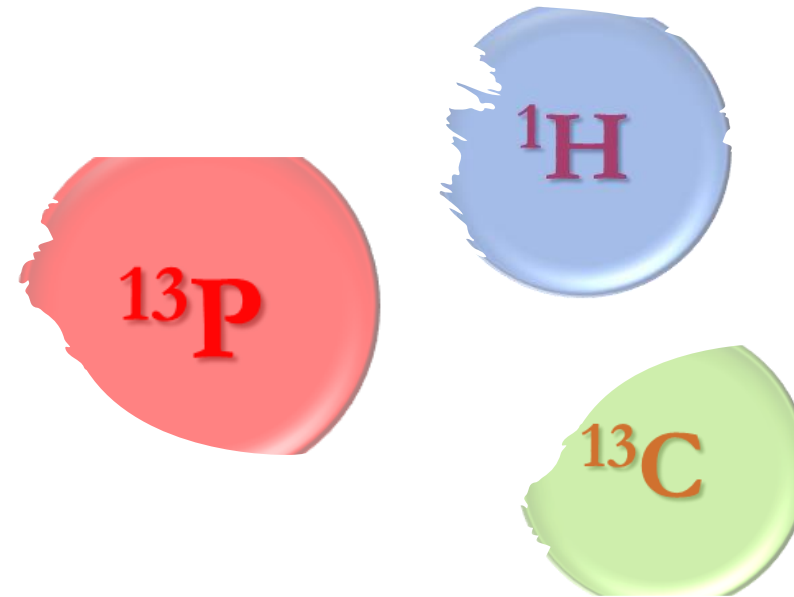


Lipid fraction



Lipid component

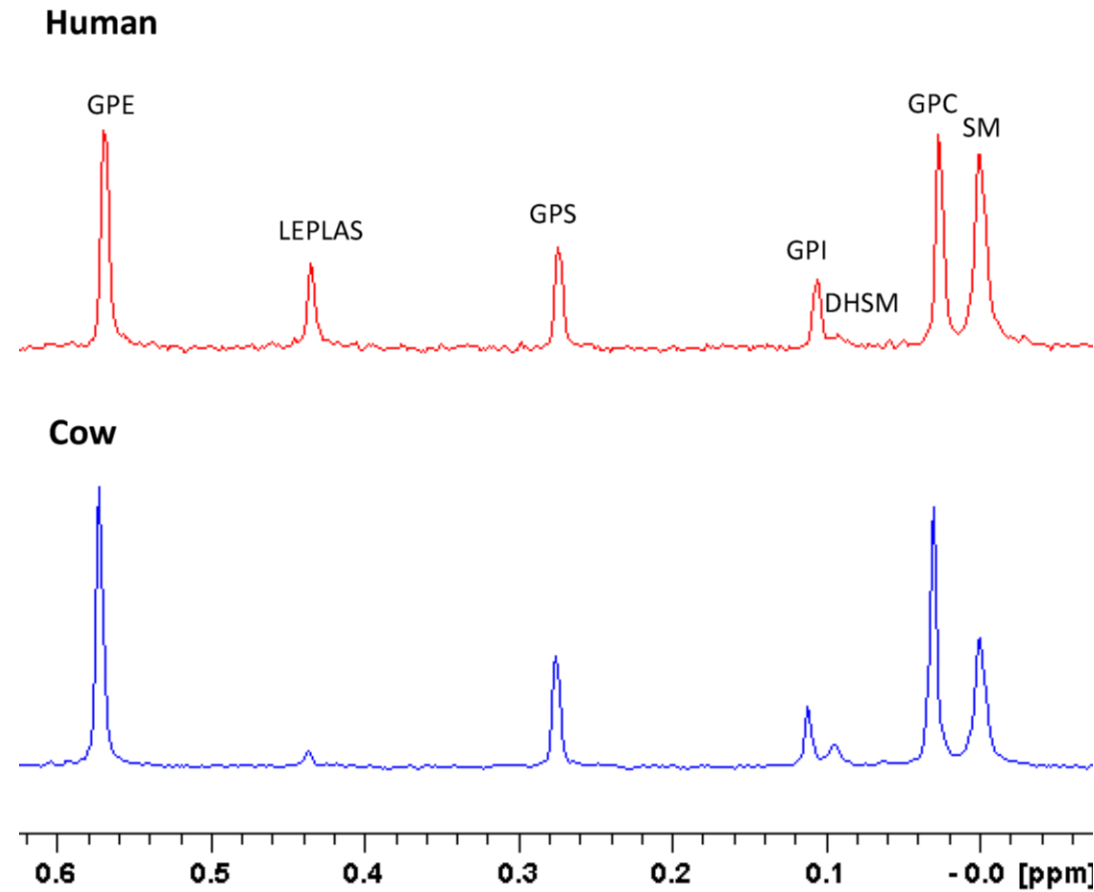
- Triacylglycerols (>95%)
- Phospholipids
- Diacylglycerols
- Monoacylglycerols
- Free Fatty Acids



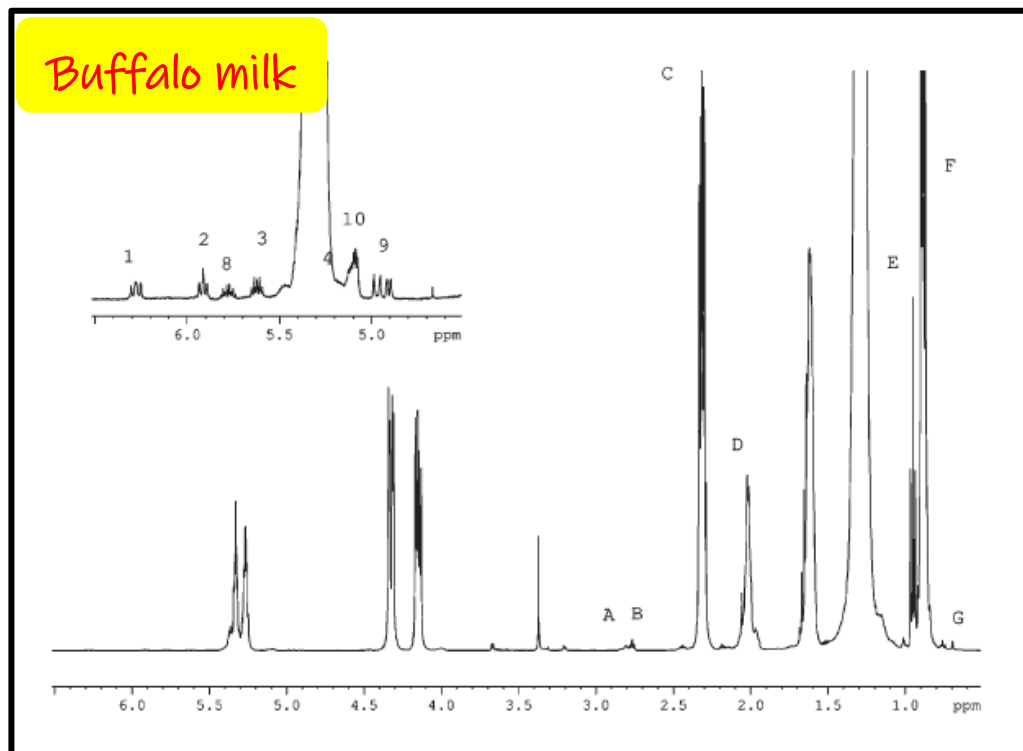
Milk fatty acids

- *short chain fatty acids (C2:C6)*
- *medium chain fatty acids (C7-C12)*
- *long chain fatty acids (C14:C18)*
- *Saturated fatty acids (SFA)*
- *Unsaturated fatty acids (UFA)*
- *Monounsaturated fatty acids (MUFA)*
- *Polyunsaturated fatty acids (PUFA)*

