

## Nutritional Metabonomics: Applications and Perspectives

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Nowadays, nutrition focuses on improving health of individuals through diet. Current nutritional research aims at health promotion, disease prevention, and performance improvement. Modern analytical platforms allow the simultaneous measurement of multiple metabolites providing new insights in the understanding of the functionalities of cells and whole organisms. Metabonomics, “the quantitative measurement of the dynamic multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modifications”, provides a systems approach to understanding global metabolic regulations of organisms. This concept has arisen from various applications of NMR and MS spectroscopies to study the multicomponent metabolic composition of biological fluids, cells, and tissues. The generated metabolic profiles are processed by multivariate statistics to maximize the recovery of information to be correlated with well-determined stimuli such as dietary intervention or with any phenotypic data or diet habits. Metabonomics is thus uniquely suited to assess metabolic responses to deficiencies or excesses of nutrients and bioactive components. Furthermore, metabonomics is used to characterize the metabolic phenotype of individuals integrating genetic polymorphism, metabolic interactions with commensal and symbiotic partners such as gut microflora, as well as environmental and behavioral factors including dietary preferences. This paper reports several experimental key aspects in nutritional metabonomics, reviews its applications employing targeted and holistic approach analysis for the study of the metabolic responses following dietary interventions. It also reports the assessment of intra- and inter-individual variability in animal and human populations. The potentialities of nutritional metabonomics for the discovery of new biomarkers and the characterization of metabolic phenotypes are discussed in a context of their possible utilizations for personalized nutrition to provide health maintenance at the individual level.

**Keywords:** metabonomics • metabolomics • nutrition • biomarker • NMR • MS • chemometrics

### Introduction

While traditional nutrition research has dealt with understanding nutrient content in order to nourish populations, it nowadays focuses more and more on improving health of individuals through diet. Modern nutritional research is aiming at health promotion, disease prevention and performance improvement. The concept of developing nutritionally enhanced or functional foods requires the understanding of the mechanisms of prevention and protection, the identification of the biologically active molecules and the demonstrated efficacy of these molecules. The scientific community already recognizes the presence of a genetic component within the dietary response of individuals. However, the complex nutrient-gene interactions at the molecular level are far from being fully understood.

The development of genetic, transcriptomic, proteomic, and metabonomic technologies has provided data generation ca-

pabilities to enable better understanding of biological functions through different levels of biomolecular organization. The concept of systems biology has then been developed and related to the integration of all information at the different levels of genomic expression (mRNA, protein, and metabolite). Thus, systems biology generates pathway information and provides the capacity to measure subtle perturbations of pathways resulting from nutritional effects. The integration of systems biology into nutritional research led to the development of nutrigenomics. Nutrigenomics tackles how diet influences gene transcription, protein expression, and metabolism.<sup>1–3</sup> In addition, nutrigenetics addresses how individual genetic disposition affects susceptibility to diet.<sup>4–7</sup> The objective of nutrigenomics then is to integrate genomics (gene analysis), transcriptomics (gene expression analysis), proteomics (global protein analysis) and metabonomics (metabolite profiling) to define a “healthy” phenotype.

The human biological system is very extensive, and the functional integrity of human physiology ultimately reflected in the phenotype depends not only on nucleotide polymorphisms but also on many external factors like environmental

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and behavioral factors, even including other genomes from symbiotic organisms, i.e., the gut microflora.<sup>8–10</sup> Gene expression and proteomic data might only indicate the potential for physiological changes because many pathway feedback mechanisms are simply not reflected in protein concentration or gene expression. On the other hand, metabolite concentrations and their kinetic changes in cells, tissues, and organs represent real end-points of physiological regulatory processes.<sup>8</sup> Metabonomics is defined as “the quantitative measurement of the dynamic multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification”.<sup>11</sup> The concept of metabonomics has arisen from various applications of high-resolution nuclear magnetic resonance (NMR) spectroscopy to study the multicomponent metabolic composition of biological fluids, cells, and tissues.<sup>11–15</sup> Metabonomics provides a systems approach to understanding global metabolic regulation of organism and its commensal and symbiotic partners.<sup>8</sup> In addition, metabolomics has been defined as the comprehensive analysis of the whole metabolome under a given set of conditions.<sup>16</sup> Metabolomics focuses on the measurements of metabolite 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 metabolic activity.<sup>8</sup> Metabonomics and metabolomics strategies aim thus at detecting changes in the distribution and concentration of a broad range of metabolites in biological matrices and tissues mainly by the use of NMR<sup>12,17–20</sup> and mass spectrometry (MS).<sup>21–26</sup> Even if these two disciplines and their definitions are highly convergent, metabonomics can be regarded as a systems biology approach. It is not only concerned with static cellular and biofluid concentrations of endogenous metabolites but also perturbations of metabolites with time, including molecules that arise from chemical rather than enzymatic processing and molecules that occur as a consequence of intergenome interaction, such as the gut bacterial populations. In both metabonomics and metabolomics, advanced statistical and bioinformatic tools are then employed to maximize the recovery of information and to aid in the interpretation of the very large datasets that are generated.<sup>18,27–29</sup>

