The Metabolome of Breast Milk and Its Potential Long-Term Effects on the Child

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1 Introduction

Current recommendations suggest that the optimal way to feed a neonate is by breastfeeding alone. Human breast milk supplies the complete nutritional requirements of the rapidly growing and developing infant up to the age of 6 months [1]. In accordance with this view, the recommendations from both the World Health Organisation (WHO) and the United Nations Children's Fund (UNICEF) are for infants up to the age of 6 months to receive exclusively breast milk, with other foods being gradually introduced alongside continuing breast milk until the child's second birthday. The time from initial conception up to the second birthday, often referred to as the first 1000 days, is a vital period influencing the metabolic programming of the child thereafter. During the period for which the mother is lactating, the composition of breast milk alters significantly, which may reflect the changing nutritional needs of the child [2].

Breast milk has been the subject of intensive research for several decades now. It is known to contain both the macro- and micronutrients necessary for infant growth as well as other molecules which are biologically active. The constituents of breast

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milk supply the basic molecular building blocks for the child's development [3] and provide a suitable environment in the maturing gut for an optimal microbiome to establish [3–6].

2 The Breast Milk Metabolome

Metabolomic studies focus on the entirety of metabolites in a particular fluid. Human milk is one such fluid. The metabolites are typically of relatively low molecular mass. There have been studies of the composition of the metabolome, the intermolecular interactions and its relation to other factors, in particular gestational age, the stage of lactation and the health of the mother, including her nutritional intake, use of medication or illicit drugs. There has also been research focusing on metabolism in the infant, including metabolomic studies of the infant urine and faeces. The former is helpful in understanding the metabolic fate of milk constituents, whilst the latter provides information about microbial involvement in the overall handling of nutrients within the body [7–11].

Marincola et al. [12] were amongst the first to conduct metabolomic research into breast milk. This study relied on nuclear magnetic resonance (NMR) spectroscopy and gas chromatography-mass spectrometry techniques and was published in 2012. A more complete analysis was subsequently performed and the results published by Smilowitz et al. and Praticò et al., in 2013 and 2014, respectively [13, 14]. These last two studies both used NMR analysis and examined milk from mothers whose offspring were born at full-term. Smilowitz and colleagues analysed milk from mothers 90 days after lactation commenced and identified 65 different constituents. Praticò and colleagues used samples from mothers who had been breastfeeding for a month and identified 43 separate constituents. The constituents included a number of different amino acids, and metabolites thereof, substrates for energy generation, products of the citric acid cycle, short chain aliphatic acids, lactose and oligosaccharides. The component present in the largest quantity was lactose. Next in abundance were the human milk oligosaccharides, citric acid, urea and glutamate. It is generally agreed that the two key determinants responsible for the varied composition between breast milk from different mothers are the mother's Lewis gene type and secretor status. These differences are expressed by the number and structural type of fucosylated HMOs present in the sample [14–16].

There have also been metabolomic investigations comparing the metabolomes of breast milk and baby formula. Qian et al. [16] provide a detailed review of this research. By comparing milk in this way, it has been possible to discover how breast milk is unique. In particular, breast milk contains HMOs, decanoates, octanoates and aliphatic acids lacking esterification. Baby formula, in contrast, contains abundant sugars (fructose and glucose), benzamidoacetic acid, ethanoate, 2-oxopropanoate, phenylalanine and intermediate products of the citric acid cycle [16, 17].

Thus, in metabolomic terms, baby formula and breast milk are markedly different. The metabolomic studies comparing the consequences of breastfeeding versus use of baby formula have been reviewed twice [18, 19].

2.1 Breast Milk and Its Metabolome

In the initial months after birth, breast milk can supply the nutritional requirements of the child, whilst also nourishing a healthy gastrointestinal microbiome. Not only does milk supply the building blocks for the growing and developing infant, namely both the macro- and micronutrients, but it also delivers a range of cytokines and other immunologically active signals to the baby. These constituents are reviewed by Bardanzellu et al. [20] and Slupsky [21].

There is considerable variation in the composition of breast milk over the period of breastfeeding. Colostrum, for example, contains the highest levels of immunologically active molecules and oligosaccharides. Concentrations of these molecules reduce over time. There are also other compositional changes over the course of lactation, affecting amino acids, sugars, aliphatic acids and certain other molecules [21–25] (see Table 1).