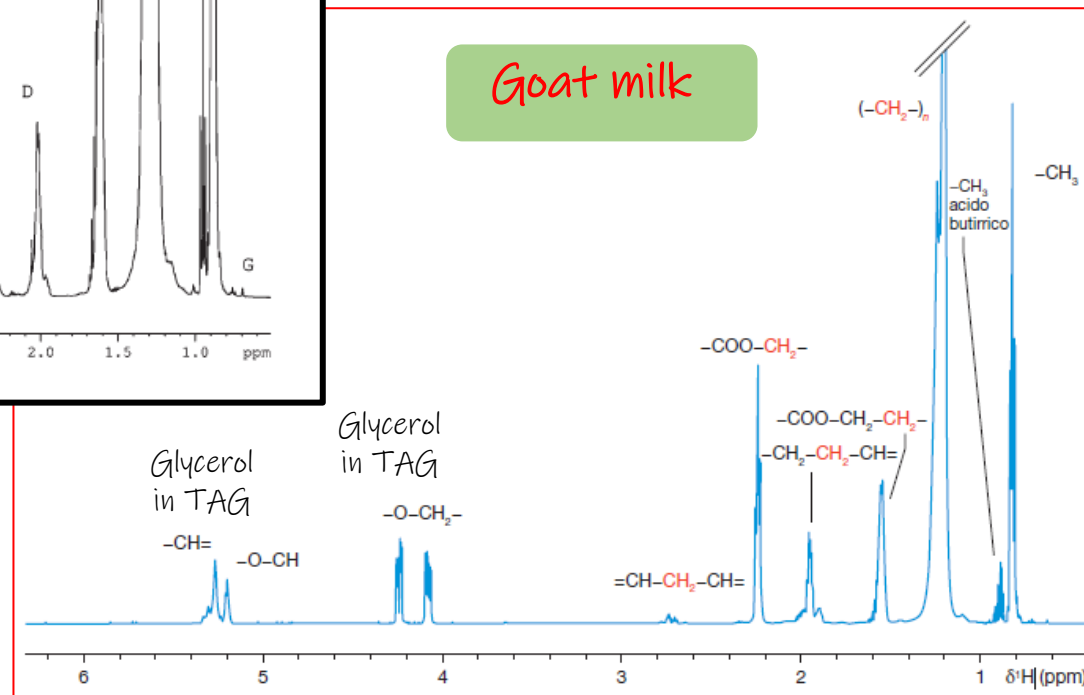
Lipid component



Lipid component

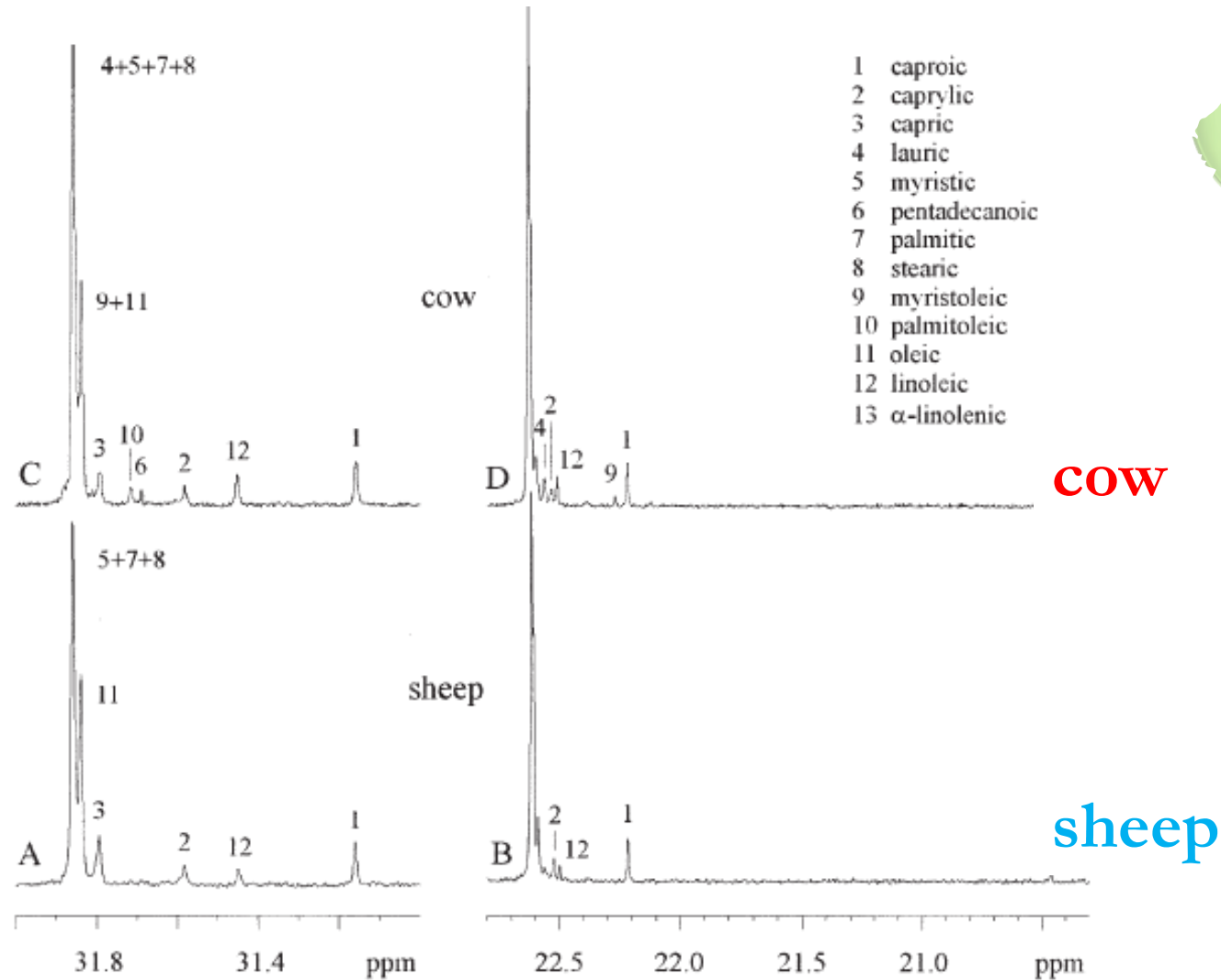


Brescia et al. *J. Am. Oil Chem. Soc.* 2004, 81, 431

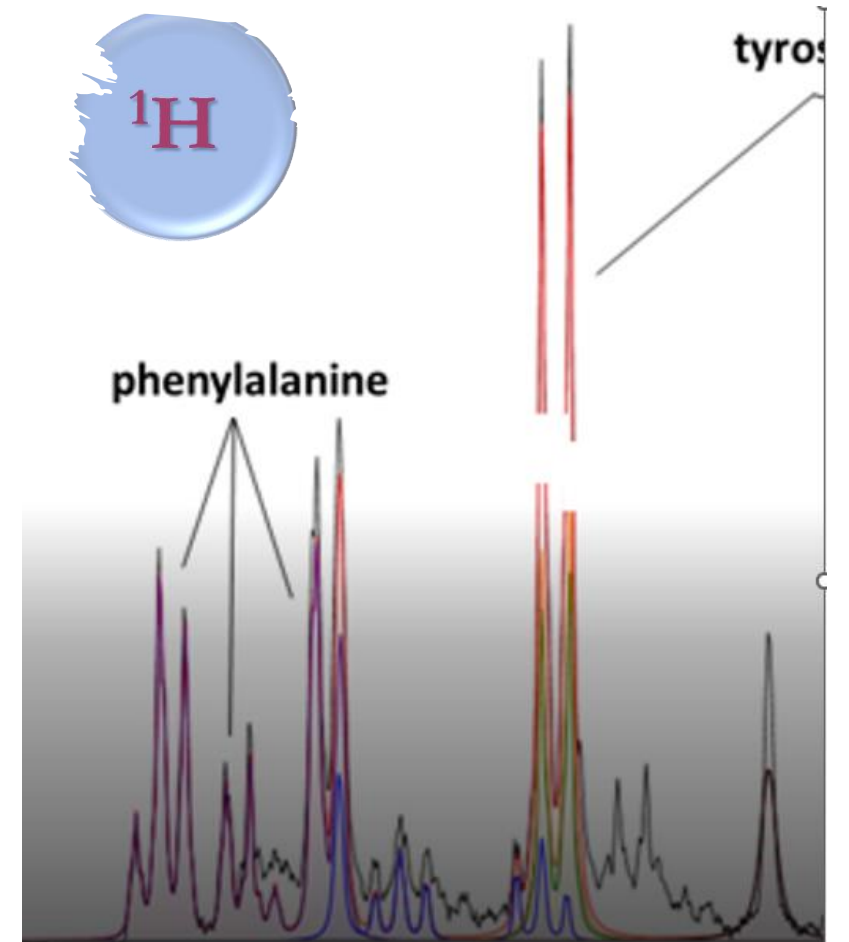


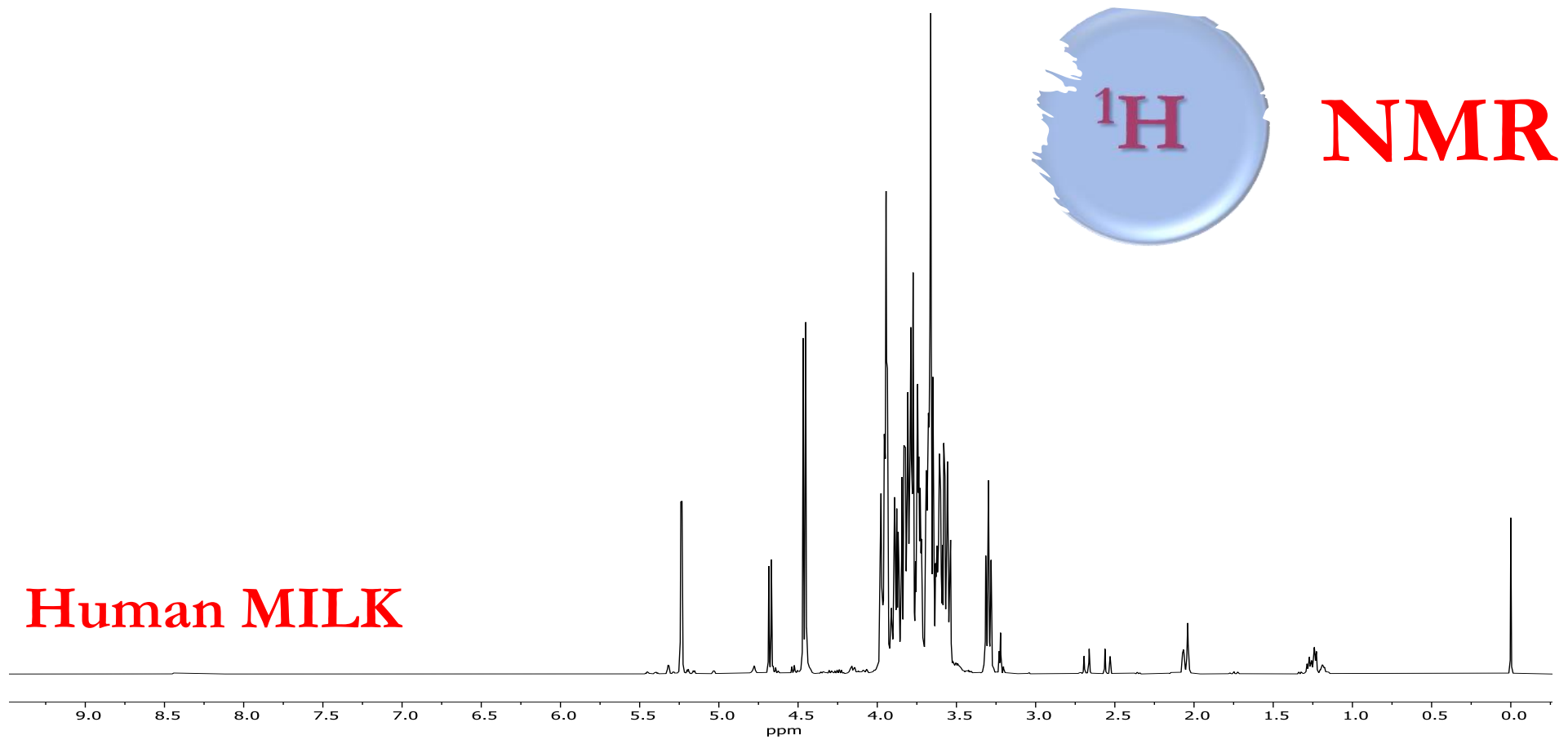
Boccia et al. *Molecules.* 2020, 25, 1491

Lipid component



Aqueous fraction

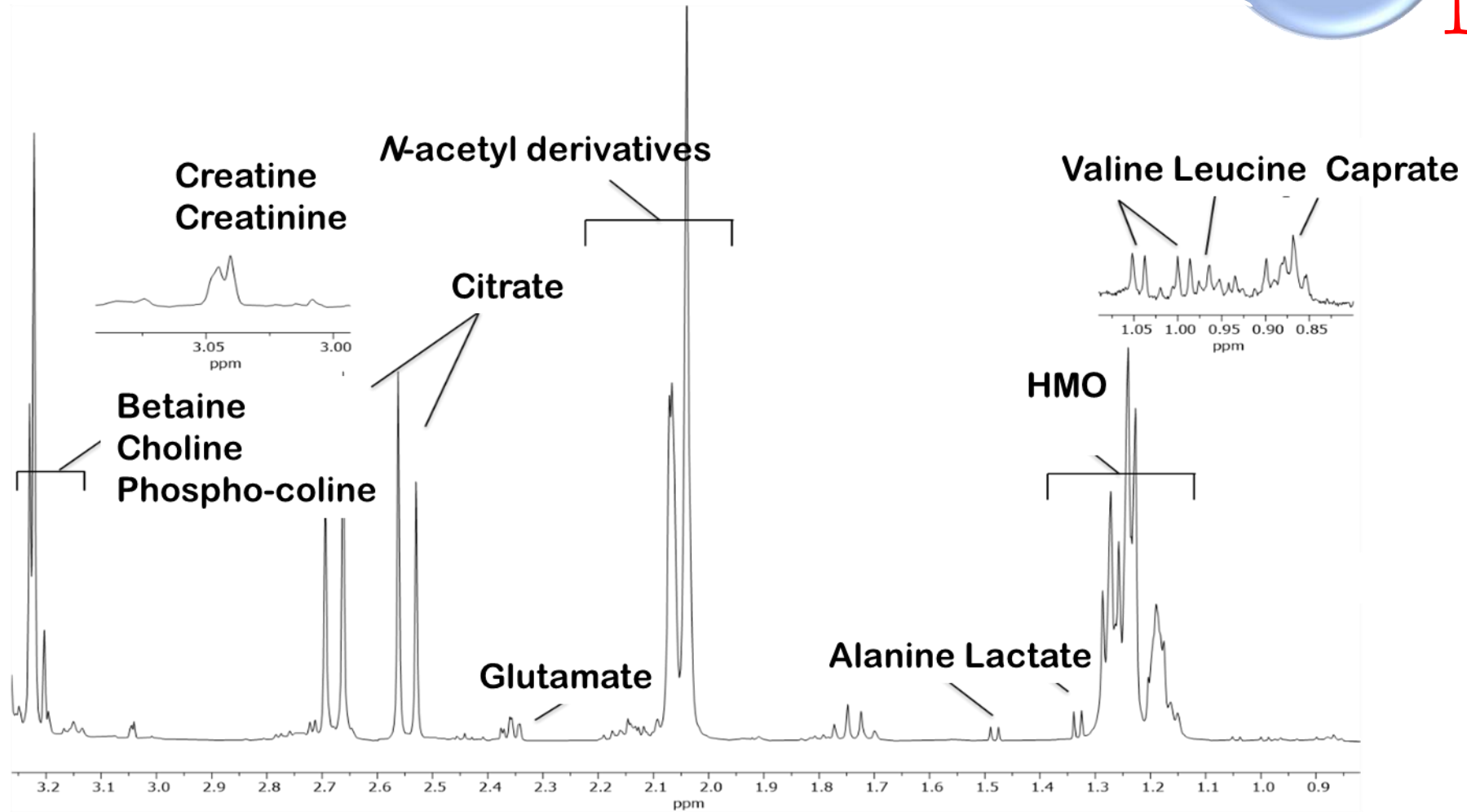




Human MILK

 ^1H

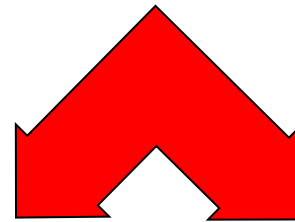
NMR



Why investigating HM?



