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Clinical impact of human breast milk metabolomics

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ABSTRACT

Metabolomics is a research field concerned with the analysis of metabolome, the complete set of metabolites in a given cell, tissue, or biological sample. Being able to provide a molecular snapshot of biological systems, metabolomics has emerged as a functional methodology in a wide range of research areas such as toxicology, pharmacology, food technology, nutrition, microbial biotechnology, systems biology, and plant biotechnology. In this review, we emphasize the applications of metabolomics in investigating the human breast milk (HBM) metabolome. HBM is the recommended source of nutrition for infants since it contains the optimal balance of nutrients for developing babies, and it provides a range of benefits for growth, immunity, and development. The molecular mechanisms beyond the inter- and intra-variability of HBM that make its composition unique are yet to be well-characterized. Although still in its infancy, the study of HBM metabolome has already proven itself to be of great value in providing insights into this biochemical variability in relation to mother phenotype, diet, disease, and lifestyle. The results of these investigations lay the foundation for further developments useful to identify normal and aberrant biochemical changes as well as to develop strategies to promote healthy infant feeding practices.

1. Introduction

It is known that human breast milk (HBM) is the only food able to satisfy all the needs of the newborn by bridging his requirements of nutrients for a correct somatic growth and by providing important functional factors whose role is performed through an enhancement of the processes of adaptation and organism growth [1]. Breast milk is a complex mixture of normal nutrients (such as carbohydrates, lipids, proteins, vitamins, and minerals) and various biologically active compounds and interactive elements (hormones, cytokines and chemokines, antimicrobial substances, etc.) that contribute to the benefic effects of breastfeeding [2]. The fascinating characteristic of HBM is the inter- and intra-variability of its composition [3]. Indeed, it changes from woman to woman and within an individual mother constantly during the entire lactation period, adapting to the needs of a growing child and potentially facilitating important physiological functions. In circumstances when breastfeeding is not possible or contraindicated, formula milk (FM) represents a safe and nutritionally adequate substitute to breast milk. The composition of infant formulae should serve to meet the particular nutritional requirements and to promote normal growth and development of the infants for whom they are intended. To this aim, since FM is usually made from cows' milk, whose composition differs widely from that of breast milk, dietary industries make use of various technological processes to make it suitable for babies. Accordingly, the formulation of products for the early childhood must follow the recommendations and standards issued proposed by the International Committees of Nutrition [4]. The variability in the ranges of nutrient levels established for the formulas destined to childhood contributes, together with the possible additions of other substances, to make extremely variable the composition of FM in commerce.

The term "metabolomics" is often used to define a scientific study that measures in a quantitative manner the metabolome, the complete set of low molecular weight metabolites in complex biological samples which are the end products of gene expression [5]. Since the metabolome can be viewed as a mirror that reflects the physiological, evolutionary, and pathological state of a biological system, metabolomics allows for a global assessment of a cellular state in relation with environment influences, particular physiological conditions, drug treatment, nutrition, lifestyle, genetic effects, etc. Metabolomics was initially applied in the fields of plant science [6] and toxicology [7], and has recently emerged as an important tool also in modern food science [8] and nutrition research [9], such as for the analysis of the chemical composition of food, the evaluation of the quality/authenticity of food products, and the monitoring of the use of nutrients in studies of nutritional interventions [10–13]. Indeed, with the introduction of powerful metabolomics platforms for the analysis of foodstuffs, the molecular understanding of food is rapidly increasing in areas aimed to assess what gives certain foods their unique flavor, texture, aroma, color, and nutritional properties.

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2. Human breast milk metabolome

To date, only a few metabolomic studies have been performed on the HBM metabolome (Table 1). To this purpose, both proton (^{1}H) nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) have being employed, providing to be incredibly useful in the chemical analyses of HBM and offering an overview of a wide range of compounds present in this food matrix [14,15]. Indeed, in combination with multivariate chemometric methods, NMR and MS techniques represent promising tools for metabolic fingerprinting of HBM and for highlighting the variations of metabolome linked to typical aspects such as mother phenotype, health, and diet.

The first metabolomic investigation of HBM was performed by Cesare-Marincola et al. in 2012 [16]. In that study, the authors tested the potential of this approach as a rapid and informative screening tool for investigating the composition of breast milk from mothers delivering a preterm low birth weight newborn during the first month of lactation. For the sake of comparison, some commercially available FM samples were also analyzed. The author attention, in particular, was focused on the hydrosoluble and fatty acid (FA) moieties that were analyzed by ^{1}H NMR spectroscopy and gas chromatography–mass spectrometry (GC–MS), respectively. Application of Principal Components Analysis (PCA) to the NMR spectra of the polar extracts of milk samples evidenced clear differences in the metabolic profiles of pre-term HBM and FM samples, the former being characterized by higher lactose concentrations, while the latter by higher levels of maltose. Significant differences in terms of oleic and linoleic acids were also observed between the two types of milk by the PCA analysis of the FA profile chromatograms.