To date, numerous successful applications of metabonomics are reported in toxicity screening, drug metabolism, and functional genomics, a major part of them involving animal models rather than human subjects due to the greater control of conditions to reduce extrinsic variability.<sup>20,30–32</sup> Although a recent introduction in the field of nutrition research, i.e., nutritional metabonomics, this approach has already delivered interesting insights for the understanding of metabolic responses of humans or animals due to dietary interventions and as well as for the definition of metabolic phenotypes. This review reports some experimental key aspects of nutritional metabonomics including the experimental design, the acquisition and statistical processing of metabolic profiles, reviews various applications in human and animal dietary interventions, and metabolic variability studies. The potential and perspectives of this approach in the fields of nutritional health and risk management, metabolic phenotype characterization, and possible applications in personalized nutrition are also addressed.

### Experimental and Technical Aspects

The Standard Metabolic Reporting Structures (SMRS) working group has recently reported a summary of draft recom-

mendations and standards in metabolic profiling.<sup>33</sup> In the specific field of Nutrition, the Long Range Planning Committee of the American Society for Nutritional Sciences has considered the issues and actions needs in metabolic data definition.<sup>34</sup> We refer thus to these two references for detailed explanations of aspects to be considered in nutritional metabonomics. We briefly discuss in this section some key issues in the three successive steps constituting the metabonomics workflow: the experimental design, the data acquisition, and the post-instrument data processing by multivariate statistical analysis.

**The Experimental Design.** In opposition to applications of metabonomics in toxicological studies where effects are generally linked to high dose of single xenobiotics, nutritional metabonomics often deals with the measurements of subtle metabolic modulations as the biochemical response against relatively low doses of bioactive food nutrients or supplements. The metabolic response is very often expressed as the product of many simultaneous regulations of biochemical pathways, which are difficult to target *a priori* as compared with conventional toxicological events. Moreover, because foods can be seen as a direct link between complex organisms and their environment and given the central role of co-metabolism from gut microflora, the observed metabolic modulations are, by default, multifactorial. With the example of urinary profiles on Sprague–Dawley rats, Nicholson and Wilson gave a representation of hypothetical “influence vectors” generating metabolic variations that could explain the specific position of an individual in the metabolic hyperspace.<sup>8</sup> The challenge is then to deconvolve specific food-induced metabolic responses within complex metabolic hyperspaces subjected to many related or nonrelated metabolic variations, the latter being seen as confounding factors. These factors are responsible of the huge inter-subject biochemical variation in metabolism, which is generally greater in humans than that observed in animal models because of their greater diversity in genetic and environmental factors, differences in diet, diurnal changes, gender and estrus cycle, health status, and a wide range of lifestyle components such as smoking, alcohol consumption, or sports activity, for instance.

As a consequence, nutritional metabonomics requires a careful experimental design. To cope with inter-subject metabolic variation, one method is to conduct carefully controlled dietary intervention studies, where each subject consumes a defined diet and participates in both a control and test intervention study period. For this purpose, a typical double crossover study design is often used. The number of participants in a study must be fixed according to statistical requirements, i.e., a sufficient number of individuals per grouping class, to deliver robust and interpretable statistical models. The criteria for the selection of participants are also fundamental. Generally, a prestudy screening using biological parameters (body mass index, blood pressure) and conventional clinical chemistry (blood cholesterol, lipid profile, glycaemia, uraemia, etc.) is carried out for participant selection. In addition, lifestyle and diet questionnaires aimed at recording additional information that could be responsible for unexpected biochemical variability can be collected. Rather than knowing the exact nutrient intake or energy expenditure, the scope is to use this information for the interpretation of unusual metabolic profiles and for the identification of foods that could have particularly influenced biofluid metabolic profiles. For instance, it is now clearly established that a large trimethylamine-*N*-oxide (TMAO) signal in urine can be associated with fish consumption.<sup>17</sup>

**Data Acquisition.** Metabonomics measures all metabolites within a biological sample and ultimately quantifies each molecule relatively (control vs treated; healthy vs diseased) or in absolute terms. To date, there is no single technological platform capable of identifying and measuring all metabolites in a single sample simultaneously. There are, however, two main basic approaches to metabonomic analysis, which both rely on spectroscopic detection.