Early infant nutrition plays important short- and long-term effects on infant health



INFANT METABOLISM

PART 2 - NMR metabolomics on HM



A metabolomic study of preterm human and formula milk by high resolution NMR and GC/MS analysis: preliminary results

Flaminia Cesare Marincola^{1,4}, Antonio Noto^{2,4}, Pierluigi Caboni³, Alessandra Reali², Luigi Barberini⁴, Milena Lussu⁵, Federica Murgia⁵, Maria Laura Santoru⁵, Luigi Atzori⁵ & Vassilios Fanos²

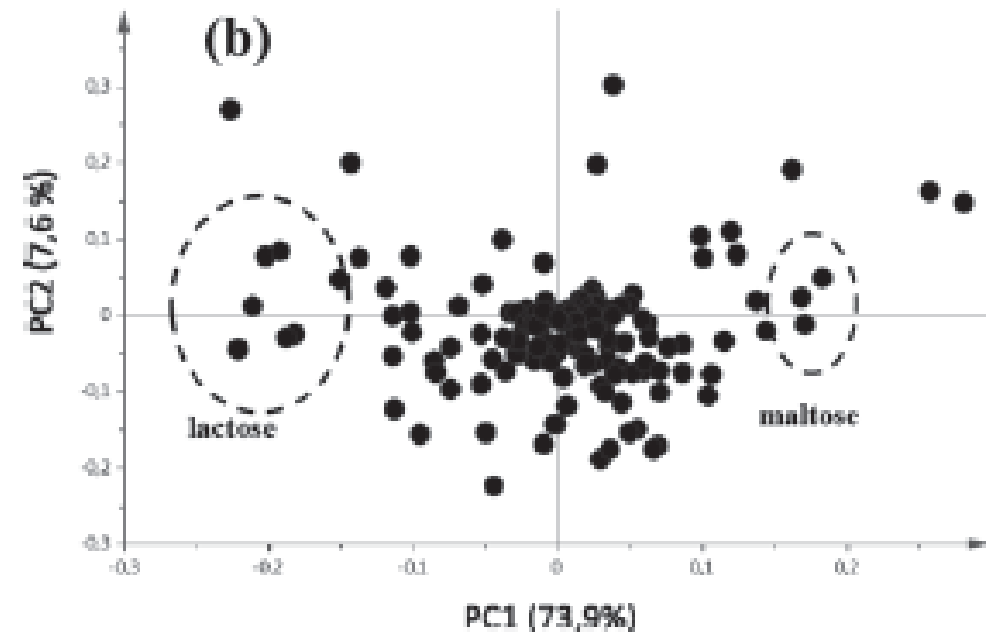
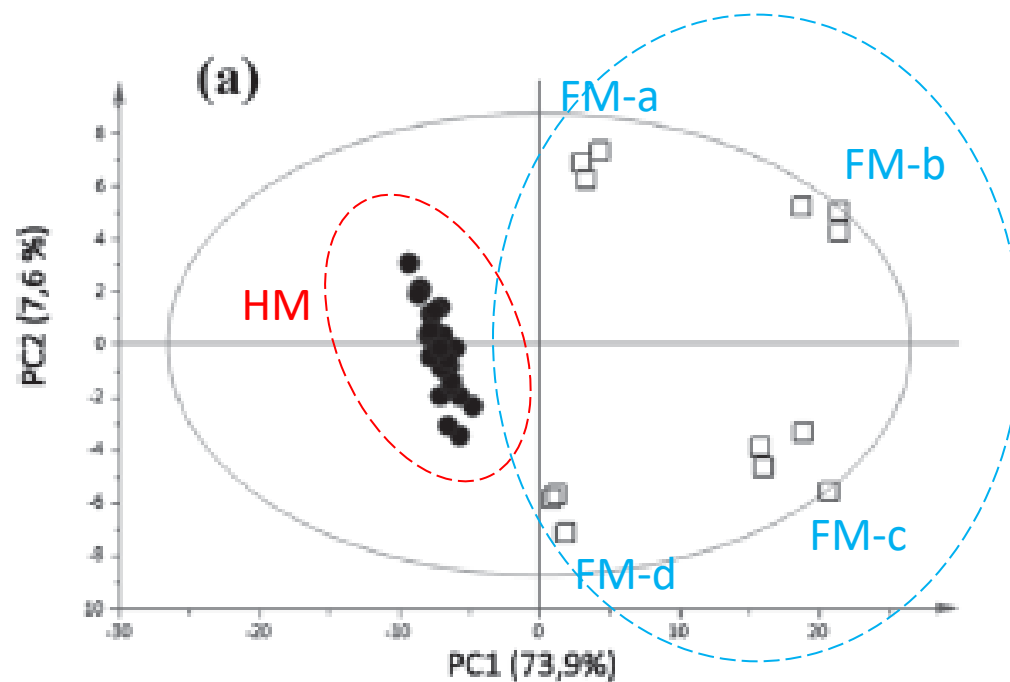
¹Department of Chemical and Geological Science, University of Cagliari, Cagliari, Italy, ²Department of Surgery, University of Cagliari, Cagliari, Italy, ³Department of Life and Environmental Sciences, University of Cagliari, Cagliari, Italy, ⁴Department of Public Health and Clinical and Molecular Medicine, University of Cagliari, Cagliari, Italy, and ⁵Department of Biomedical Sciences, University of Cagliari, Cagliari, Italy

The Journal of Maternal-Fetal & Neonatal Medicine 2012 (25), 62-67

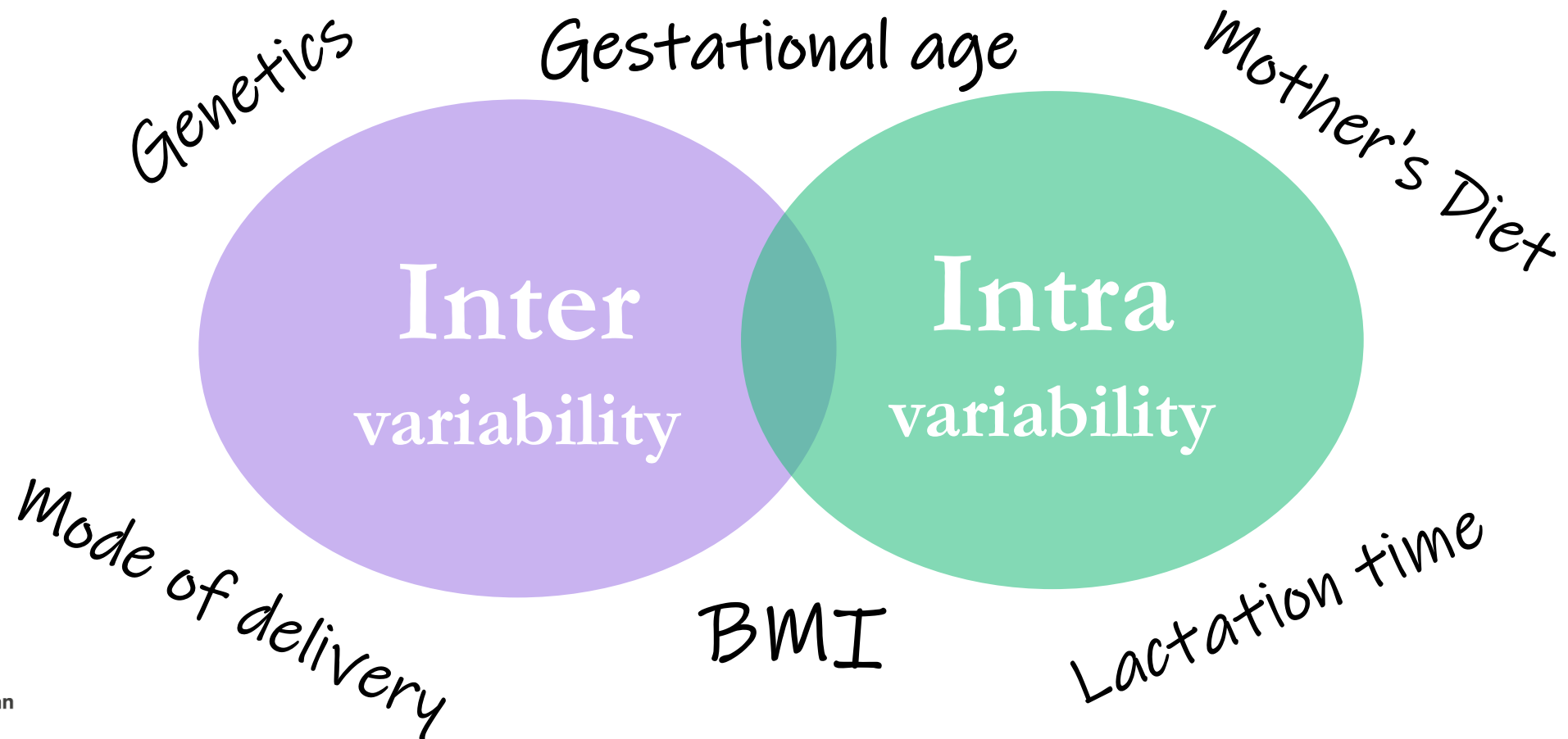
A metabolomic study of preterm human and formula milk by high resolution NMR and GC/MS analysis: preliminary results

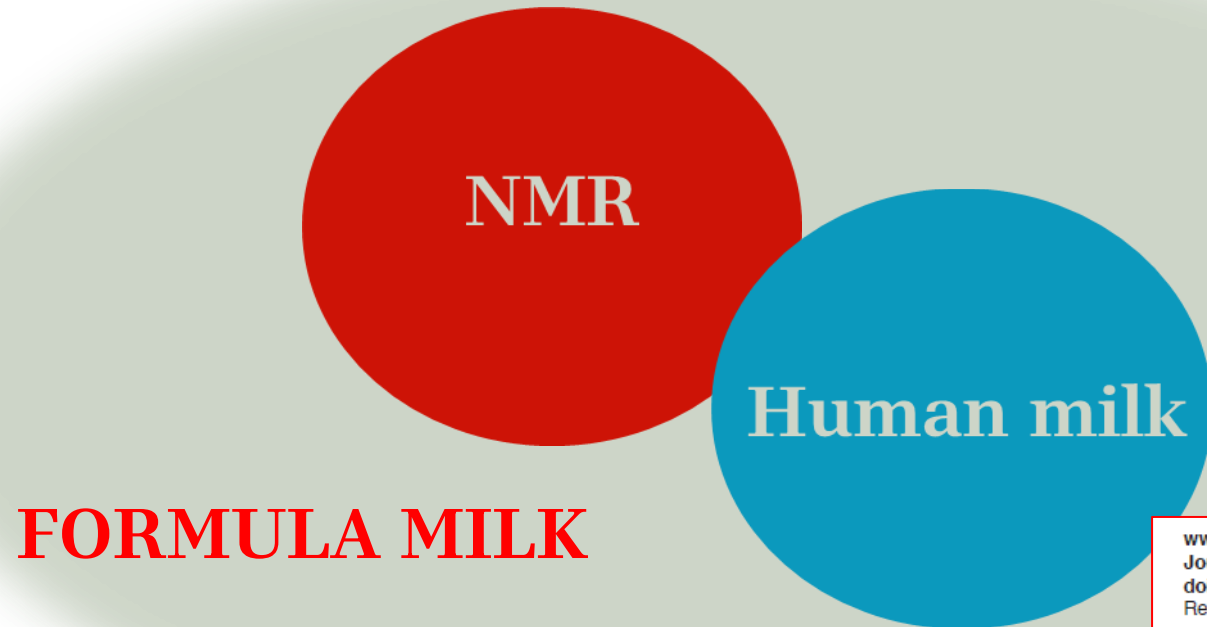
Flaminia Cesare Marincola^{1,6}, Antonio Noto^{2,6}, Pierluigi Caboni³, Alessandra Reali², Luigi Barberini⁴, Milena Lussu⁵, Federica Murglia⁵, Maria Laura Santoru⁵, Luigi Atzori⁵ & Vassilios Fanos²