A further comparison between HBM and FM has been recently performed by Longini et al. [17] that analyzed the water-soluble extract of milk samples collected within one week of delivery from mothers of preterm and term neonates. The comparison between the ^{1}H NMR spectra of breast milk samples with that of FM by using PCA evidenced that formula milk for premature infants was the most similar to breast milk of preterm babies. In addition, mother milk of very preterm babies (23–25 weeks of gestational age, GA) showed a different metabolic profile from preterm infants (GA ≥ 29 weeks) with a subsequent trend to similarity around the 30th week of post-natal age. Breast milk from preterm infants (29–34 weeks), collected up to 40 weeks of postnatal age, showed a temporal change over the first three weeks of lactation, approaching to zero with the achievement of term age.

The time-related variation of HBM metabolome composition within the first month post-partum was investigated by Villasenõr et al. using

Table 1
Summary of metabolomic studies on human breast milk (HBM).

Aim of study	Population studied	Sample type	Platform	Most variable metabolites[e]	Direction of magnitude variation	First author (year) Ref.
Comparison between the metabolic profiles of preterm HBM and formula milk (FM).	*Mothers delivering preterm infants (n = 20) *Mothers giving birth full-term infants (n = 1) *Formula milks commonly used for preterm infants (n = 13)	Hydrosoluble and liposoluble extracts[a]	^{1}H NMR GC-MS	Lactose Maltose Oleic, linoleic	↑ HMB[f] ↓ HMB[f] ↓ HMB[f]	Cesare Marincola F (2012) [16]
Influence of mother phenotype on HMO composition in HBM.	Mothers delivering term infants (n = 20)	Hydrosoluble extract[a]	^{1}H NMR	3′-FL, LNDFH II and derivatives	↑ Le-positive non-Secretors[g]	Praticò G (2014) [23]
Influence of maternal phenotype and diet on the HBM metabolome.	Mothers delivering term infants (n = 52)	Hydrosoluble extract[b]	^{1}H NMR	2′-FL, LDFT, LNFP I, fucose 3′-FL, LNFP II, LNFP III, LNT, 3′-SL, 6′-SL	↑ Secretors ↑ Non-Secretors	Smilowitz JT (2014) [24]
Development of a single-phase extraction method suitable for both GC–MS and LC–MS to characterize HBM over the first four months and characterization of differences in HBM composition within the first month post-partum	Mothers delivering term infants (n = 52)	Organic extract[c]	LC–MS GC–MS	Linoleic acid, palmitoleic acid, oleic acid, LPE, gluconic acid, hydroxyadipic acid, MG, DG, TG Lysolipids, phospholipids, α-tocopherol, cholesterol, CE, fucose, Furanose, D-glucosaminic acid	↑ At more than 26 days post-partum[h] ↓ At more than 26 days post-partum[h]	Villaseñor A (2014) [18]
Influence of chemotherapy on the microbiota and metabolome of HBM.	*Lactating woman undergoing chemotherapy for Hodgkin's lymphoma (n = 1) *Healthy lactating women (n = 8)	Alcoholic extract[d]	GC–MS	DHA, PUFA, inositol Arabinose, threitol, decanoic acid, myristic acid, 1-monopalmitin, Butanal	↓ In chemotherapy (weeks 2 to 16)[i] ↑In chemotherapy (weeks 2 to 16)[i]	Urbaniak C (2014) [19]
Comparison between the metabolic profile of HBM and formula milk (FM).	*Mothers delivering between 23 and 41 weeks of gestation (n = 20) *Formula milks recommended for newborn with different birth weight (n = 4)	Hydrosoluble extract[a]	^{1}H NMR	Lactose Galactose 1-phosphate and maltose	↑ HBM[f] ↓ HMB[f]	Longini M (2014) [17]

[a] Extraction solvent: choloform/methanol mixture.
[b] Samples were isolated from milk by filtration through 3000 molecular weight cutoff filter.
[c] Extraction solvent: methanol/methyl-tert-butyl ether mixture.
[d] Extraction solvent: methanol.
[e] List of abbreviation: CE, cholesteryl ester; DG, diglyceride; DHA, docosahexaenoic acid; 2′-FL, 2′-fucosyllactose; 3′-FL, 3′-fucosyllactose; LNDFH, lacto-*N*-difucohexaose; LDFT, lactodifucotetraose; LNFP, lacto-*N*-fucopentaose; LNT, lacto-*N*-tetraose; LPE, lysophosphatidylethanolamine; MG, monoglyceride; PUFA, polyunsaturated fatty acids; 3′-SL, 3′-sialyllactose; 6′-SL, 6′-sialyllactose; TG, triglyceride.
[f] Variation relative to formula milk.
[g] Variation relative to Le-positive Secretors.
[h] Variation relative to the first 7 days post-partum.
[i] Variation relative to week 0.

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