

# Metabonomics in neonatal nutrition research

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*From the womb to the adult*

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## Abstract

Maternal obesity and early post-natal nutrition might associate with increased obesity risk in later life. We have investigated the effect of breastfeeding and infant formulas differing in protein content on the urinary and fecal metabolism of term infants born from overweight and obese mothers using a metabonomic approach. Metabolic differences were observed between breast and formula fed infants both in urine and stool samples. Metabolic profiles of formula fed infants exhibited a distinct metabolic pattern that was associated with the processing of dietary proteins from the host and the gut microbiota. Metabonomics appears as a powerful tool to measure the physiological response to infant formula versus the gold standard breastfeeding. In future, nutritional phenotyping will combine metabonomics and nutritional profiling to study specific nutritional requirements and measure the efficacy of tailored nutritional interventions on growth and development endpoints. It will then open novel opportunities to develop targeted nutritional solutions for health maintenance and disease prevention.

## Keywords

Metabonomics, breastfeeding, formula feeding, nutritional phenotyping.

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## Perinatal nutrition: a window opportunity for investing on health potential

Immediately after birth, the neonate is submitted to a series of complex physiological and metabolic processes to adapt to extra-uterine life and acquire the needed functions for proper growth and development. It is thought that the first one thousand days are key to provide the neonate with the metabolic set-points that will determine health status later in life. Nutrition, ideally delivered through breastfeeding in a first time and later on by weaning foods, has a key role to play to fulfil the nutritional requirements for water, macro-, and micronutrients. From an evolutionary perspective, breast milk can be considered as one of the most evolved nutritional matrix due to its unique composition and structure that have resulted from constant Darwinian selective pressure to ensure infant survival and through this the entire reproduction of the species. Up to today, breastfeeding is considered the gold standard nutrition for infants of healthy mothers. Infant formulas have been developed on the concept of mimicking the physiological response to human milk intake. On another hand, the rise of maternal obesity [1] points towards a plausible link with increased incidence of overweight and obesity in childhood. Indeed, neonates have shown increased adiposity and heavier weight when born from overweight or obese mothers relatively to infants of lean women. This observation was related to possible increased risk for obesity and impaired metabolic health in adolescents. In such a context, the understanding of how perinatal nutrition can modulate healthy growth becomes of paramount importance to contribute reducing the burden of overweight, obesity and associated metabolic disorders later in life. Yang et al. have reviewed associations between perinatal nutrition with later

obesity from childhood through adulthood [2]. By doing so, it was highlighted that adulthood obesity associated with insufficient prenatal dietary intakes of energy, protein and micronutrients and that breastfeeding, together with timely introduction of complementary feeding, showed a protective effect to later obesity. However, obesity and increased body fat mass were linked to high-protein nutrition in early childhood. Yet, proteins are essential to sustain organ growth and ensure metabolic functions. We have investigated the effect of three feeding regimens including breastfeeding (control), low-protein formula (LF, 1.65 g protein/100 kcal formula) and high-protein formula (HF, 2.7 g protein/100 kcal formula) on the metabolism of infants from the age of 3 months onwards and born to overweight or obese mothers using the metabonomic analysis of urine and stool samples [3].

## Nutrimetabonomics as a systems biology approach to understanding mammalian metabolism

Metabonomics studies the concentration changes of the endpoints of physiological regulatory processes, the metabolites, in biological samples such as blood plasma, urine and stool samples. The approach is based on the exploration of high dimension metabolic profiles using sophisticated univariate and multivariate statistical data analysis methods, or a combination of both, with the aim to extract metabolic features that are differently expressed between the investigated conditions. As biological fluids carry metabolic information on the whole system, metabonomics owns the potential to depict the ultimate phenotypic expression that captures biochemical processes resulting from multiple biological determinants including genetics, meta-genetics (gut microbiota), nutrition, lifestyle, and the complex molecular networks between them. Nutritional metabonomics offers thus unique scientific perspectives to underpin the molecular processes linking dietary nutrients and key physiological processes [4]. Of particular interest is the ability of the metabonomics approach to shed light into the complex molecular interactions between the host metabolism and the functional ecology of the gut symbiotic partners, i.e. the gut microbiota [5, 6]. For these reasons, metabonomics is increasingly gathering scientific interest in neonatal and pediatric research due to its inherent potential to open a systems biology

window using a relatively simple and non-invasive approach when analyses are carried out on urine or stool samples [7, 8]. By doing so, it provides an unprecedented research means to study developmental physiology, metabolic programming, and nutritional requirements. Various analytical approaches are nowadays well established for the measurement of metabolites in biological samples. They can roughly be classified into untargeted, i.e. with no *a priori* selection of measured metabolites, and targeted approaches. Untargeted methods are dominated by high resolution Nuclear Magnetic Resonance spectroscopy, mainly proton-based ( $^1\text{H}$  NMR), and mass spectrometry techniques. So-called targeted approaches are usually chosen to deliver quantitation of specific metabolites using tandem mass spectrometry coupled to high resolution liquid chromatography for instance. Here, we sought to capture metabolic variations relative to the feeding modes (breastfeeding, LF, HF) of infants born from overweight and obese mothers using  $^1\text{H}$  NMR analysis of urine and stool samples.