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Human milk metabolome



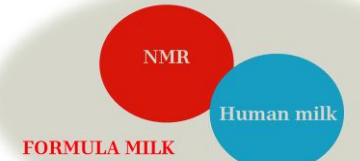


www.jpnm.com Open Access eISSN: 2281-0692
Journal of Pediatric and Neonatal Individualized Medicine 2019;8(2):e080228
doi: 10.7363/080228
Received: 2019 Aug 14; accepted: 2019 Sept 24; published online: 2019 Oct 23

Original article

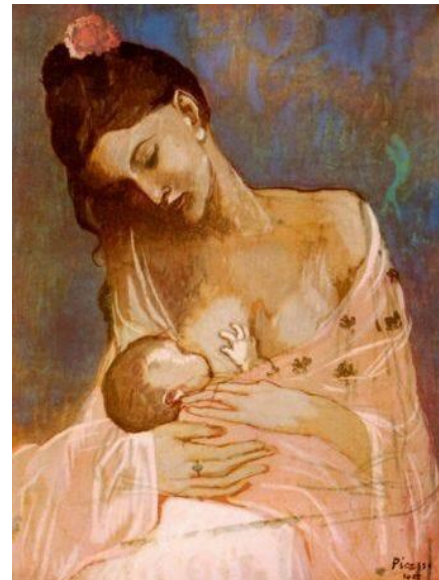
NMR-based metabolomics analysis of organic and conventionally produced formula milk: preliminary results

Sara Corbu¹, Roberta Pintus², Angelica Dessi², Melania Puddu², Flaminia Cesare Marincola¹, Vassilios Fanos²



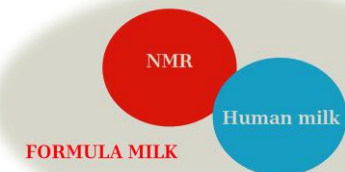
Breastfeeding reduces the baby's risk of developing various illnesses such as:

- ✓ Cold and infections
- ✓ Allergic diseases
- ✓ Gut infections
- ✓ Diabetes
- ✓ Inflammatory bowel diseases
- ✓ Childhood leukaemia



The Breastfed Child and Motherhood
by Pablo Picasso, 1905,

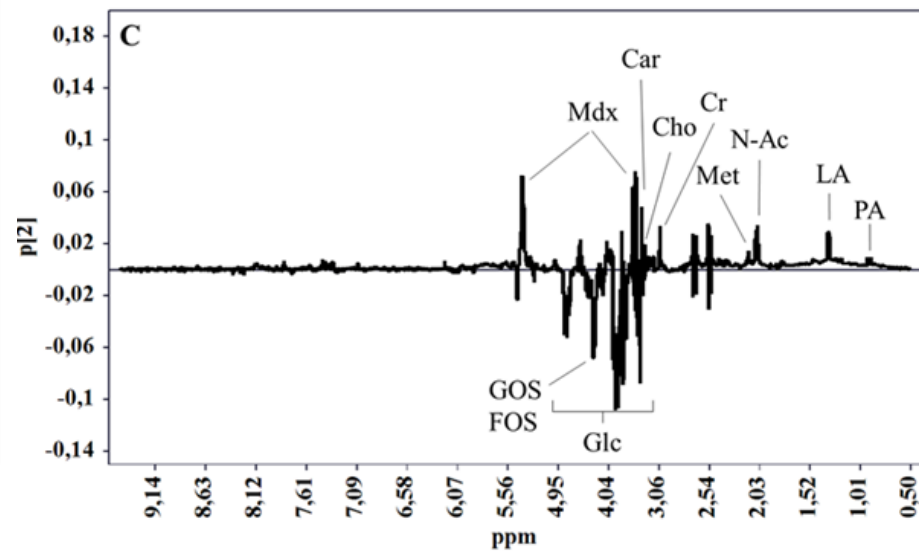
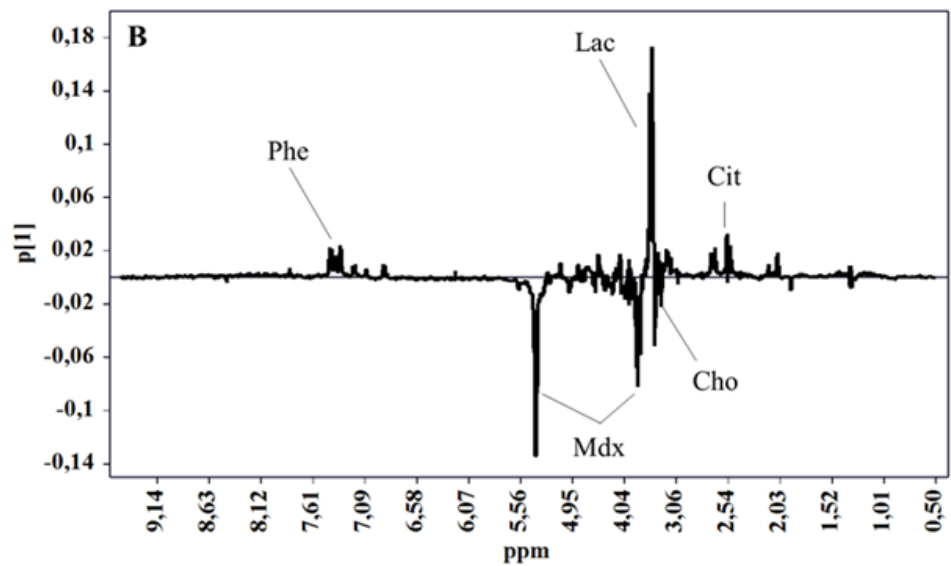
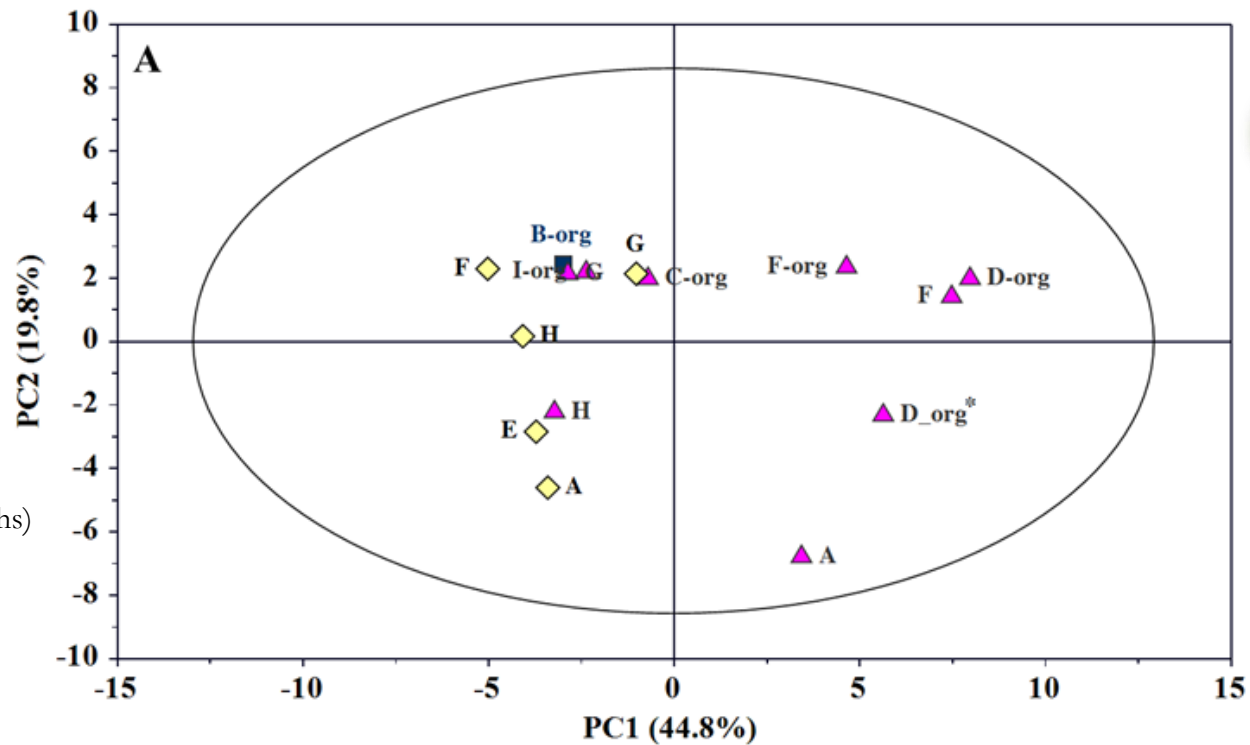


