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Nutrimetabonomics: nutritional applications of metabolic profiling

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ABSTRACT

An individual's metabolic phenotype, and ultimately health, is significantly influenced by complex interactions between their genes and the diet. Studying these associations and their downstream biochemical consequences has proven extremely challenging using traditional hypothesis-led strategies. Metabonomics, a systems biology approach, allows the global metabolic response of biological systems to stimuli to be characterised. Through the application of this approach to nutritional-based research, nutrimetabonomics, the biochemical response to dietary inputs is being investigated at greater levels of resolution. This has allowed novel insights to be gained regarding intricate diet-gene interactions and their consequences for health and disease. In this review, we present some of the latest research exploring how nutrimetabonomics can assist in the elucidation of novel biomarkers of dietary behaviour and provide new perspectives on diet-health relationships. The use of this approach to study the metabolic interplay between the gut microbiota and the host is also explored.

Keywords: *nutrimetabonomics, nutrition, gut microbiota, metabolic profiling*

Glossary

Exposome: The exposome has been first described as the "life-course environmental exposures (including lifestyle factors), from the prenatal period onwards"[1]. Here we use a more limited definition that excludes all endogenous processes. We consider the exposome to encompass only exposure to external molecules and distinguish it from exposure to external stresses referred to as "lifestyle".

Phenotype: The set of observable characteristics of an individual.

Genotype: The set of genes

Homeostasis: The maintenance of a physiological equilibrium.

Metabolome: The full set of metabolites produced by a biological system (cell, organ, multi cellular organism).

Metabolomics: The measurement of metabolites concentrations, fluxes and secretions in cells and tissues in which there is a direct connection between the genetic activity (gene expression), protein activity (proteome) and the metabolic activity itself[30].

Metabonomics: "The quantitative measurement of the dynamic multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification"[31].

Mass spectrometry: An analytical technique that measures the mass and concentration of molecules based on the detection of ionised particles.

Nuclear magnetic resonance: An analytical technique that measures the absorption of electromagnetic radiation by a nucleus in an external magnetic field.

Pattern recognition: Learning machine and statistical tools that enable detection of common features within a set of observations.

1. The challenge to monitor metabolic homeostasis

An individual's phenotype is the product of elaborate interactions between their genes and the environment. These environmental factors include those related to lifestyle (*i.e.* physical activity, psychological, and other external stimulations) and a set of pressures collectively termed the exposome**.** Although the exposome was first defined by Wild as the "life-course environmental exposures (including lifestyle factors), from the prenatal period onwards"[1], we consider here a more refined definition that excludes all endogenous processes. In this context, the exposome encompasses only exposure to exogenous molecules, such as those originating from the diet or other external sources

such as environmental pollutants, drugs, and the biochemical outputs of indigenous microorganisms (Figure 1). These inputs and their interactions with the genome add another layer of biocomplexity to the mammalian biological system and can have an important influence on an individual's phenotype, and ultimately health. To unravel the complexity of biological systems and investigate the impact of exposome–genome interactions, powerful systems biology approaches are being increasingly applied. Such interactions occur at all tiers of the gene–protein–metabolite cascade but the end-point is a set of metabolites collectively termed the metabolome. This metabolome contains the substrates and products of multi-faceted cellular activity and provides a measure of the overall metabolic status of the individual. This reflects the effort of the system to maintain metabolic homeostasis, which is achieved by modulating gene transcription, protein expression and enzymatic activity (Figure 1).

Metabolic profiling, also known as metabolomics, applies modern molecular phenotyping tools to assess such metabolic balance. Using untargeted quantification of

