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Defining Personal Nutrition and Metabolic Health Through Metabonomics

S. Rezzi, F-P.J. Martin, S. Kochhar(✉)

BioAnalytical Science, Metabonomics and Biomarkers, Nestlé Research Center,
PO Box 44, 1000 Lausanne 26, Switzerland
email: *sunil.kochhar@rdls.nestle.com*

Abstract. A major charter for modern nutrition is to provide a molecular basis for health outcome resulting from different food choices and how this could be designed to maintain individual health free of disease. Nutrigenomic techniques have been developed to generate information at various levels of biological organization, i.e. genes, proteins, and metabolites. Within this frame, metabonomics targets the molecular characterization of a living system through metabolic profiling. The metabolic profiles are explored with sophisticated data mining techniques mainly based on multivariate statistics, which can recover key metabolic information to be further linked to biochemical processes and physiological events. The power of metabonomics relies on its unique ability to assess functional changes in the metabolism of complex organisms stemming from multiple influences such as lifestyle and environmental factors. In particular, metabolic profiles encapsulate information on the metabolic activity of symbiotic partners, i.e. gut microflora, in complex organisms, which represent a major determinant in nutrition and health. Therefore, applications of metabonomics to nutrition sciences led to the nutrimetabonomics approach for the clas-

sification of dietary responses in populations and the possibility of optimized or personalized nutritional management.

1 Metabonomics Technology Set-up

High-resolution proton nuclear magnetic resonance (^{1}H NMR) spectroscopy is an efficient and non destructive tool for generating data on a multitude of metabolites in biofluids or tissues. However, it is inherently less sensitive than mass spectrometry. When coupled to a liquid chromatography system, mass spectrometry (MS) provides a rapid platform for metabolite profiling at a concentration range of a few nM to pM. With the advent of ultraperformance liquid chromatography (Acquity UPLC system, Waters, Milford, MA, USA) combined with a time-of-flight mass spectrometer (MicroMass LCT-primers, Waters, Milford, MA, USA) equipped with an electrospray interface, complementary data to ^{1}H NMR profiling can be generated in 15–30 min per sample, thus enlarging the metabolite window for biomarker extraction (see Fig. 1 for a typical metabonomic analytical platform). The acquired spectral profile of a biofluid such as urine, plasma or saliva reflects the metabolic status of a living organism. Information recovery, in terms of relationships between the NMR, MS spectral profiles and their biochemical interpretation, can be maximized by applying multivariate statistical tools to analyze the information-rich spectroscopic data (see Fig. 2 for a typical data workflow). ^{1}H NMR and/or MS spectroscopy of complex biological mixtures combined with multivariate statistical analysis provides better visualization of the changing endogenous metabolite profile in response to physiological challenge or stimulus such as a disease process, administration of a xenobiotic, environmental stress, genetic modification, changes in nutrition and other physiological effects.

2 Nutrimetabonomics

Over the past decade, metabonomics has been shown to be a powerful tool to assess metabolic effects associated with toxicological insults (Coen et al. 2007; Ebbels et al. 2007; Skordi et al. 2007; Yap et al.