Oligosaccharide levels follow a general trend of reduction over time, whereas lactose, a number of amino acids and free aliphatic acids of various lengths all tend to increase. Thus, milk does not consist of a fixed ratio of components. The regulation of which molecules are present and at what level lies with the breast tissue and depends on maternal genetic factors. The alterations in composition are continuous in response to the infant's changing need for particular metabolites and is instrumental in preparing the growth of a healthy microbiome, potentially throughout life. There is a much greater degree of similarity in the microbial flora and faecal metabolites of different infants fed artificial milk than between infants breastfed by different mothers. This dissimilarity stems from the differences in the contents of breast milk over the course of breastfeeding. The composition of artificial milk is fixed, however. The choice of breastfeeding or artificial milk seems to play a vital role in determining the eventual composition of the gut microbiome [20, 25].

Table 1 How the composition of milk varies at different stages of lactation in mothers with a secretor phenotype (adapted from reference [21])

	Molecules, the concentration of which rises during the period of breastfeeding	Molecules, the concentration of which falls during the period of breastfeeding	Molecules, the concentration of which exhibits no significant alteration during the period of breastfeeding
Sugars	Lactose 3-Fucosyllactose Glucose	2′-Fucosyllactose 3′-Galactosyllactose 3′-Sialyllactose 6′-Sialyllactose Fucose Lacto-N-tetraose Lacto-N-neotetraose Lacto-N-fucopentaose I Lacto-N-fucopentaose III Sialic acid Myoinositol	Galactose Lactodifucotetraose Lacto-N-fucopentaose II
Amino acids	Alanine Glutamate Glutamine Phenylalanine Threonine Valine	Leucine Lysine Proline	Asparagine Aspartate Histidine I Soleucine Methionine Tyrosine
Free aliphatic acids and derivatives thereof	Ethanone Azelaic acid Butyrate Carnitine Decanoate Octanoate	Ethanoate	
Glycolysis		Lactate pyruvate	
Intermediate products of citric acid cycle	*Cis*-Aconitate	Citric acid Fumarate Succinate	2-Oxoglutarate
Others	2-Aminobutyrate Choline Glycerophosphocholine Urea	Ascorbate Betaine Creatine Methanoic acid Benzamidoacetic acid Hypoxanthine phosphocholine Taurine	Pantothenate Methanol Creatine phosphate creatinine Ethanolamine Uridine

3 Human Milk Responds to the Needs of the Neonate

Breast milk has a unique composition exactly tailored to the requirements for growth in the neonate. When breast milk is substituted with milk from other species, this deeply affects the metabolism of the neonate. For instance, baby formula-fed

infants have raised circulating levels of amino acids and this high level potentially interferes with normal signalling by insulin [26]. The persistently high levels of plasma amino acids may prevent the mitochondria in the liver from functioning properly. This then potentially causes obesity, insulin insensitivity and dyslipidaemias [27].

The molecules within breast milk may also produce direct interactions with particular metabolic processes. An example is modulation of the action of the mechanistic target of rapamycin (mTOR) molecule, which guides optimal development [28]. mTOR forms a complex (mTORC1) which performs a kinase function depending on nutrient levels and regulates various processes affecting cellular growth, protein manufacture and lipid assembly, alongside deposition of fat within adipose tissues. MTORC1 is especially significant in regulating growth and metabolism of osseous tissues, voluntary muscle, the brain and spinal cord, gut, blood cellular components and certain other systems [29]. The amino acid level also controls the activity of mTORC1. The level of leucine in the whey portion of breast milk correlates with the circulating level of leucine in breastfed children [30]. In fruit flies (Drosophila spp.), the way nutrient levels can be detected by the TOR system is also affected by the presence of *Lactobacillus plantarum*, amongst other bacteria. A recent study found that *L. plantarum* may actually be passed from the mother to the infant during feeding [31, 32]. It seems likely, therefore, that the microbiome in the infant has an effect on the way the mTOR network functions, although this has not yet been decisively proven.

4 Metabolites Originating from the Microbiota at Different Times

The effect of breast feeding or bottle feeding at different points on how the gut flora metabolises amino acids with aromatic residues is another topic to be considered.

5 Being Bottle-Fed Results in More Secondary Metabolites of Bile Salts Produced by Microbial Action

There is a significant effect of being bottle-fed versus breastfed on the bile salts identifiable in faeces. Approximately 20 metabolites of bile salts have been detected, as well as cyprinol sulphate, the metabolic precursor. Bile acids exhibiting primary and secondary conjugation, as well as bound to glycine, taurine and sulphate moieties have been noted. The bile compound most strongly associated with breastfeeding is cyprinol sulphate, the faecal level of which rises up to the age of 7 months in exclusively breastfed infants. The molecule glycochenodeoxycholate is present in characteristic amounts depending on the type of feeding, as some bile acids are

conjugated to a sulphate group, such as sulphated chenodeoxycholate. Surprisingly, however, the metabolite lithocholate was barely detectable in faeces from infants below the age of 1 year. In any case, secondary bile metabolites are present in lower levels in children's faeces than in those of adults [33].