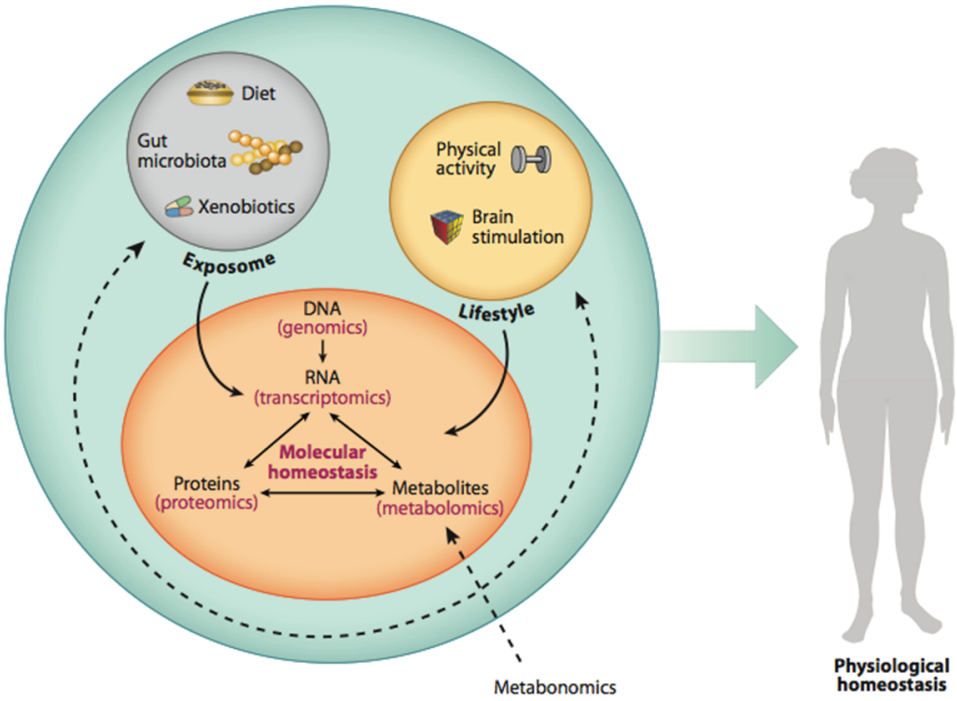

Figure 1 *The molecular homeostasis of an organism is the product of intricate interactions between RNA, proteins, and metabolites. This biological system is constantly shaped by environmental pressures that can be classified into two main categories: the exposome and lifestyle. The former includes all external factors, such as diet, gut microbial activity, and xenobiotics, including food pollutants, to which an individual may be exposed. The latter includes physical activity, brain stimulation, and any emotional stress. All of these elements need to be well balanced to achieve physiological homeostasis. By measuring the metabolic fingerprint of this system, metabonomics provides an accurate assessment of the global metabolic state of an organism, providing a cumulative measure of all interactions. Solid arrows indicate the impact of environmental factors on the organism, whereas dashed arrows indicate what can be directly or indirectly measured using metabonomics. From Claus and Swann[2], reproduced with permission.*

small molecular weight metabolites, it provides a snapshot of the metabolic status. The distinction is made between metabolomics and metabonomics when repeated distinct measures of several biological replicates are combined with multivariate statistics, also known as pattern recognition tools. This latter approach enables metabolic responses to perturbations or stimuli to be studied. This systems biology approach has been successfully applied to various research fields, from plant and agricultural science to research on human diseases. In particular, it has proved extremely useful in nutrition research where diet-induced modulations can result in subtle metabolic effects that would otherwise escape detection by traditional hypothesis-led approaches. Indeed, the power of the metabolic profiling approach lies in its departure from the restrictive identification of a single biomarker, but rather a multitude of various markers (*i.e.* metabolites) that in combination indicate the fluctuations in an individual's metabolic signature. This holistic profiling strategy bypasses the need for specific hypotheses, shaped by existing knowledge, and instead asks open questions allowing novel perspectives to be gained.

Two dominant analytical platforms are currently used to comprehensively evaluate the metabolome. These are liquid chromatography or gas chromatography coupled with mass spectrometry (LC-MS and GC-MS, respectively) and ^{1}H nuclear magnetic resonance (NMR) spectroscopy. A detailed description of the use of these analytical platforms for nutrimetabonomics studies falls outside the scope of this mini review but they are described by Claus and Swann[2].

2. Nutrimetabonomics in practice

The number of nutrimetabonomic studies is steadily growing and there are many successful examples of metabolic profiling being applied to nutrition research. One valuable nutrimetabonomic application has been to discover novel nutritional biomarkers that can evaluate eating patterns in free-living populations. This offers an attractive alternative to traditional questionnaire-based approaches to monitor dietary behaviour, as these subjective methods are susceptible to reporting bias. In contrast, these biomarkers provide an objective measure for accurately monitoring dietary intake, in turn improving our ability to investigate causal relationships between diet and health. In one study, Heinzmann *et al.* used a NMR-based nutrimetabonomic approach to identify urinary proline–betaine as a reliable marker of citrus fruit consumption[3]. This was later confirmed by targeted MS detection of proline–betaine and its derivatives[4]. Recent studies have corroborated these findings with flavanone glucuronides also identified as markers of citrus fruit consumption[5]. A similar ^{1}H NMR spectroscopy-based approach identified urinary *S*-methyl-ι-cysteine sulfoxide and its related metabolites as biomarkers of cruciferous vegetable intake[6]. Given the inverse association between cruciferous vegetable intake and many human diseases, an accurate biomarker of intake has potential to improve the real-world study of these components and our understanding of the mechanisms behind their beneficial properties. Large-scale population studies have also proven useful for extracting biochemical fingerprints of dietary intake. Characterisation of the urinary metabolic phenotypes from the large-scale INTERMAP (INTERnational collaborative study of Macronutrients, micronutrients and blood Pressure) study found that individuals with a high fish intake excreted greater amounts of trimethylamine-*N*-oxide (TMAO) than those with a high meat intake. TMAO is an oxidative product of trimethylamine found in large concentrations in fish[7]. Consistent with this finding, TMAO excretion has

also been observed following consumption of smoked salmon in addition to anserine and 1-methylhistidine[8].

The biomolecular impact of nutritional components has also been studied using nutrimetabonomics (extensively reviewed here[2,9]). This includes the biochemical perturbations associated with the intake of various food groups including lean and fatty-fish intake[10], whole-grain cereals[11], and vegetarian, low- and high-meat diets[12] and specific dietary components such as polyphenols, flavonoids, and isoflavones[13–16]. Using nutrimetabonomics in a piglet study, the importance of early-life nutritional events on the metabolic system has been highlighted. Here, different weaning diets (soya- or egg-based) were observed to differentially modulate the urinary metabonome of piglets. Interestingly, these early metabolic alterations persisted even after both groups were transferred to a fish-based diet[17].