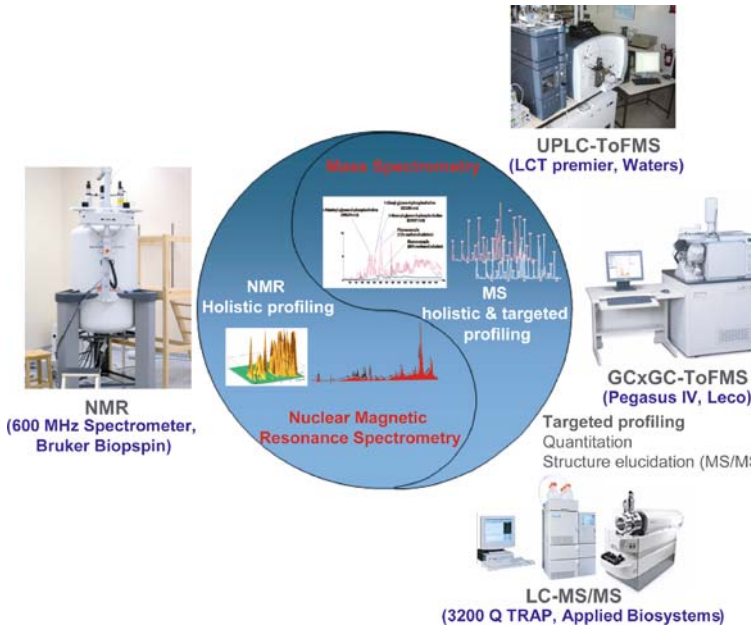

Fig. 1. Schematic drawing describing typical NMR- and MS-based metabonomic analytical platform

2006) and pathophysiological states (Brindle et al. 2002, 2003; Odunsi et al. 2005; Wang et al. 2005; Yang et al. 2004). Nutrimetabonomics provides a systems approach to characterize metabolic health and phenotypes of individuals. An individual human phenotype is determined by a complex interplay between genes, environmental and lifestyle factors, as well as intestinal symbionts (Gavaghan et al. 2004; Martin et al. 2007a; Nicholson et al. 2004; Nicholson and Wilson 2003). Recently, nutrimetabonomic strategies were successfully applied to classify dietary responses in populations and personalized nutritional management (Rezzi et al. 2007a).

Application of metabonomics to nutrition sciences, i.e. nutrimetabonomics, attempt to decipher metabolic effects of specific ingredients or diets in healthy human populations. This makes the task of nutrimetabonomics even more challenging than pharmaceutical or clinical metabo-

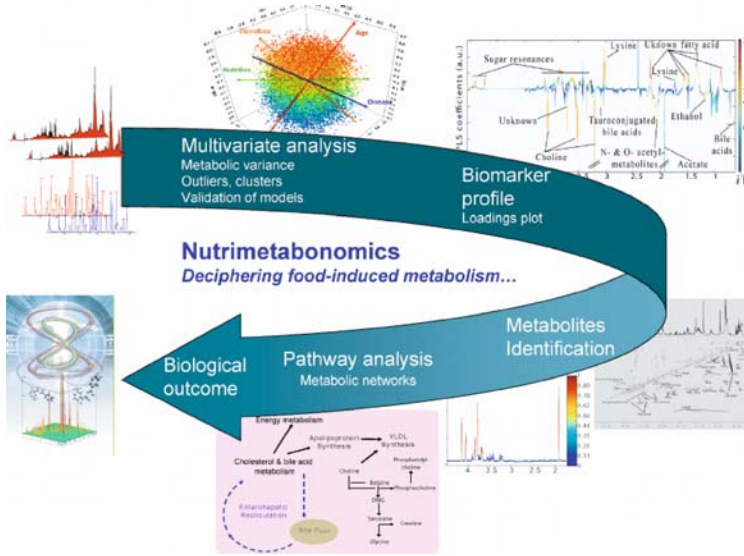

Fig. 2. Schematic description of a typical metabonomics data analysis workflow

nomics, since food-induced metabolic modulations appear as a result of complex interactions between the food and consumer metabolomes. In nutrition, effects indeed cannot be reduced to the effect of a single active molecule and are of low amplitude when compared to toxicological and clinical stressors. An additional source of complexity is attributable to inherent intra- and inter-individual variability that is reflected in the metabolism as a consequence of circadian rhythms, ovarian cycle, genetic polymorphism, different gut microflora activity, environmental and lifestyle components, and age. These variability sources, seen as confounding factors, can led to artifactual interpretation and therefore need to be controlled through an appropriate experimental design. In terms of analytical strategy, nutrimetabonomics requires well-established technical approaches most of the time based on NMR spectroscopy and MS in combination with multivariate statistics (Lindon et al. 2006).