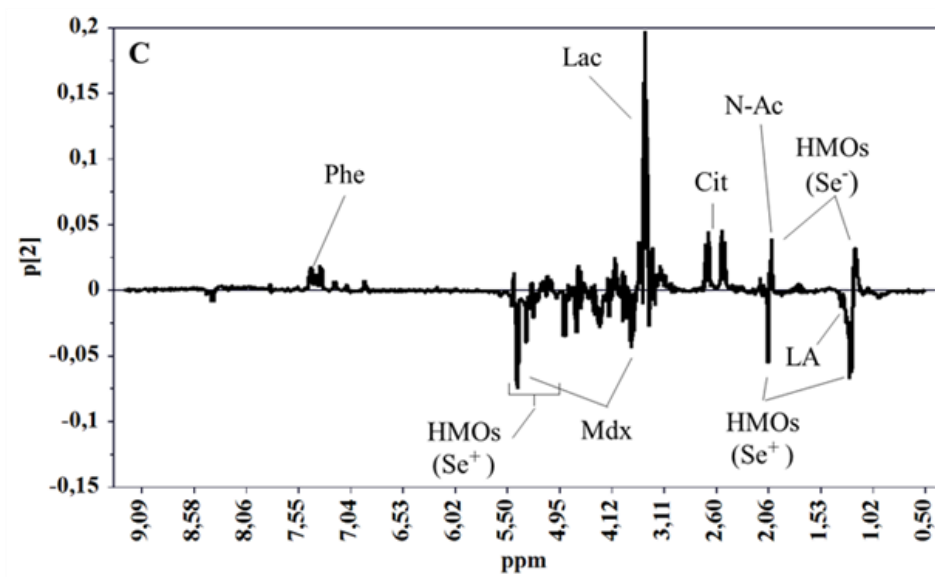
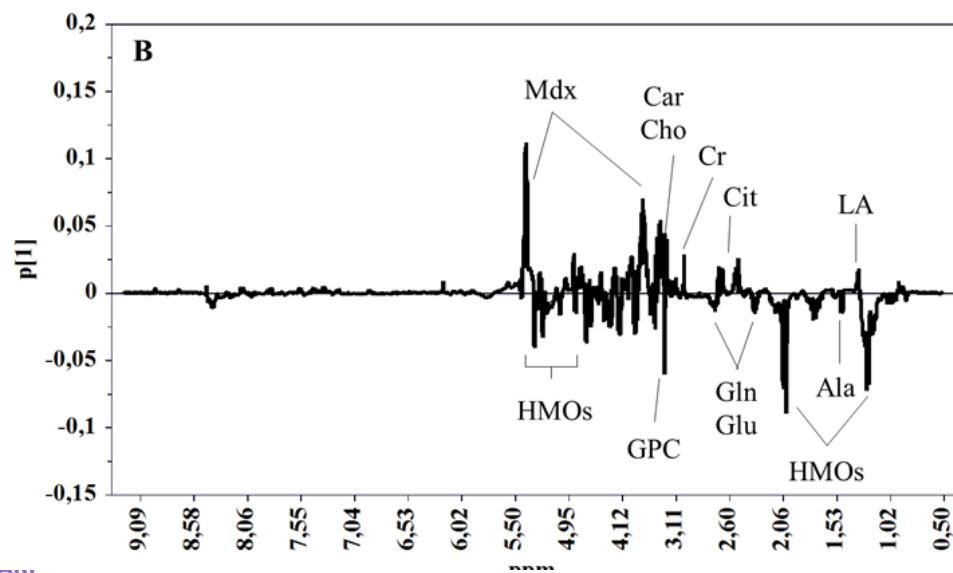
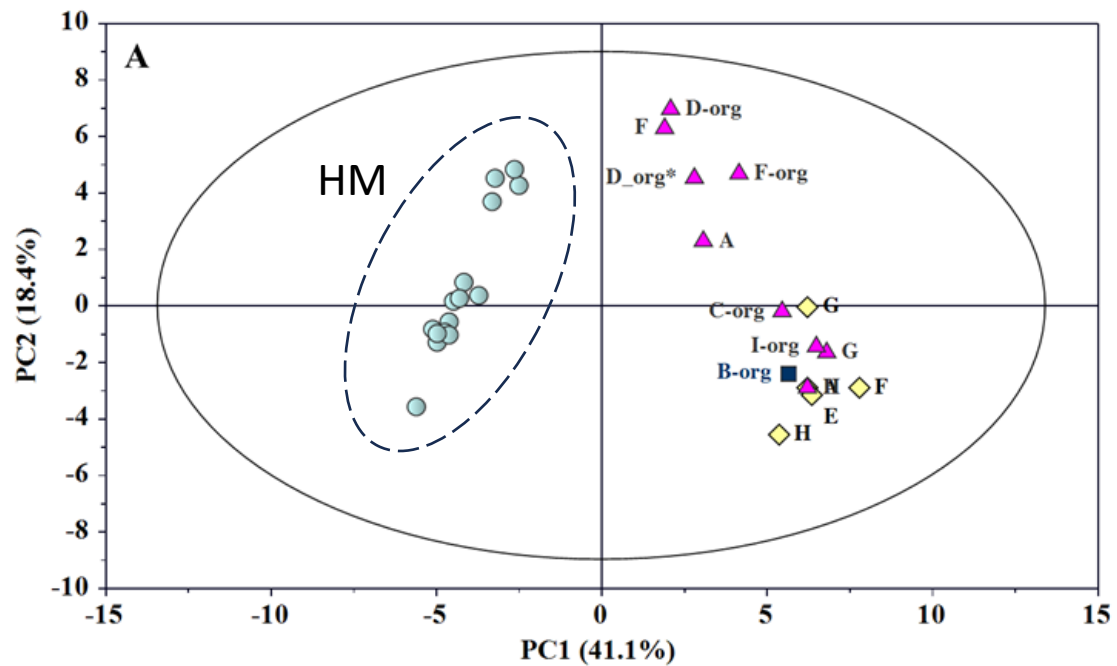
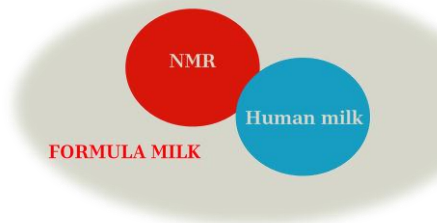


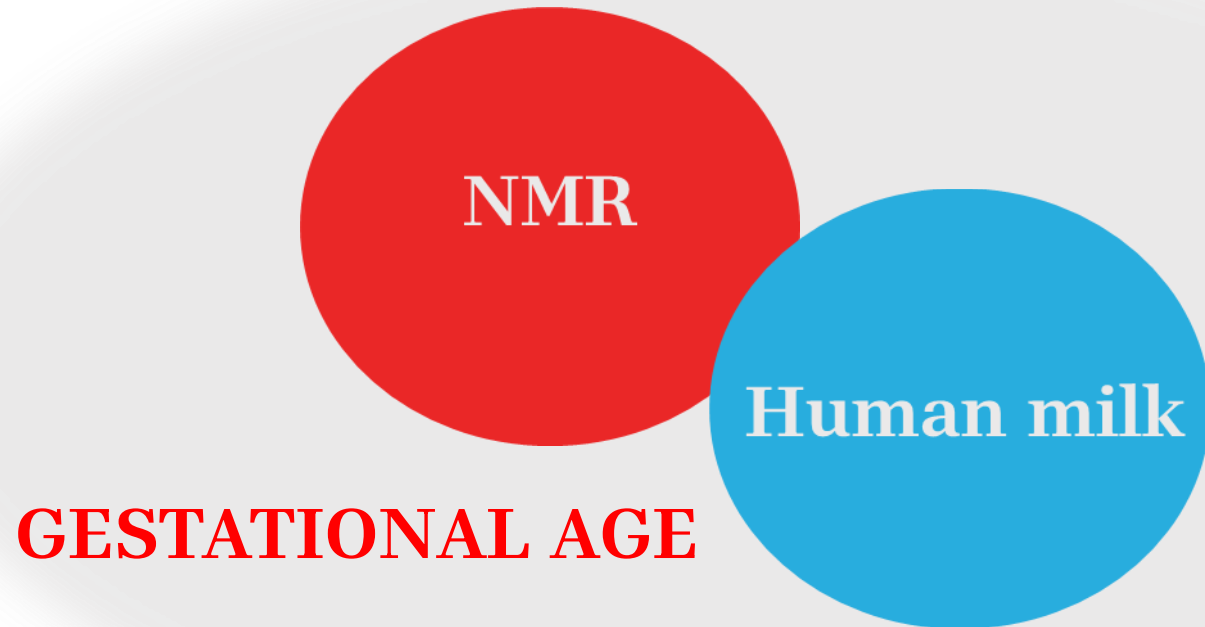
□ Formula milk 0-12 months

▲ First infant milk (from birth to 6 months)

◇ Follow-on milk (6 months and older)











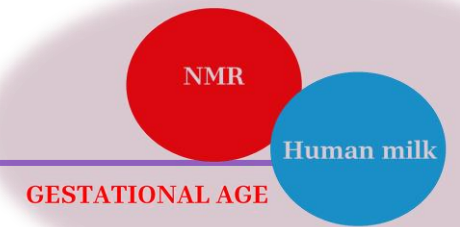
Foods **2022**, 11, 345. <https://doi.org/10.3390/foods11030345>



Article

NMR Metabonomic Profile of Preterm Human Milk in the First Month of Lactation: From Extreme to Moderate Prematurity

Chiara Peila ¹, Stefano Sottemano ¹, Flaminia Cesare Marincola ^{2,*}, Matteo Stocchero ^{3,4,*}, Nicoletta Grazia Pusceddu ², Angelica Dessì ⁵, Eugenio Baraldi ^{3,4}, Vassilios Fanos ⁵ and Enrico Bertino ¹



PRETERM INFANTS



Donor-expressed milk processed by human milk banks has been used to provide preterm infants with breast milk when there are circumstances that preclude the use of mother's own milk.

Since there are differences between term and preterm milk, a complete understanding of the differences between milk from women delivering term and preterm infants over time, particularly in the first month postpartum, has clinical relevance.



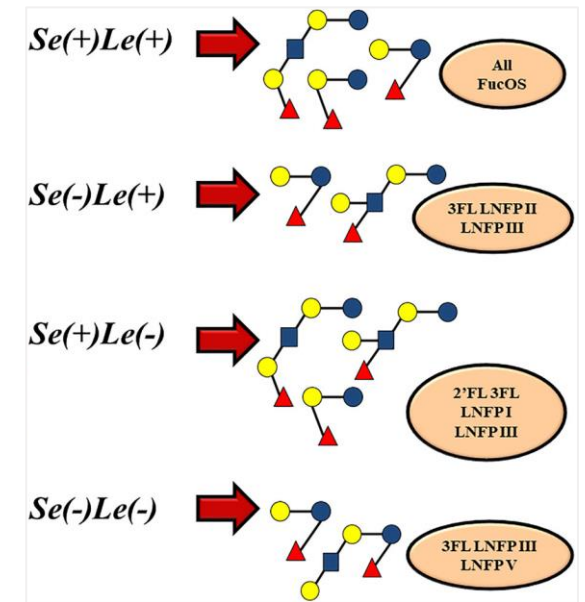
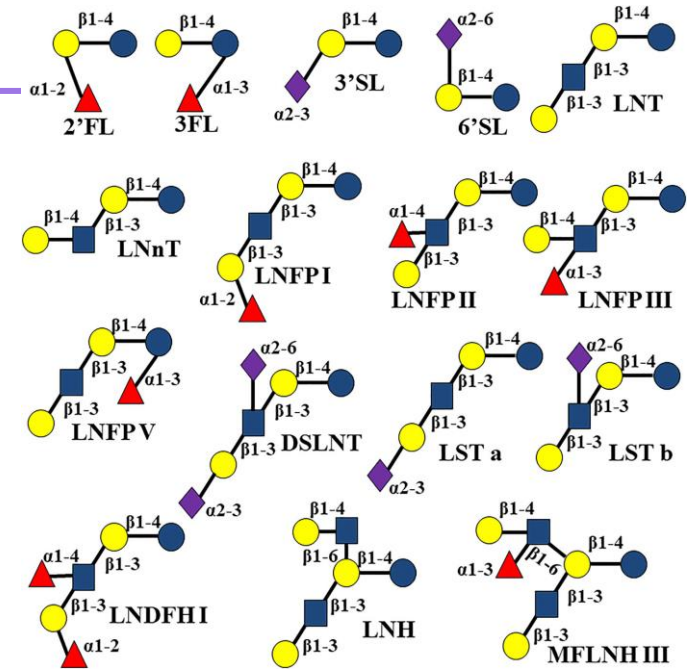
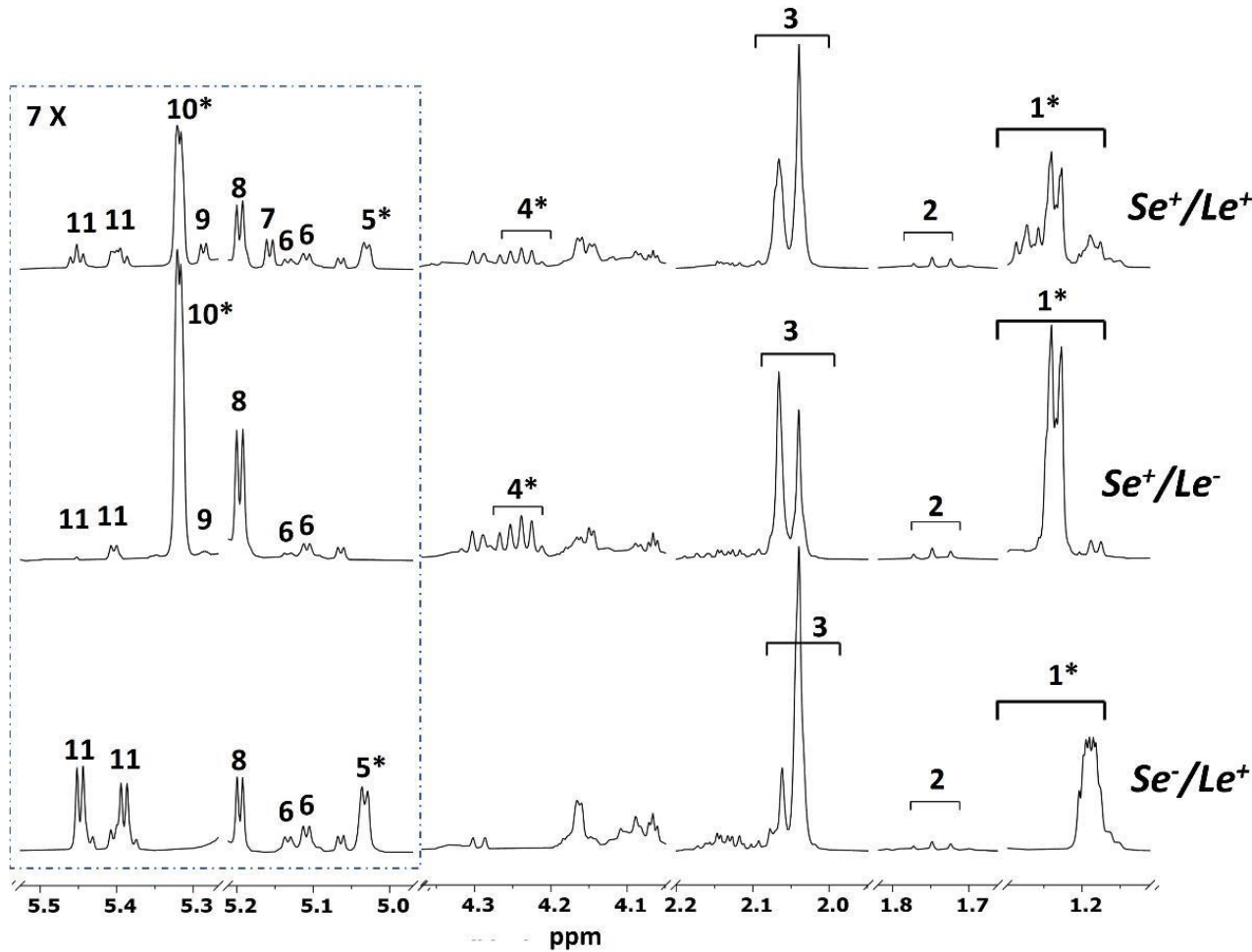
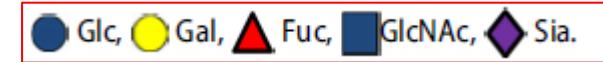
NMR