Infants who are breastfed have higher levels of 4-hydroxyphenyllactate and indolelactate in their faeces up to the age of 1 year. There is no difference in the levels of these compounds between infants who are exclusively breastfed and those who receive supplemental formula milk; however, children who are given additional supplementation with bifidobacteria have been shown to have higher levels. It has been noted in the literature already that the amino acids possessing an aromatic residue (phenylalanine, tyrosine and tryptophan) are metabolised by bifidobacteria to phenyllactate, 4-hydroxyphenyllactate and indolelactate, respectively [34–36]. Bifidobacteria represent the most important bacterial flora in children receiving breast milk. It is not clear what happens to the aromatic amino acids in the gut by assessing their concentrations in faeces, and this likely results from their having multiple metabolic fates. Phenyllactate stands out in this respect. The infant diet does not appear to affect its level, which always appears to increase as the infant gets older. One explanation for this is colonisation of the gastrointestinal tract by other bacterial genera that are also capable of converting phenylalanine to phenyllactate. In contrast, once weaning had begun at the second birthday, the levels of 4-hydroxyphenyllactate and indolelactate became virtually undetectable. Brink et al. have noted that tryptophan can be metabolised into kyneurate, indole-3-lactate and indole-3-ethanoate. These products were detected at higher levels in faecal samples from children receiving breast milk compared to those consuming dairy or soy milk formula [37].

Children aged between 1 and $1\frac{1}{2}$ years are already colonised by bacterial species capable of synthesising lithocholate by 7α-dehydroxylation of bile acids [38]. Indeed, according to Hammons et al., lithocholate can even be detected in faeces from 3 months old children, albeit the levels vary considerably [39]. It was more common in faecal samples from these very young children to identify other secondary metabolites of bile salts, such as 7-keto lithocholate, 3-dehydrochenodeoxycholate, Ursodeoxycholate, 7,12-dioxolithocholate, 7-ketodeoxycholate, 3-dehydrocholate and 7-epicholate. The existence of unexpected metabolites in faeces has already been noted by Lester et al., in reference to ursodeoxycholate [40]. Infants who received formula alone or with breast milk had higher faecal levels of 7-keto lithocholate, 3-dehydrochenodeoxy-cholate, Ursodeoxycholate and 7-epicholate. These metabolites may be indicative of a more varied gut microflora in children fed formula than in those exclusively fed breast milk [41]. Hammons *et al.* have also noted that secondary metabolites of bile salts were more abundant in infants receiving baby formula [39]. In research using young pigs, the faeces also contained raised amounts of the secondary metabolite, deoxycholate.

6 Conclusion

The microbial flora of the gastrointestinal tract, metabolism and the immune system all undergo important alterations in response to a person's diet. The fact that breast milk affects all these processes suggests that it is a highly evolved and sophisticated nutritional and communication system that exists to offer the neonate the greatest chance to be healthy. The alterations in the constituents of breast milk over the course of lactation are probably reflective of the infant's developing requirements and the evolutionary advantage of a particular type of gut flora. Metabolomics is an important type of phenotypical investigation and is vital in any research examining the metabolic consequences of changes in diet. Metabolomic studies on faeces help to establish the roles played by microbial gut flora. If values are established in healthy individuals, metabolomic analysis of peripheral blood, urine and faeces can be used to assess short- and long-term consequences of dietary interventions.

There is a need for further research on various aspects of breast milk on the child's growth and development. This research should also encompass metabolic programming and immunological effects. The health status of the mother and the effects of the environment also need to be factored in. In order to fully comprehend how a dietary change affects specific individuals and their microbiome, there is a need to also consider genetic and epigenetic aspects. The application of nutrigenomics and microbiomics together will elucidate how milk and the various compounds it contains modulate genetic expression in the infant, either directly or via microbial activity. This is an ambitious aim, and will take many more years, but ultimately such research can only lead to a much more effective use of dietary manipulations to promote health.