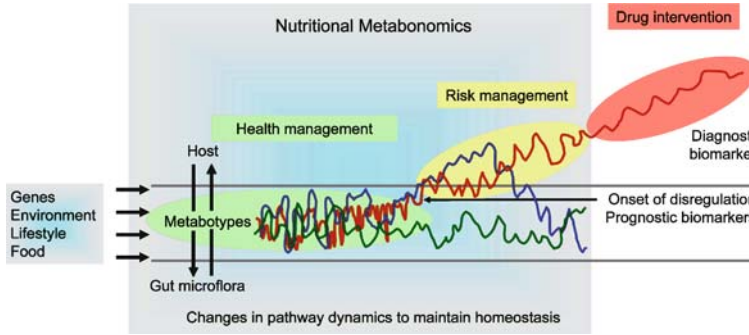

Fig. 3. Schematic representation of nutritional metabonomics concept. Different individual metabolic phenotypes (represented by *colored lines*) are under homeostatic controls maintaining metabolic variability within a healthy range (*green line*). Metabolic profiling will enable the prediction of individual susceptibility to develop well-defined diseases and optimizing nutrition for health maintenance and to restore homeostasis, as illustrated by the *blue line*. (Reproduced with permission from Rezzi et al. 2007a)

The sheer complexity of the web of interactions between host, gut microorganisms and the complex food metabolome is an important determinant to the response of the organism to a stressor or intervention that can easily be captured using metabolic profiling (Fig. 3). We have exemplified this nutrimetabonomic concept in a recent study where spectroscopically generated metabolic phenotypes obtained from healthy human volunteers were correlated with behavioral or psychological dietary preferences (Rezzi et al. 2007b). Dietary preferences and habits, which are predominantly cultural in origin, affect the health of both individuals and populations. In this study, we reported that metabolic profiling of urine and plasma samples revealed differential profiles for lipoproteins, basal energy metabolism and human-gut–microbial metabolic interactions. The observed metabolic imprinting findings provide evidence for a link between specific dietary preferences and metabolic phenotypes in both human basal metabolism and gut microbial activity, which in turn may have long-term health consequences.

These data suggested that gut microbial metabolism in humans may be modulated by the diet more than previously thought, which was previously suggested in previous studies. For instance, the changes in the metabolism of human subjects caused by variations in their vegetarian, low-meat, and high-meat diets were investigated in a crossover design (Stella et al. 2006). Individuals consuming the vegetarian diet showed metabolic signatures indicative of an altered metabolic activity of gut bacteria with variations in the urinary levels of 4-hydroxyphenylacetate and *p*-cresol sulfate. The authors reported that conversion of 4-hydrohyphenylacetate to *p*-cresol was carried out by a limited number of microbial species and could be used to assess changes in gut bacterial activities in response to nutritional interventions. More recently, potential metabolic imprinting in the bacterial activities in response to aging and specific dietary intervention were exhaustively described in a long-term study on caloric restriction (CR) in dog (Wang et al. 2007). Metabolic profiling of urine samples revealed signatures related to aging and growth-related biological processes as well as CR-induced effects on basal energy metabolism. The authors highlighted that both aging and CR led to differential profiles of mammalian-microbial cometabolites, such as aromatic derivates, i.e. hippurate, 3-hydroxyphenylpropioniate, as well as aliphatic methylamines, which may indicate altered activities of the gut microbiome.

3 The Complex Host–Microbiome Interactions: A Challenge for Nutrition

The recent discovery of the contribution of gut microbiota in the predisposition to gastritis and obesity has raised new interest in gut microbial activities of human and their implications in future nutritional healthcare. The gut microbiome-mammalian superorganism (Lederberg 2000) represents a level of biological evolutionary development in which there is extensive transgenomic modulation of metabolism and physiology that is a characteristic of true symbiosis (Martin et al. 2007c). The gut microbial community contains multiple cell types providing an extended genome interacting with a number of important mammalian metabolic regulatory functions. As the microbiome interacts strongly

with the host to determine the metabolic phenotype (Dumas et al. 2006; Martin et al. 2007a) and metabolic phenotype influences on the outcomes of drug interventions (Clayton et al. 2006; Nicholson et al. 2004), there is clearly an important role of understanding these interactions as part of personalized healthcare solutions. Several mammalian–microbial associations, both positive and negative, have already been studied with metabonomics (Dunne 2001; Gill et al. 2006; Ley et al. 2006; Nicholson et al. 2005; Verdu et al. 2004).