Human milk

GESTATIONAL AGE

	Extremely Preterm (<i>n</i> = 14)	Very Preterm (<i>n</i> = 11)	Moderately Preterm (<i>n</i> = 11)
Mothers			
Maternal age, y (ANOVA, <i>p</i> = 0.48)	34.6 ± 5.1	34.6 ± 4.3	35.2 ± 3.4
Maternal BMI, kg/m ² (ANOVA, <i>p</i> = 0.41)	23.4 ± 3.9	22.9 ± 3.6	24.6 ± 6.9
Type of pregnancy (Singleton/Twins) (Chi-squared test, <i>p</i> = 0.69)	12/2	9/2	8/3
Mode of delivery (vaginal/casarean section) (Chi-squared test, <i>p</i> = 0.006)	10/4	2/9	2/9
Infants			
Gender (Male/Female) (Chi-squared test, <i>p</i> = 0.70)	6/10	6/7	5/9
Birth weight, g (ANOVA, <i>p</i> = 0.002)	977 ± 233	1382 ± 357	1369 ± 375
Gestational age, wk [min–max]	26 [23–28]	30 [29–31]	33 [32–33]
Milk Samples			
Colostrum (3–6 lactation days)	12	9	11
Transitional milk (7–15 lactation days)	14	11	11
Mature milk (16–26 lactation days)	12	8	9
Lewis (Le) and Secretor (Se) phenotype of mothers² (Chi-square test, <i>p</i> = 0.33)			
Se ⁺ /Le ⁺	11	9	6
Se ⁻ /Le ⁺	1	1	4
Se ⁺ /Le ⁻	2	1	1

Mother phenotypes and human milk oligosaccharides

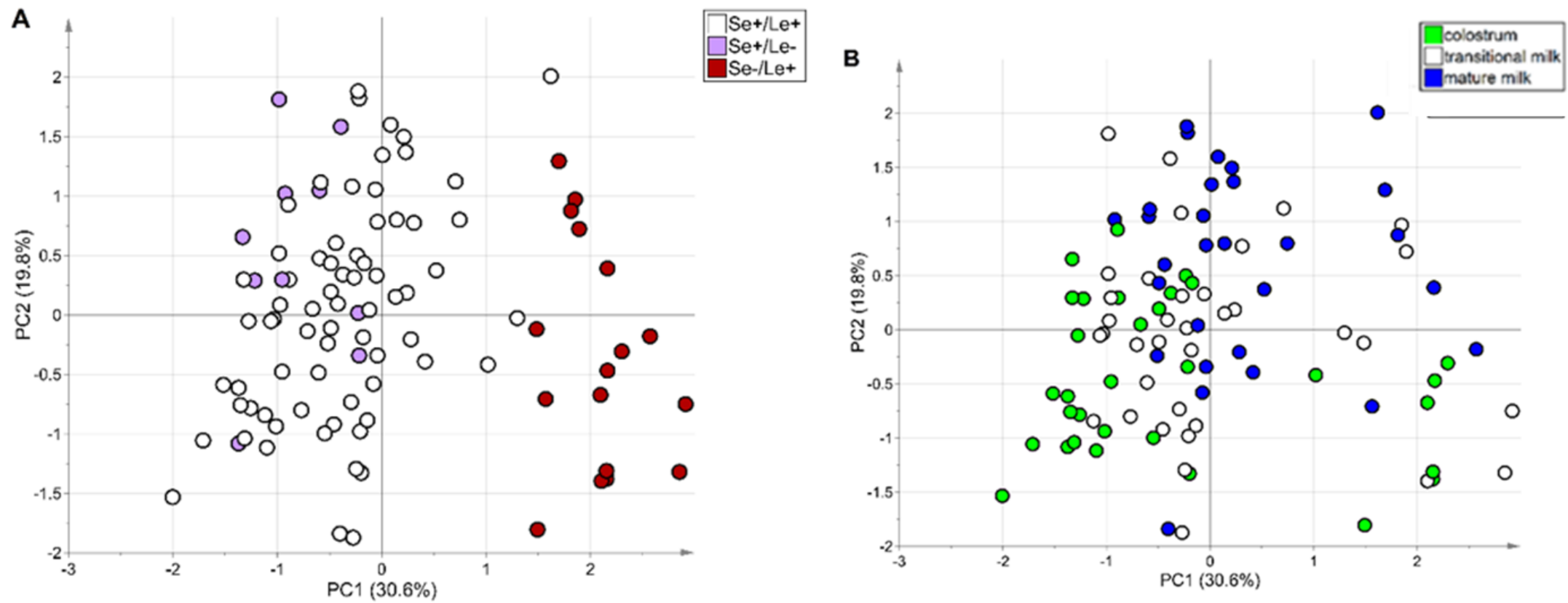


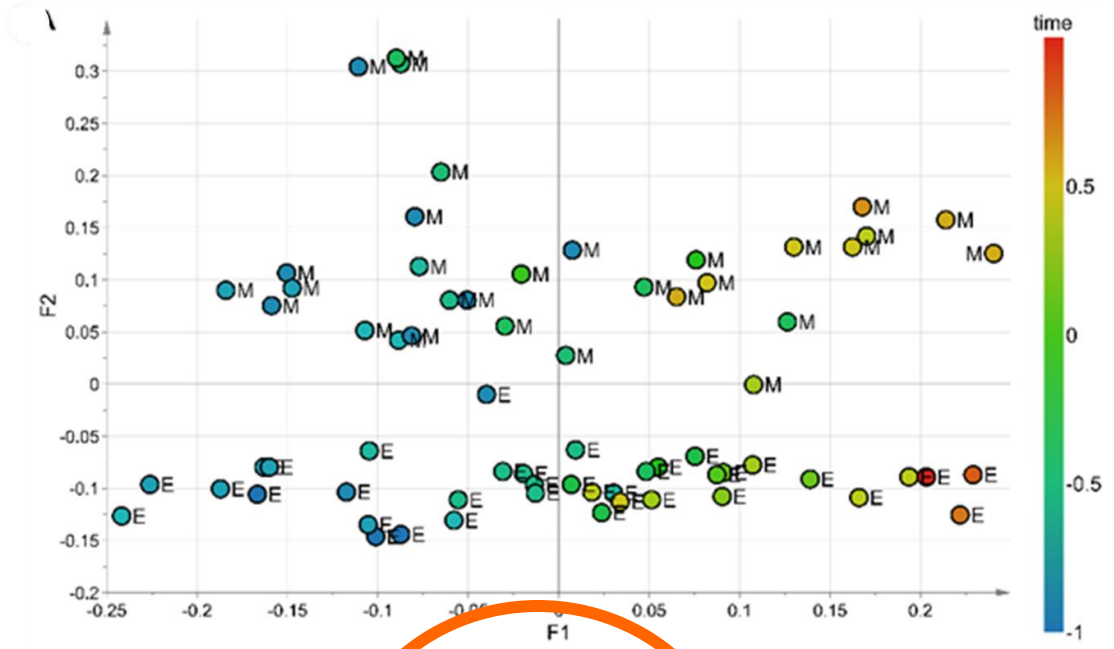
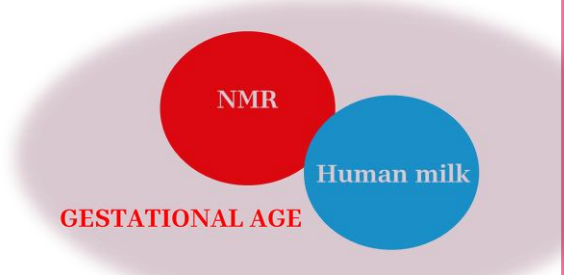
HMO: prebiotics, promoting the growth and activity of beneficial bacteria in the infant's gut ; role in supporting brain development

NMR

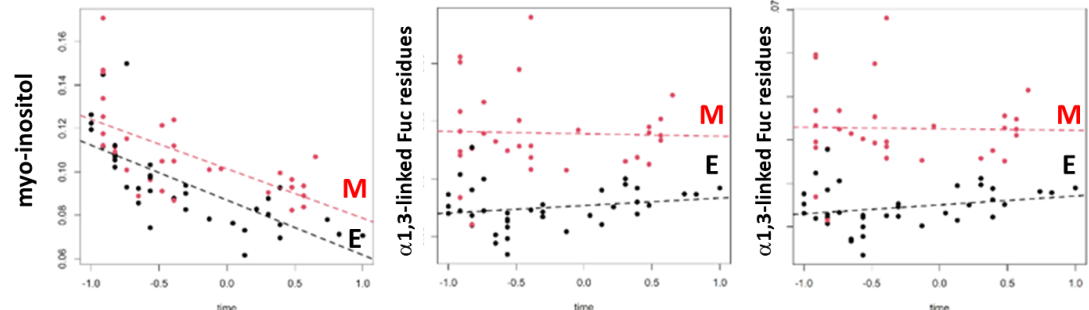
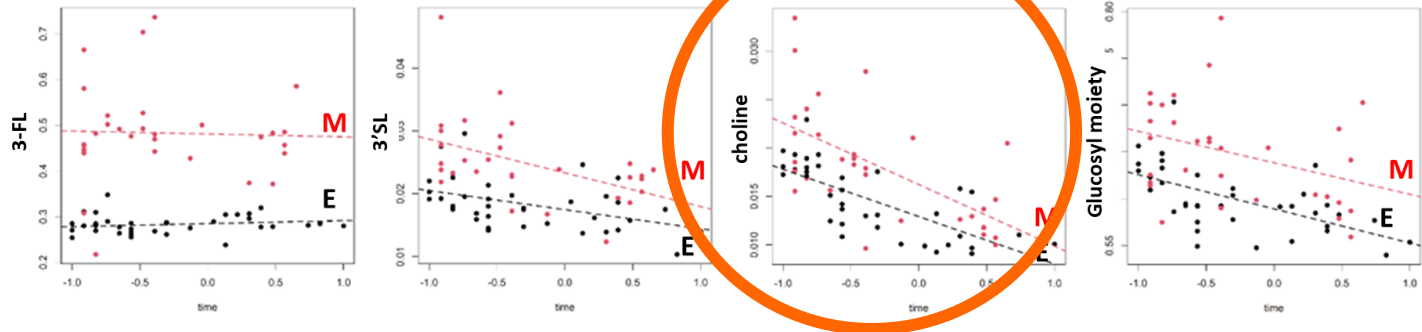
Human milk

