

Miracles and mysteries of breast milk: from Egyptians to the 3 M's (Metabolomics, Microbiomics, Multipotent stem cells)

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*“... Pray for us now
and at the hour of our birth.”*
T.S. Eliot, *Animula*

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Introduction

The ancient Egyptians considered breast milk to be extremely precious, miraculous, and capable to cure every disease. They believed that it was the nectar of Gods that could give life, strength and ensure a very long existence [1].

Nowadays, it is well known that breast milk is a dynamic bioactive mixture that is tailored upon the needs of the neonates, since it varies from one feed to another and the composition differs depending on the gestational age. The first study to investigate the metabolome of maternal milk, according to our knowledge, was performed by Cesare Marincola et al. in 2012 [2]. Breast milk contains nutritional substances (such as lipids, carbohydrates, proteins, vitamins and minerals), bioactive substances (such as hormones, cytokines, chemokines, immunoglobulins, leucocytes), and, according to the most recent research, bacteria (microbiome of maternal milk) and multipotent stem cells [3].

Therefore, due to its composition, breast milk plays a major role in the programming of the neonates; for instance, it improves the immune system, as seen for example in a significant lower incidence of necrotizing enterocolitis (NEC) in breastfed infants, and it ameliorates the long-term neurocognitive development. It can therefore be said that the first meal is as important as the first breath [4].

Moreover, several studies demonstrate that breast milk is beneficial even in newborns with neonatal abstinence syndrome (NAS): neonates fed with breast milk experience less severe NAS (its outcome is postponed) and need fewer pharmacological therapies [5]. Furthermore, according to Baudesson de Chanville et al., when it comes to preterm neonates, the odor of maternal milk is sufficient to exert an analgesic effect on these infants [6].

Metabolomics and breast milk: unraveling the mysteries

In order to understand the reasons why breast milk is like the food of Gods, i.e. the golden standard for neonatal feeding, investigators applied metabolomics to study this biofluid and its effects on neonatal metabolism.

Metabolomics is one of the newest “omics” sciences that make it possible to have a snapshot of the metabolic state of an individual through the analysis of body fluids such as urine, saliva or

blood. It is also possible to analyze the composition of human milk with techniques such as nuclear magnetic resonance (NMR), liquid or gas chromatography coupled with mass spectrometry (LC-MS or GC-MS).

The latest metabolomics studies concerning maternal milk confirmed the hypothesis that the composition varies in time after delivery, in particular in the first 3 months of lactation [7].

Metabolomics also allowed to observe that the metabolites levels differ among colostrum, transition milk and mature milk [8].

Furthermore, since preterm babies have different necessities with respect to at term neonates, it is amazing to see that the breast milk of mothers of preterm babies differs from that of full-term neonates, showing higher protein levels [9]. Besides, preterm milk metabolome varies in the 5-7 weeks post partum in order to become as much similar as possible to at term milk, independently of gestational time at delivery [10].

According to Dessì et al. and Acharjee et al., it is also possible to distinguish breastfed infants from those who receive formula milk through metabolomic analysis of neonatal blood and urine, [10, 11]. Acharjee and colleagues, in particular, set up a panel of biomarker of lipids metabolites that are peculiar of breastfed infants. On the other hand, Sachse et al. observed in 2014 that this technology allows identifying the mothers who breast fed from those who don't by the analysis of their urine [12].

The most recent metabolomics studies are summarized in **Tab. 1** [7, 8, 11-18].

Miraculous breast milk: the maternal milk microbiota

Human breast milk was mistakenly thought to be sterile for almost a century, but nowadays using the modern technologies it is well known that it is “contaminated”. In fact, investigators referring to the bacteria that can be found in breast milk talk about maternal milk microbiota. A breastfed baby is thought to ingest up to 10 millions of live bacteria per day. There are as much as 600 species and the most abundant genera are *Corynebacteria*, *Bradyrhizobiaceae*, *Streptococcus*, *Serratia*, *Ralstonia*, *Propionibacteria*, *Pseudomonas*, *Staphylococcus* and *Sphingomonas*, while *Bifidobacteria* and *Lactobacillus* are less abundant and less common [19]. Their origin is both inside the breast and the intestine of the mothers and/or outside. The mode of delivery is the event that mostly shapes the

Table 1. Most relevant metabolomics studies concerning human milk and its effects on metabolism published from 2014 to 2017 (continues on the next page) [7, 8, 11-18].