Recently, the contribution of the gut microflora on mammalian metabolism was revisited using metabolic profiling. The importance of the metabolic relationship between gut microflora and host physiology was demonstrated in a study assessing the effect of dietary changes, i.e. switching from a 5% control low-fat diet to a 40% high-fat diet, on the metabolic status of insulin-resistant mice (Dumas et al. 2006). In this experiment, mice showed a significant association between a specific metabolic phenotype, e.g. low plasma phosphatidylcholine and high urinary methylamines, and genetic predisposition to high-fat diet-induced dyslipidemia and nonalcoholic fatty liver. The urinary excretion of methylamines was directly related to microflora metabolism, suggesting that conversion of choline into methylamines by microbiota in this experimental design mimics the effect of choline-deficient diets, causing nonalcoholic fatty liver disease. These data also indicate that gut microbiota may play an active role in the development of insulin resistance.

The intimate relationship that animals have with the organisms inhabiting their guts is also very well illustrated by Martin et al., who describe the results of a top-down view of these microbial–mammalian interactions, showing the effects of different gut flora on the metabolic profiles of the mouse organism (Martin et al. 2007a). This study aimed at assessing the metabolic effects of inoculating germ-free mice with human-derived flora (nonadapted microflora) or exposing them to conventional mice (re-conventionalization), enabling the acquisition of a normal mouse microbiome. Metabolic profiling revealed that reconventionalized mice tend to converge metabolically and ecologically toward conventional mice with a healthier physiology. Inoculation of germ-free mice with a nonadapted microflora modifies the physiology of the murine host toward a prepathologic state and maintains the gut

tract and the liver outside a sustainable mouse ecological equilibrium. It was shown that a nonadapted gut microbiota is critically involved in supplying host calorific requirement via reprocessing of bile acids. This is part of what Martin et al. have termed the microbiome–host metabolic axis, i.e. "the multi-way exchange and co-metabolism of compounds between the host organism and the gut microbiome resulting in transgenomically regulated secondary metabolites which have biological activity in both host and microbial compartments." Understanding the effects of bacterial metabolism on the balance of bile acids in enterohepatic recirculation is a major challenge due to the implications of microbiota in fat absorption, lipid metabolism, drug therapeutic or toxic effects as well as direct effects within the gastrointestinal tract and its contents. In that regard, recent advances in microbial and metabolic profiling make possible the multicompartment study of bile acids and their effects on intermediary metabolism.

Much attention has recently been focused on the use of probiotic supplements as a means to promote gut health, thus preventing the incidence of allergies and inflammatory states. A probiotic is generally defined as a "live microbial feed supplement added to appropriate food vehicles," which is expected to benefit intestinal microbial balance and consequently the host physiology (Gibson and Fuller 2000). In a follow-up study, Martin et al. have assessed the transgenomic metabolic effects of exposure to either *Lactobacillus paracasei* or *Lactobacillus rhamnosus* probiotics in this humanized microbiome mouse model (Martin et al. 2008). The authors have illustrated the robust capabilities of metabonomics to capture subtle metabolic fluctuations in diverse biological compartments including biofluids, tissues, and cecal content in relation to modulation of microbial population. The authors exemplified how multicompartmental transgenomic metabolic interactions could be resolved at the compartment and pathway level. They have described how probiotic exposure exerted microbiome modification with subsequent alteration of lipid metabolism and glycolysis. Probiotic treatments also altered a diverse range of pathways, including metabolism of amino acids, methylamines and short-chain fatty acids. These integrated system investigations demonstrate the usefulness of a top-down systems biology concept based on metabolic profiling to investigate the mecha-

nistic bases of probiotics and the monitoring of their effects on the gut's microbial activity.

These investigations bring further evidence on the crucial role of the gut microbial activity on human health that have previously been raised in studies exploring the physiological effects of probiotic interventions in different mouse models. To begin with, the effects of a therapeutic intervention with *L. paracasei* probiotic on normalizing the metabolic disorders have been assessed in a model of *Trichinella spiralis*-induced irritable bowel syndrome (Martin et al. 2006). Both systemic and tissue-specific metabolic changes were captured using a metabonomic approach, which were consistent with an increase in energy requirement due to muscular hypercontractility and hypertrophy, inflammation and alteration of gut microbial activities. The authors illustrated partial regression of the metabolic disorders achieved by intervention with a *L. paracasei* probiotic. The probiotic treatment moved the energetic metabolism toward normality, reduced the gut microbiota disturbances and might contribute to normalization of the late inflammatory markers.