References

1. Poulsen KO, Sundekilde UK. The metabolomic analysis of human milk offers unique insights into potential child health benefits. Curr Nutr Rep. 2021;10(1):12–29. https://doi.org/10.1007/s13668-020-00345-x. Epub 2021 Feb 8.
2. Ballard O, Morrow AL. Human milk composition. Nutrients and bioactive factors. Pediatr Clin N Am. 2013;60:49–74.
3. Pacheco AR, Barile D, Underwood MA, Mills DA. The impact of the milk glycobiome on the neonate gut microbiota. Annu Rev Anim Biosci. 2015;3:419–45.
4. Kunz C, Rudloff S, Baier W. Oligosaccharides in human milk: structural, functional, and metabolic aspects. Annu Rev Nutr. 2000;20:699–722.
5. Ten-Doménech I, Ramos-Garcia V, Piñeiro-Ramos JD, Gormaz M, Parra-Llorca A, Vento M, et al. Current practice in untargeted human milk metabolomics. Meta. 2020;10:43.
6. Sundekilde UK, Downey E, O'Mahony J, O'Shea C-A, Ryan C, Kelly A, et al. The effect of gestational and lactational age on the human milk metabolome. Nutrients. 2016;8:304.
7. Spevacek AR, Smilowitz JT, Chin EL, Underwood MA, German JB, Slupsky CM. Infant maturity at birth reveals minor differences in the maternal milk metabolome in the first month of lactation. J Nutr. 2015;145:1698–708.

8. Shoji H, Shimizu T. Effect of human breast milk on biological metabolism in infants. Pediatr Int. 2019;61:6–15.
9. Martin FP, Sprenger N, Montoliu I, Rezzi S, Kochhar S, Nicholson JK. Dietary modulation of gut functional ecology studied by fecal metabonomics. J Proteome Res. 2010;9:5284–95.
10. Nicholson JK, Holmes E, Kinross J, Burcelin R, Gibson G, Jia W, et al. Host-gut microbiota metabolic interactions. Science (80-). 2012;336:1262–7.
11. Urbaniak C, McMillan A, Angelini M, Gloor GB, Sumarah M, Burton JP, et al. Effect of chemotherapy on the microbiota and metabolome of human milk, a case report. Microbiome. 2014;2:2.
12. Marincola FC, Noto A, Caboni P, Reali A, Barberini L, Lussu M, et al. A metabolomic study of preterm human and formula milk by high resolution NMR and GC/MS analysis: preliminary results. J Matern Fetal Neonatal Med. 2012;25:62–7.
13. Smilowitz JT, O'Sullivan A, Barile D, German JB, Lönnerdal B, Slupsky CM. The human milk metabolome reveals diverse oligo- saccharide profiles. J Nutr. 2013;143:1709–18.
14. Praticò G, Capuani G, Tomassini A, Baldassarre ME, Delfini M, Miccheli A. Exploring human breast milk composition by NMR-based metabolomics. Nat Prod Res. 2014;28:95–101.
15. Dessì A, Briana D, Corbu S, Gavrili S, Marincola FC, Georgantzi S, et al. Metabolomics of breast milk: the importance of phenotypes. Meta. 2018;8:8. https://doi.org/10.3390/metabo8040079.
16. Qian L, Zhao A, Zhang Y, Chen T, Zeisel SH, Jia W, et al. Metabolomic approaches to explore chemical diversity of human breast-milk, formula milk and bovine milk. Int J Mol Sci. 2016;17:17. https://doi.org/10.3390/ijms17122128.
17. Scano P, Murgia A, Demuru M, Consonni R, Caboni P. Metabolite profiles of formula milk compared to breast milk. Food Res Int. 2016;87:76–82.
18. Phan M, Momin SR, Senn MK, Wood AC. Metabolomic insights into the effects of breast milk versus formula milk feeding in in- fants. Curr Nutr Rep. 2019;8:295–306. https://doi.org/10.1007/s13668-019-00284-2.
19. Li K, Jiang J, Xiao H, Wu K, Qi C, Sun J, et al. Changes in the metabolite profile of breast milk over lactation stages and their relationship with dietary intake in Chinese women: HPLC-QTOFMS based metabolomic analysis. Food Funct. 2018;9:5189–97.
20. Bardanzellu F, Fanos V, Reali A. "Omics" in human colostrum and mature milk: looking to old data with new eyes. Nutrients. 2017;9:843.
21. Slupsky CM. Metabolomics in human milk research. Nestle Nutr Inst Workshop Ser. 2019;90:179–90. https://doi.org/10.1159/000490305. Epub 2019 Mar 13.
22. Munblit D, Peroni DG, Boix-Amorós A, et al. Human milk and allergic diseases: an un- solved puzzle. Nutrients. 2017;9:894.
23. Smilowitz JT, O'Sullivan A, Barile D, et al. The human milk metabolome reveals diverse oligosaccharide profiles. J Nutr. 2013;143:1709–18.
24. Spevacek AR, Smilowitz JT, Chin EL, et al. Infant maturity at birth reveals minor differ- ences in the maternal milk metabolome in the first month of lactation. J Nutr. 2015;145:1698–708.
25. Sundekilde UK, Downey E, O'Mahony JA, et al. The effect of gestational and lactational age on the human milk metabolome. Nutrients. 2016;8:E304.
26. Hyde R, Taylor PM, Hundal HS. Amino acid transporters: roles in amino acid sensing and signalling in animal cells. Biochem J. 2003;373:1–18.
27. Morán-Ramos S, Ocampo-Medina E, Gutierrez-Aguilar R, et al. An amino acid signature associated with obesity predicts 2-year risk of hypertriglyceridemia in school-age children. Sci Rep. 2017;7:5607.
28. Melnik BC. Milk—a nutrient system of mammalian evolution promoting mTORC1-dependent translation. Int J Mol Sci. 2015;16:17048–87.
29. Semba RD, Trehan I, Gonzalez-Freire M, et al. Perspective: the potential role of essential amino acids and the mechanistic target of rapamycin complex 1 (mTORC1) pathway in the pathogenesis of child stunting. Adv Nutr. 2016;7:853–65.