Authors	Patients/samples	Biofluids	Methods	Metabolite results	Biological hypothesis
Acharjee 2017 [11]	85 human milk, 87 formula, 67 mixed fed neonates at 3 months of age	Blood	H-NMR	Phosphocholine and sphingomyelins	Biomarkers for infant nutrition during early development that can be used to determine whether young infants (3-6 months) are breastfed or receive formula milk.
Qian 2016 [13]	30 human samples, 20 formula and 20 bovine samples	Milk	GC-TOFMS, UPLC-Q-TOF MS	↑ non esterified fatty acids in human milk ↓ TCA cycle intermediates in human milk	Human milk displays higher lipid content since lipids are a major energy source for infants and play several important physiological roles in their development.
Cesare Marincola 2016 [14]	11 breastfed, 24 enriched formula, 25 standard formula fed infants	Urine	NMR	Similar temporal trends of choline, betaine, myoinositol, taurine, and citrate	Choline and betaine showed similar temporal modifications in all three groups, since in the healthy infant there is a great excretion of betaine from birth that decreases in time. Taurine: the similarity is due to the fact that it is an essential metabolite; if it is absent, long-term developmental problems are possible. Thus, most manufacturers enriched their formula milks with it. The reason why there is an increase of citrate urinary content is still unknown.
Wu 2016 [7]	15 mothers, morning and evening samples on days 9, 12, 24, 31, 60, 85, 86 and 87 post-delivery	Milk	NMR	↑ lactose, choline, alanine, glutamate, and glutamine ↓ citrate, phosphocholine, glycerophosphocholine, and N-acetylglucosamine in late lactation stages	Milk metabolites content changes substantially not only during the colostrum and transition milk phases but even in the mature milk lactation phase. Lactose was the most abundant component and its levels tended to decrease in time, probably due to modifications in carbohydrate biosynthesis processes and the breakdown of oligosaccharides occurring in the mammary glands. Decrease in phosphocholine and glycerophosphocholine concentrations and the augmentation of choline are due to the necessities of the latest for a proper neonatal development. Glutamate and glutamine were the most abundant amino acids in human milk, important for neurodevelopment and proper colonization of gut by the microbiota. A significant decrease in citrate content in the late lactation stages could be due to the increased lactose secretion in order to maintain a constant osmotic pressure.
Sundekilde 2016 [15]	15 mothers of preterms, 30 mothers of term neonates	Milk	NMR	↑ valine, leucine, betaine, creatinine, in colostrum from term mothers compared with mature milk ↓ glutamate, caprylate, caprate in colostrum compared with mature milk at term ↑ oligosaccharides, citrate, creatinine increased in preterm colostrum ↑ caprylate, caprate pantothenate, valine, leucine, glutamate increase with time post-partum	Milk metabolites concentration is modified according to gestational age and lactation stage. The major modifications in preterm milk occur within 5-7 weeks post partum in order to be like the term milk, independently from time of gestation at delivery.

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Liu 2016 [16]	120 infants between 9 and 70 days of age (40 breastfed, 40 standard formula and 40 enriched formula fed neonates) vs rats	Human milk, neonatal feces, rat milk, plasma, cecal and colon tissue	NMR	↑ lactose, acetic acid ↓ fat, protein in human milk and feces	The fecal short-chain fatty acids (acetic acid concentration) in exclusively breastfed and in enriched formula fed babies are similar and they are biomarkers of microbial metabolism, while their concentration is lower in standard formula fed babies, indicating a different microbial activity.
Spevacek 2015 [8]	15 term neonates, 13 preterms	Colostrum, transition and mature milk	¹ H-NMR	↓ 3'-galactosyllactose, 2-hydroxybutyrate, methionine, acetoin in term milk	The metabolome of breast milk varies during the first month of lactation, and preterm milk seems to vary more than at term milk.
Andreas 2015 [17]	57 healthy term-born infants	Hindmilk between 2 and 80 days post-birth	LC-MS, GC-MS, CE-MS, ¹ H-NMR	↑ fucose and triacylglycerols, short-chain fatty acids during lactation	These metabolites increase in order to match the energy demand of the neonates.
Longini 2014 [18]	46 mothers at different GA from 23 weeks of gestation until term-equivalent age	Human milk, 4 formulas	MRS	↑ lactose ↓ maltose, galactose 1-phosphate in breast milk	Very preterm milk metabolome (23-25 weeks) is different from that of preterm neonates (≥ 29 weeks) but they become similar at around 30 weeks after delivery. Once the term age is reached there are no more modifications.
Sachse 2014 [12]	326 breastfed, 156 partially breastfed, 67 formula fed infants	Urine from mothers	NMR	Metabolites peculiar of breastfeeding mothers: lactose, creatinine, glycine and creatine	The breastfeeding mothers were distinguishable from those who didn't breastfeed.

TCA cycle: tricarboxylic acid cycle; GA: gestational age.

milk microbiota composition, i.e. the taxa present in the milk microbiota. It is interesting to note that urgent C-section shapes the microbiota more similarly to spontaneous delivery than programmed C-section; this is probably due to stress hormones. Another factor that can influence the microbiome is obesity or fast weight gain in pregnancy [20]. One of the physiological roles hypothesized for these microbial communities is to modulate and improve the immune system of neonates. In fact, breastfed infants are less susceptible of allergies, asthma, and even NEC [21]. Another role is that of favoring the colonization of neonatal guts. Indeed, according to Liu et al., breastfed neonates have different intestinal microbial activity with respect to those who are formula fed, and this might be due both to the composition of the milk and its microbiota [16].

An ace up its sleeve: human milk multipotent stem cells

Breast milk contains billions of non-immune cells and the first evidence of the presence of multipotent stem cells or progenitors-like cells came from Cergan et al. in 2007 [22]. A breastfed infant may ingest up to millions of maternal stem

cells per day. The most fascinating property of these cells is that they seem to have the ability to cross the intestinal barrier, survive in the main blood stream and reach several organs. Once they arrive at their destination they seem to proliferate and differentiate and integrate in the target tissue as another form of microchimerism. For instance, they appear to have the capability to cross the blood-brain barrier and differentiate into neurons, astrocytes and oligodendrocytes. In cell cultures studies they differentiate into adipogenic, chondrogenic and osteogenic lineages, while in animal studies they seems to differentiate into liver cells [23]. It also seems that stem cells differs in preterm milk compared to at term milk meaning that once again that the mother's milk is personalized to match the necessities of her baby. Nevertheless, their specific role need further investigations. The possible future applications of these discoveries are endless: developing a tailored regenerative medicine with fewer ethical problems and improved outcomes for patients.

Declaration of interest

The Authors declare that there is no conflict of interest.

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