GESTATIONAL AGE

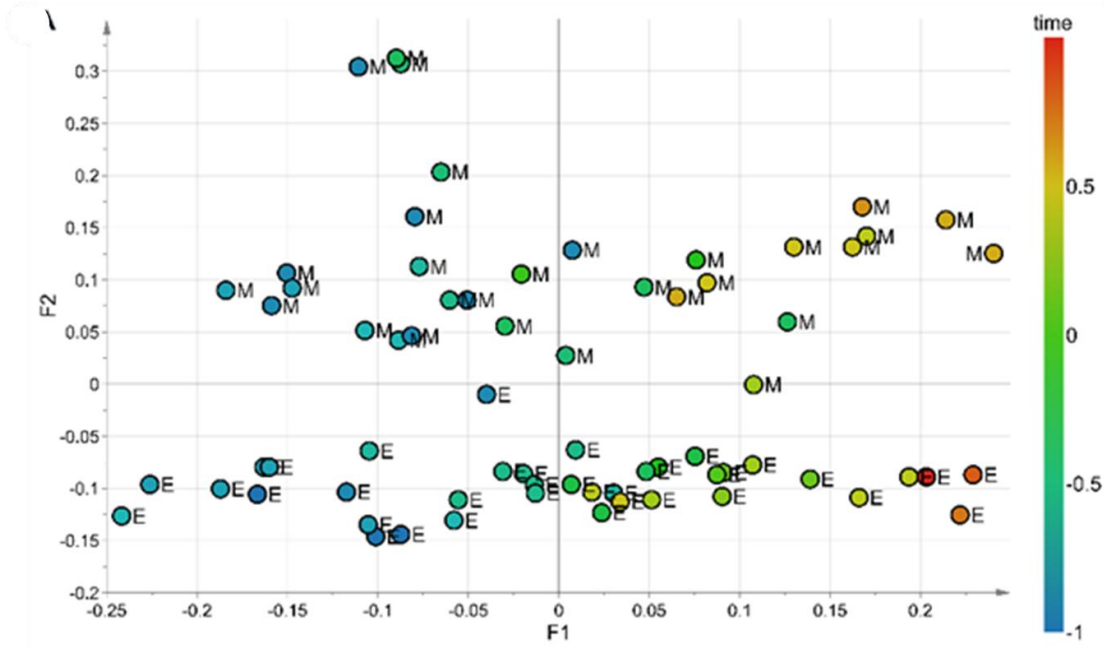
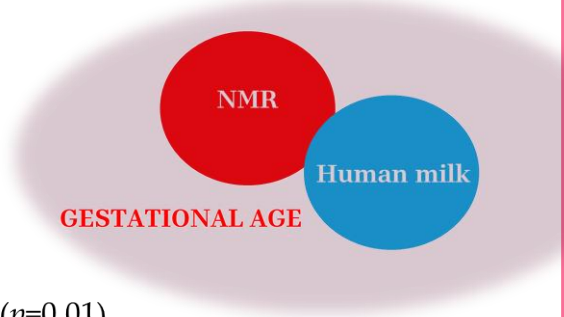




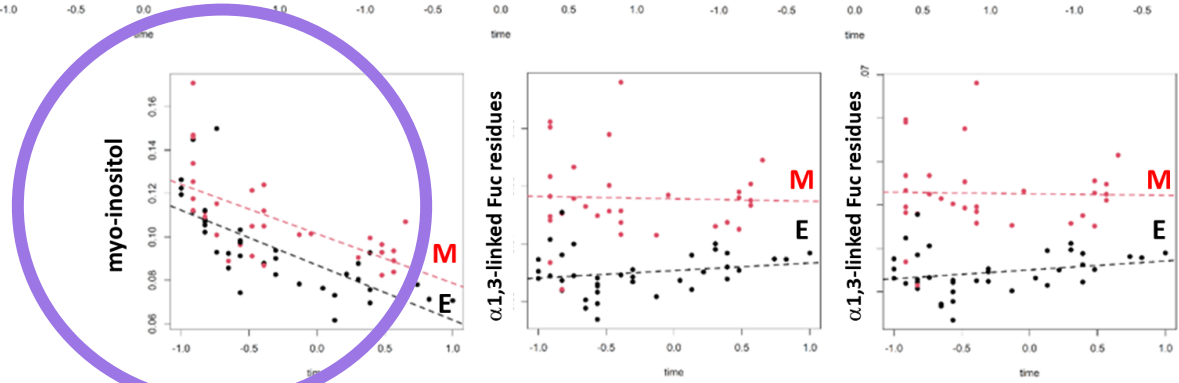
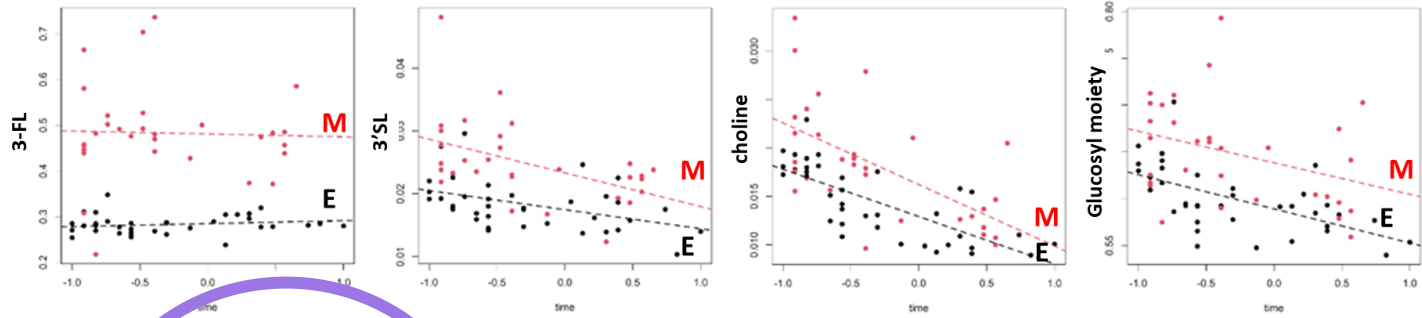
$R^2_{\text{time}}=0.76$ ($p=0.01$)
 $Q^2_{\text{time}}=0.40$ ($p=0.01$)
 $R^2_{\text{prematurity}}=0.80$ ($p=0.01$)
 $Q^2_{\text{prematurity}}=0.43$ ($p=0.05$)



- component of **phospholipids**, which are crucial for the structure and integrity of cell membranes
- a vital component in the synthesis of **acetylcholine**, a neurotransmitter essential for brain development and function



$R^2_{\text{time}}=0.76$ ($p=0.01$)
 $Q^2_{\text{time}}=0.40$ ($p=0.01$)
 $R^2_{\text{prematurity}}=0.80$ ($p=0.01$)
 $Q^2_{\text{prematurity}}=0.43$ ($p=0.05$)



- a structural component of cell membranes, contributing to their integrity and function
- role in improving insulin sensitivity and glucose metabolism



NMR

Human milk

NUTRITIONAL EFFECTS

Impact of Early Postnatal Nutrition on the NMR Urinary Metabolic Profile of Infant

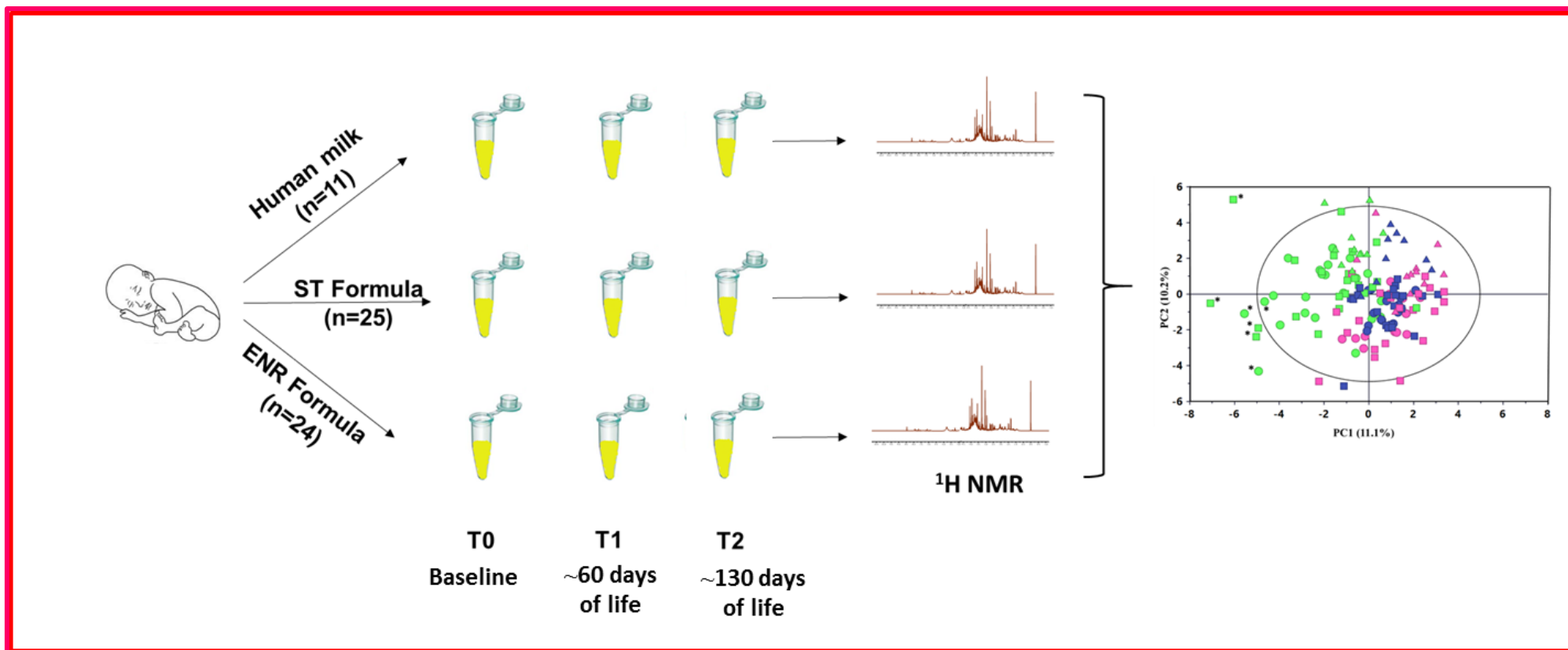
Flaminia Cesare Marincola,^{*,†} Sara Corbu,[†] Milena Lussu,[‡] Antonio Noto,[‡] Angelica Dessì,[‡] Stefania Longo,[§] Elisa Civardi,[§] Francesca Garofoli,[§] Beatrice Greci,[§] Elisa Mongini,[§] Andrea Budelli,^{||} Alessia Grinzato,[⊥] Francesca Fasano,[⊥] Vassilios Fanos,[‡] and Mauro Stronati^{§,#}