In other investigations, we have also explored the role of single probiotic inoculation on physiological status of different sections of the gastrointestinal tract of animals that were raised without any resident microorganisms (Martin et al. 2007b). Metabolic signatures reflecting the structure and function of the different compartments were obtained with variations in concentrations of amino acids, antioxidants, osmolytes and creatine. For instance, jejunum and colon showed metabolic signatures ascribed to lipogenesis and fat storage. More interestingly, metabonomic allowed capturing region-dependent metabolic changes triggered by ingestion of live *L. paracasei* in the upper gut, consistent with modulation of intestinal digestion, absorption, amino acid homeostasis, lipid metabolism and protection against oxidative stress. Contrary to the effects induced by live *L. paracasei*, no changes were seen with supplementation of irradiation-killed bacteria, which suggested that the differential metabolism observed with live bacteria is probably due to genuine host–bacteria interactions.

4 Characterizing the Metabolic Status of Individuals: A Step Toward Personalized Nutrition

As the microbiome interacts strongly with the host to determine the metabolic phenotype, metabolic health and nutritional status, there is clearly an important role of understanding these interactions as part of optimized nutrition. Nutrimetabonomics provides a system approach that is potentially able to assess metabolic status of individuals considering their specificity in terms of genetic and environmental factors, gut microbiota activity, lifestyle and food habits.

The characterization of the metabotype of individuals could open access to important information on dietary variations in humans and on the degree of response to dietary modulations. This may ultimately provide new insights into the role of diet and nutrition for health maintenance and personalized healthcare nutrition programs. Personalization of nutrition is the outcome for individuals who will adapt their diet and lifestyle according to knowledge about their current and future health status, and their subsequent nutritional requirements (see Fig. 4 for the conceptualization of nutritional metabonomics for health and risk management). This implies the development of analytical strategies leading to the characterization of the initial nutritional status on which diet or lifestyle recommendations could be applied to maintain or even improve metabolic health. In this way, the concept of "pharmaco-metabonomics" developed by Clayton et al. is interesting because providing a means to predict the metabolic response of a living organism from a simple preintervention metabolic profile (Clayton et al. 2006). Transposed to nutrition, such a concept could be used to optimize dietary recommendations for individuals. In order to achieve this goal, information contained in complex metabolic profiles would need to be validated for well-determined physiological and nutritional outcomes. In such cases, a dietetics professional could use this metabolic information to develop coordinated approaches to optimize and maintain metabolic health, proposing nutritional solutions consistent with the metabotype of individuals considering their lifestyle and health aspirations.

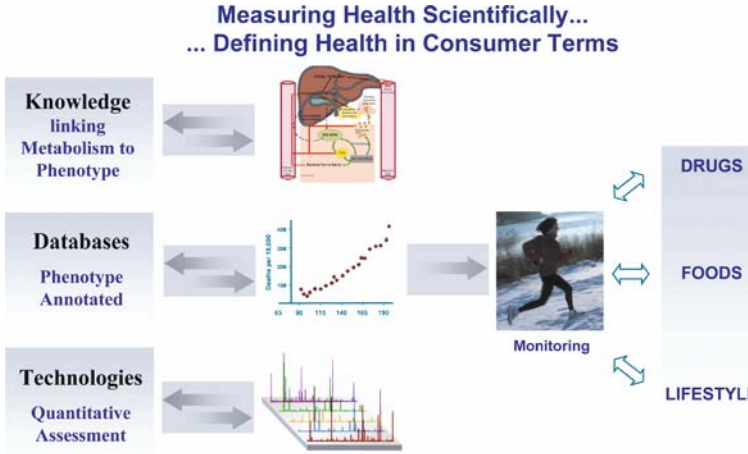

Fig. 4. Metabolic profiling as an approach to personalized health and nutrition

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