#### **Metabolic effects of breast, low and high protein formula feeding in urine and stool samples**

Inspection of urinary  $^1\text{H}$  NMR profiles with supervised multivariate statistical analysis, i.e. orthogonal projection to latent structure discriminant analysis (O-PLS-DA), showed significant metabolic variations according to age and the type of feeding. Metabolic profiles were significantly different at 1 year of age and between breast and formula fed infants at 3 and 6 months. Furthermore, results indicated that time associated metabolic differences between 3 and 6 months were relatively less pronounced in samples from breastfeeding group. Metabolite set enrichment analysis, a method used to detect coordinated concentration changes of metabolites within metabolic pathways, was performed to facilitate data interpretation. Significantly highlighted pathways included proteins, amino acids, ketone bodies and fatty acid oxidation. Statistical analysis of stool  $^1\text{H}$  NMR spectra revealed variations overtime, with more pronounced changes observed at 1 year. The metabolic profiles significantly varied in formula fed groups when switching from started formula at month 3 to high and low protein formulas at month 6. Furthermore, stool metabolic profiles varied between formula fed and breastfeeding groups at 3 and 6 month but not at

1 year. Interpretation of O-PLS-DA coefficients revealed that high concentration of fucosylated oligosaccharides and lactate differentiated the stool metabolic profiles of breast fed infants. The formula fed groups were characterized by higher concentrations of proteolytically derived short-chain fatty acids (propionate, butyrate, acetate, 5-amino-valerate) as well as free amino acids (phenylalanine, tyrosine, leucine, isoleucine). These metabolic findings suggest either increased proteolytic activity or excess of amino acid intake in formula fed infants.

The deployment of metabonomics in this clinical study has thus contributed to shed new light onto the physiological regulatory processes related to different infant nutrition during the first year of life. The study highlighted several metabolic differences, particularly relative to protein metabolism, between formula fed infants relatively to breastfeeding. It illustrates the potential of metabonomics to measure physiological equivalence of formula feeding with the gold standard breastfeeding. However, leveraging the displayed metabolic differences consecutive to the feeding regimen into potentially novel formulation that aims at further mimicking the physiological response to breastfeeding would require a deeper investigation on how systemic metabolites, which are conventionally measured by metabonomics, correlate with nutritional status and nutrient intakes of the infant. The recently developed nutritional profiling approach [9] opens new research opportunity to measure the nutritional status at the molecular level through the quantitation of a broad range of nutritional organic and inorganic nutrients and micronutrients in biological samples. Integrated with metabonomics, it provides a molecular linkage between nutrient intake and the systemic metabolic footprint in response to nutrition intervention. Therefore, nutritional profiling increments metabonomics with a new dimension that enables to explore the cross-talk between nutritional phenotype, nutrient intake, host genetics, symbiotic partners and lifestyle factors. Interestingly, such an integrated approach can be extended to the mother's nutrition and health before, during and after pregnancy. Deployed both in observational and nutrition intervention studies, this next generation of nutritional phenotyping is foreseen to deliver new quantitative knowledge on the specific nutritional requirements of individuals. As such, this integrated approach will become instrumental to build the scientific foundations of

future targeted nutrition that will aim at matching specific nutritional requirements of individuals to promote healthy growth and development, and thus to prevent the onset of nutrition-associated metabolic disorders later in life.

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### Declaration of interest

SR, FPM, SM, IM, SC, LDS and MK are employed by the for-profit Nestlé Institute of Health Sciences. PS is employed by the for-profit Nestlé Health Sciences.

### References

1. Sebire NJ, Jolly M, Harris JP, Wadsworth J, Joffe M, Beard RW, Regan L, Robinson S. Maternal obesity and pregnancy outcome: a study of 287,213 pregnancies in London. *Int J Obes Relat Metab Disord.* 2001;25:1175-82.
2. Yang Z, Huffman SL. Nutrition in pregnancy and early childhood and associations with obesity in developing countries. *Matern Child Nutr.* 2013;9(Suppl 1):105-19.
3. Martin F-P, Moco S, Montoliu I, Collino S, Da Silva L, Rezzi S, Prieto R, Kussmann M, Inostroza J, Steenhout P. Impact of breastfeeding and high- and low-protein formula on the metabolism and growth of infants from overweight and obese mothers. *Pediatr Res.* 2014;75(4):535-43.
4. Rezzi S, Ramadan Z, Fay LB, Kochhar S. Nutritional metabolomics: applications and perspectives. *J Proteome Res.* 2007;6(2):513-25.
5. Nicholson JK. Global systems biology, personalized medicine and molecular epidemiology. *Mol Syst Biol.* 2006;2:52.
6. Martin FP, Collino S, Rezzi S, Kochhar S. Metabolomic applications to decipher gut microbial metabolic influence in health and disease. *Front Physiol.* 2012;3:113.
7. Moco S, Collino S, Rezzi S, Martin FP. Metabolomics perspectives in pediatric research. *Pediatr Res.* 2013;73(4 Pt 2):570-6.
8. Antonucci R, Atzori L, Barberini L, Fanos V. Metabolomics: the “new clinical chemistry” for personalized neonatal medicine. *Minerva Pediatr.* 2010;62:3(Suppl 1):145-8.
9. Rezzi S, Collino S, Goulet L, Martin F-P. Metabonomic approaches to nutrient metabolism and future molecular nutrition. *TrAC – Trends in Analytical Chemistry.* 2013;52:112-9.