30. Melnik BC. Excessive leucine-mTORC1-signalling of cow milk-based infant formula: the missing link to understand early childhood obesity. J Obes. 2012;2012:197653.
31. Storelli G, Defaye A, Erkosar B, et al. Lacto-bacillus plantarum promotes drosophila systemic growth by modulating hormonal signals through TOR-dependent nutrient sensing. Cell Metab. 2011;14:403–14.
32. Sillner N, Walker A, Lucio M, Maier TV, Bazanella M, Rychlik M, Haller D, Schmitt-Kopplin P. Longitudinal profiles of dietary and microbial metabolites in formula- and breastfed infants. Front Mol Biosci. 2021;8:660456. https://doi.org/10.3389/fmolb.2021.660456.
33. Huang CTL, Rodriguez JT, Woodward WE, Nichols BL. Comparison of patterns of fecal bile acid and neutral sterol between children and adults. Am J Clin Nutr. 1976;29:1196–203. https://doi.org/10.1093/ajcn/29.11.1196.
34. Beloborodov NV, Khodakova AS, Bairamov IT, Olenin AY. Microbial origin of Phenylcarboxylic acids in the human body. Biochem Mosc. 2009;74:1350–5. https://doi.org/10.1134/s0006297909120086.
35. Beloborodova N, Bairamov I, Olenin A, Shubina V, Teplova V, Fedotcheva N. Effect of phenolic acids of microbial origin on production of reactive oxygen species in mitochondria and neutrophils. J Biomed Sci. 2012;19:89. https://doi.org/10.1186/1423-0127-19-89.
36. Aragozzini F, Ferrari A, Pacini N, Gualandris R. Indole-3-lactic acid as a tryptophan metabolite produced by Bifidobacterium Spp. Appl Environ Microbiol. 1979;38:544–6. https://doi.org/10.1128/aem.38.3.544-546.1979.
37. Brink LR, Mercer KE, Piccolo BD, Chintapalli SV, Elolimy A, Bowlin AK, et al. Neonatal diet alters fecal microbiota and metabolome profiles at different ages in infants fed breast Milk or formula. Am J Clin Nutr. 2020;111:1190–202.
38. Eyssen H. Role of the gut microflora in metabolism of lipids and sterols. Proc Nutr Soc. 1973;32:59–63. https://doi.org/10.1079/pns19730016.
39. Hammons JL, Jordan WE, Stewart RL, Taulbee JD, Berg RW. Age and diet effects on fecal bile acids in infants. J Pediatr Gastroenterol Nutr. 1988;7:30–8. https://doi.org/10.1097/00005176-198801000-00008.
40. Lester R, Pyrek JS, Little JM, Adcock EW. Diversity of bile acids in the fetus and newborn infant. J Pediatr Gastroenterol Nutr. 1983;2:355–64. https://doi.org/10.1097/00005176-198302020-00026.
41. Bazanella M, Maier TV, Clavel T, Lagkouvardos I, Lucio M, Maldonado-Gòmez MX, et al. Randomized controlled trial on the impact of early-life intervention with bifidobacteria on the healthy infant fecal microbiota and metabolome. Am J Clin Nutr. 2017;106:1274–86.