J. Proteome Res. 2016, 15, 3712–3723

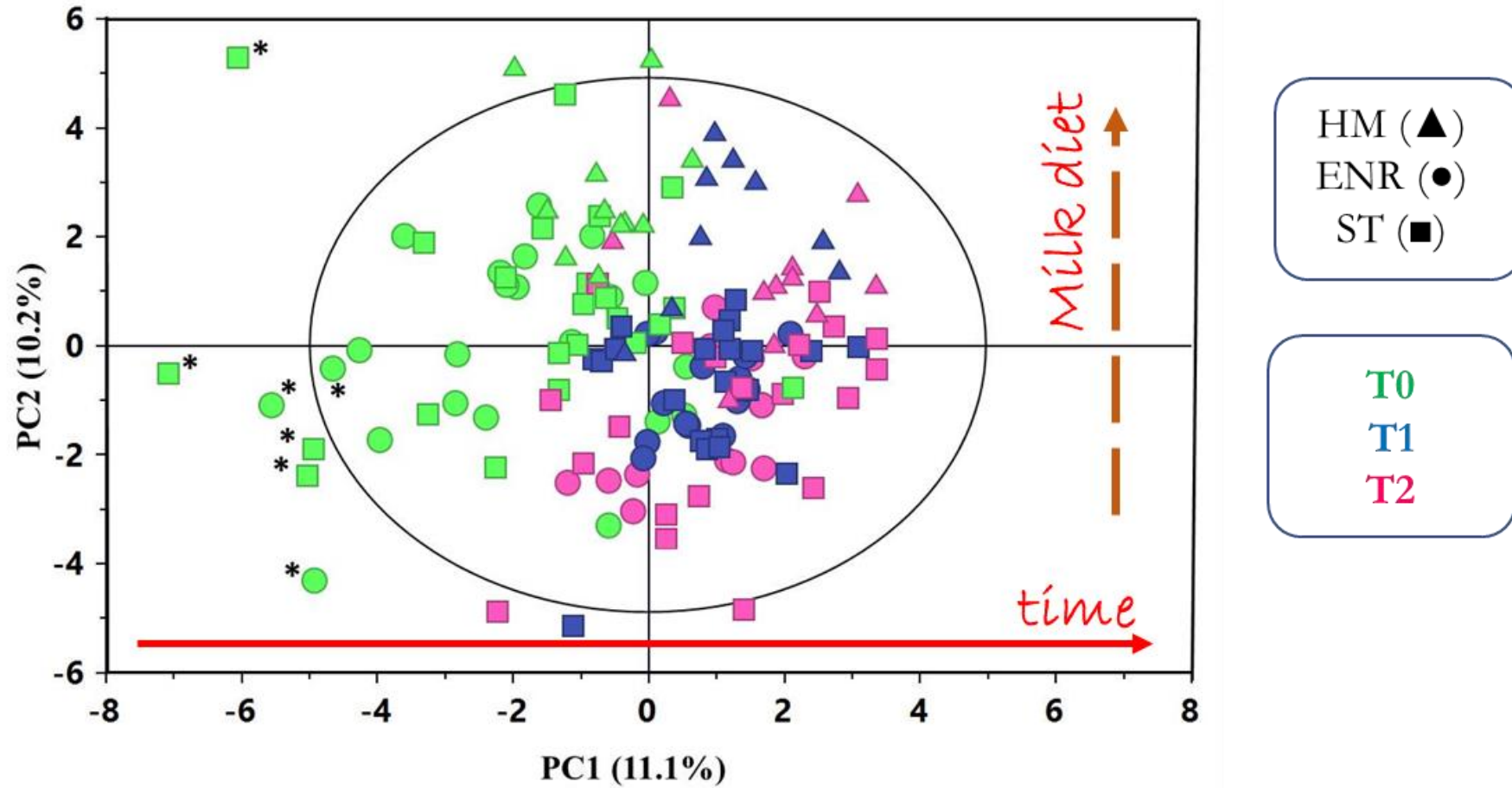
NUTRITIONAL EFFECTS

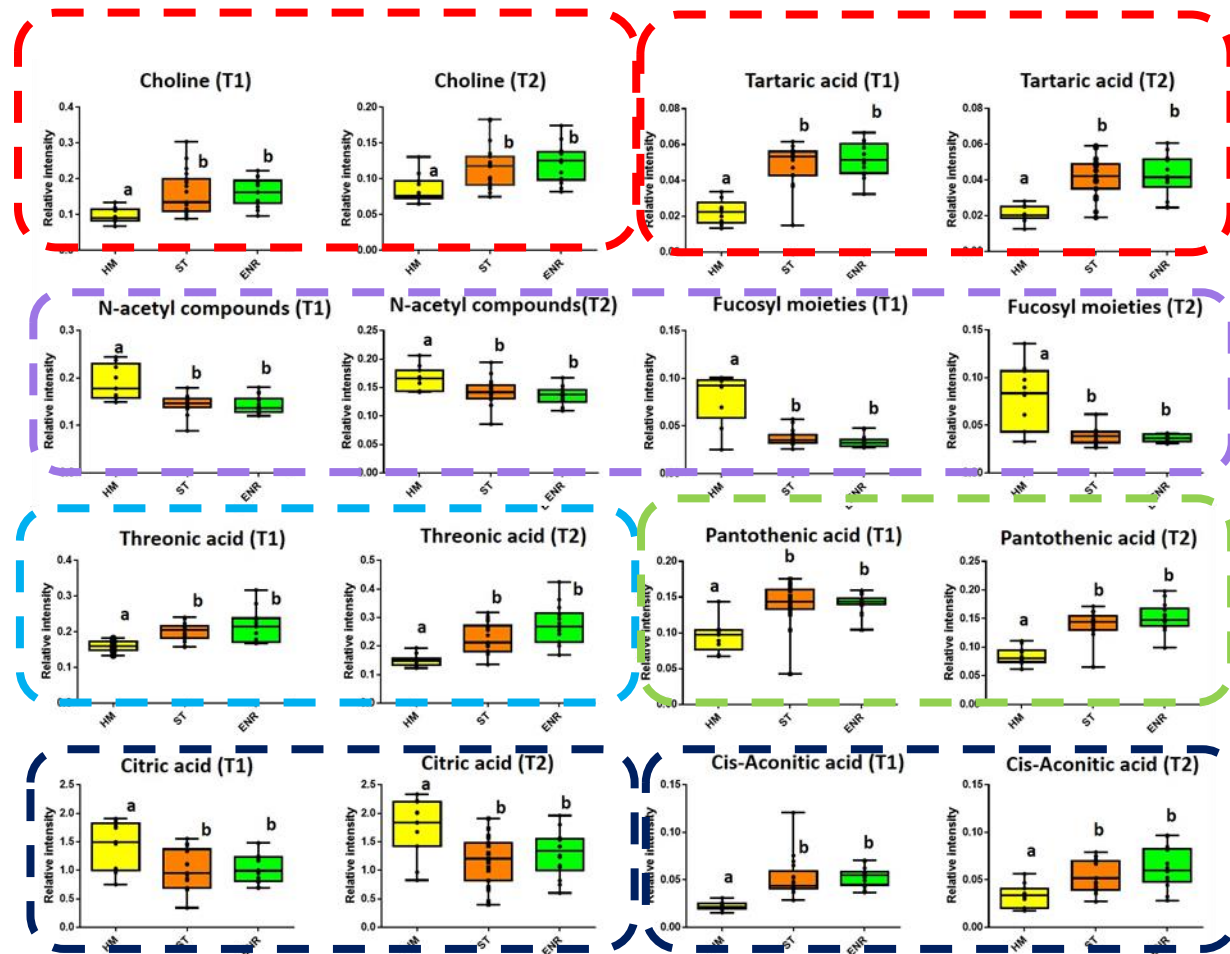
NMR

Human milk



PCA





Choline metabolism/dietary supply

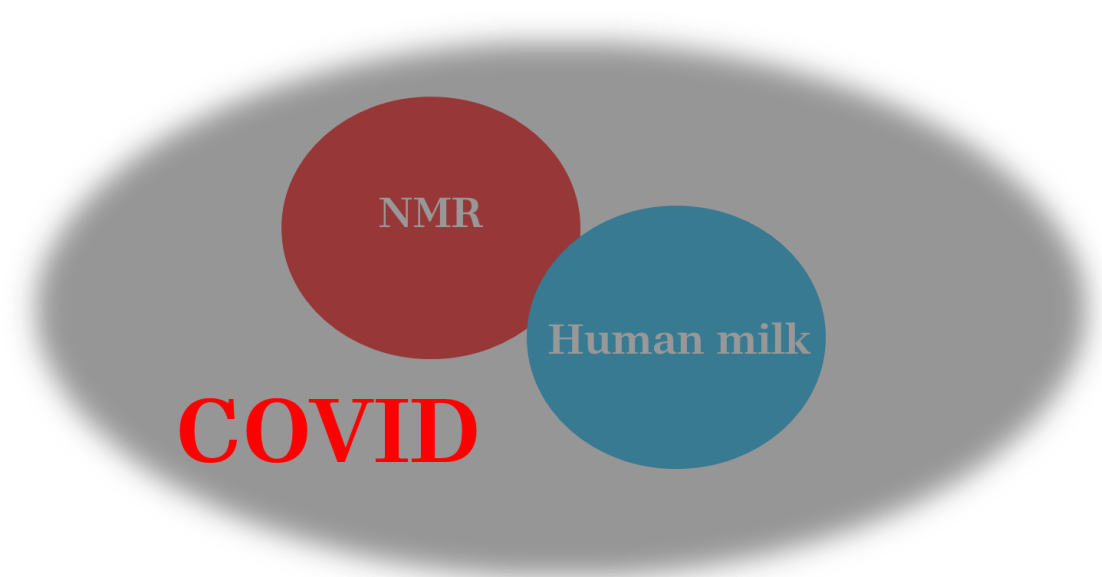
HMO
(prebiotic, anti-infective functions)

Threonic acid: from oxidation of ascorbic acid

Pantothenic acid: dietary supply/energy metabolism

TCA cycle activity

Letters labeled above the boxes denote no significant difference ($P > 0.05$) if they are identical and a significant difference ($P < 0.05$) if they are different.



Conclusions

- **Comprehensive Insight:** Metabolomics offers **a holistic view of the molecular composition of human milk**, providing a comprehensive understanding of its complex nature.
- **Dynamic Composition:** Enables **the identification and quantification of a diverse range of metabolites**, showcasing **the dynamic and variable nature of human milk composition**.
- **Nutritional Significance:** Facilitates **the exploration of nutritional components**, aiding in the elucidation of factors influencing infant health and development.
- **Biomarker Discovery:** Allows for **the discovery of potential biomarkers**, contributing to the identification of specific metabolic signatures associated with maternal and infant health.
- **Personalized Nutrition:** Paves the way for **personalized nutrition strategies** by uncovering individual variations in human milk composition, considering the unique needs of both mothers and infants.
- **Health Outcomes:** Provides insights into **the impact of maternal diet, lifestyle, and environmental factors** on human milk composition, ultimately influencing infant health outcomes.
- **Advancing Research:** Contributes to **advancing research in the field of lactation**, fostering a deeper understanding of the intricate interplay between maternal physiology and infant nutrition.

Thank you



Full Professor of Pediatrics, University of Cagliari.

- Director of Neonatal Intensive Care Unit, Azienda Ospedaliero Universitaria and University of Cagliari.