Nutrimetabonomics has proven particularly effective in studying the metabolic functionality of the gut microbiome and its biochemical dialogue with the host. The importance of the gut microbiome in defining host health is now well recognised and metabolic profiling has been extensively applied in animal models to study gut microbiota-host interactions[18–21]. Indeed, the urinary output of the mammalian host contains products of both microbial metabolism, including the short chain fatty acids, and microbial-host co-metabolism, such as hippurate and phenylacetylglutamine[22]. The diet is known to be a major factor in shaping the gut microbiota and subsequently, the global host metabolic system[23]. A recent study integrated molecular microbial profiling (DNA-based metagenomic sequencing) and metabolic phenotyping to better understand the role of these commensal microorganisms in the context of undernutrition[24]. Here, Smith *et al.*[24] showed in a population of Malawian twin pairs discordant for kwashiorkor (a symptom of severe acute malnutrition), that the gut microbiota interact with the local diet to trigger severe weight loss. The metabolic profiles of these individuals revealed that the activity of the gut microbiota was altered during treatment, particularly in the biochemistry of amino acids. This suggests that modulation of gut microbial functionality, rather than modification of the microbial ecosystem *per se*, is involved with recovery. This highlights the need to consider the gut microbiota, along with the diet, to restore an individual back to their optimal metabolic disposition (Figure 2).

Conversely, metabolic profiling has been applied to characterise the consequences of a high fat diet on mammalian biochemistry[25]. A recent study feeding obese individuals with the prebiotic inulin correlated plasma and urinary metabolic profiles with the gut microbiota[26]. Although the prebiotic did not significantly reduce body weight in this study, it was found to induce systemic modulations in energy metabolism. Interestingly, metabolic signatures associated with obese individuals and those with type I diabetes contain gut microbial-host co-metabolites indicating variation in microbe-host relationships with these phenotypes[27,28]. In another metabonomic study, the protective effect of two fermentable carbohydrates (inulin and β-glucan) on weight gain in mice fed high-fat diets was studied. Both prebiotics were found to reduce body weight gain but different mechanisms were identified. Appetite regulation was modified in those mice fed β-glucan while a greater effect on adipose tissue was observed in those receiving inulin. Examination of the faecal metabolic profiles revealed that both prebiotics resulted in the greater excretion of energy metabolites, amino acids, and lactate. These findings suggest a lower utilization of energy-related compounds reflecting greater faecal energy loss with both prebiotics[29].

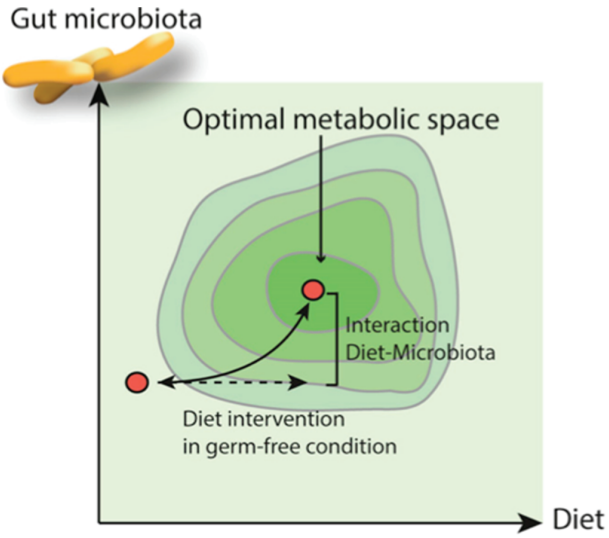

Figure 2 *Interaction between gut microbiota and diet in the context of undernutrition. Diet and gut microbiota are two interdependent parameters that are crucial for an individual's health, which is determined by his metabolic status (red dots). A healthy situation is obtained when the metabolic status moves around an optimal metabolic space, represented at the top of a topographic map (dark green). The more an individual drifts from his optimum, the more he tends toward disease. Although other factors (*e.g.*, genes, pathogens, stress,* etc.*) can be critical, the interaction between diet and gut microbiota needs to be considered in addition to a dietary intervention alone in order to maintain homeostasis and long-term health.*

3. Nutrimetabonomics in the future

As shown by this brief mini-review, nutrimetabonomics is an emerging field that has experienced a growth in success over the last few years. This can partly be explained by the increasing interest in personalised nutrition. To achieve a tailored diet, one must be able to evaluate the global parameters that affect an individual's metabolism in response to environmental modulation, including nutritional variation. This represents a major challenge for the 21st century, especially as we aim at targeting large-scale populations. This can only be achieved using a relatively simple method to measure the output of the sum of all these parameters on the metabolism and monitor the individual's metabolic homeostasis. In combination with other profiling tools, the so-called 'omics' technologies, we will be able to decipher the mechanisms involved and achieve an improved nutrition for a long-term health.

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