NMR spectroscopy allows the simultaneous analysis of a wide range of low molecular weight metabolites directly in complex mixtures.<sup>14,15</sup> The major advantages of NMR over other assays are that little or no pretreatment of the samples is required and that information about a range of metabolites can be obtained in a single experiment. In proton NMR spectroscopy (<sup>1</sup>H NMR), all covalently attached protons from mobile molecules within a very high dynamic range of concentrations, i.e., from millimolar to nanomolar scales, if using modern cryogenic probes, are scanned simultaneously thus providing a biochemical fingerprint of an organism. <sup>1</sup>H NMR-based metabonomics is generally preferred to other nuclei like carbon-13 because of its highest sensitivity and the relative short experimental time needed to acquire metabolic profiles. Indeed, thanks to the development of flow NMR technology, <sup>1</sup>H NMR has emerged as a high throughput system allowing the acquisition of hundreds of metabolic profiles per day in a complete automated fashion.<sup>35</sup> However, resonances of metabolites may be highly overlapped within the proton resonance window. In such case, ultrahigh magnetic field and/or two-dimensional (2D) NMR spectroscopy can be used to resolve resonances overlapped in 1D proton spectra.<sup>15,36–38</sup> Online coupling of NMR with high-pressure liquid chromatography (HPLC) has also been developed and applied to biological fluids providing an efficient separation of metabolites before NMR analysis.<sup>39,40</sup> Finally, NMR may also be used effectively to generate metabolic profiles from intact tissue using high-resolution magic angle spinning spectroscopy.<sup>13,41</sup>

Advances in MS techniques, particularly when linked to liquid chromatography, have resulted in the development of robust methods for the screening of low molecular mass metabolites in biological matrices.<sup>22–24,42–46</sup> The main advantage of MS techniques lies with its high sensitivity and its structure-rich information. However, as opposed to <sup>1</sup>H NMR where there is no need to preselect analytical conditions, the presence of mobile and slowly exchanging protons being sufficient, MS detection implies that the analyte be ionized. Preliminary steps of sample preparation such as deproteinization are also needed in most of cases. Furthermore, because biological samples contain a very wide range of molecular species including non-ionic, acidic, basic, and amphoteric substances, a MS analysis using both positive and negative ionization techniques is often mandatory to ensure the best possible chance of detecting the maximum number of metabolites. One major drawback of direct-injection MS approaches is linked to ion suppression problems, which have been substantially solved by the MS analysis being linked to a robust generic gradient HPLC separation. Moreover, the introduction of the HPLC systems using sub-2  $\mu\text{m}$  packing columns combined with high operating pressures (UPLC technology) results in higher peak capacity, greater resolution, and increased sensitivity compared to a conventional 3–5  $\mu\text{m}$  material. This technology improvement allows a drastic decrease of the analysis time and has opened the possibility of high throughput screening MS for metabonomics. Several successful applications have recently validated

these technological improvements and revealed the great potential of MS in metabonomics.<sup>24,43–46</sup>

NMR and MS can then be seen as complementary analytical platforms capable of producing complex metabolic profiles of living organisms in a relatively short time. The combination of NMR with MS has already been illustrated in metabonomics studies.<sup>21,47,48</sup> As valid for other “omics” disciplines, metabolite profiling, identification, and quantitation face the challenge of pronounced inter-individual variability. A crucial issue in metabonomics is therefore the quality assessment of analytical data including the accuracy and the reproducibility of measurements. To date, the inter-platform and inter-laboratory variability of NMR-based metabolic profiling is generally lower than MS. A recent study on a large-scale population phenotyping via urine profiling showed that the multivariate analytical reproducibility of the NMR screening platform was >98%, which testifies of the robustness of the NMR approach.<sup>49</sup> Improvements of the quality of measurements are expected via the definition and the validation of international standards.<sup>33</sup>

**Data Analysis.** Pattern recognition (PR) and related multivariate statistical tools are used to extract maximum information in complex spectroscopic data.<sup>29</sup> There are many multivariate methods for clustering, classifying, and modeling metabonomics (<sup>1</sup>H NMR, LC–MS, GC–MS, etc.) datasets.<sup>50</sup> Simple unsupervised clustering algorithms (requiring no *a priori* knowledge as to the class of the sample) such as principal components analysis (PCA) enable visualization of biological data sets based on the inherent similarity/dissimilarity of samples with respect to their biochemical composition. It is usually applied in the initial stages of an investigation to present an overview of the information contained in the data set. PCA creates a condensed summary of the data, which can be analyzed graphically by means of two types of plots, the scores plot and the loadings plot. The scores plot is a summary of the relationship between the observations (i.e., spectra, *m/z* listings, chromatograms, etc.) and can be used to establish any significant similarities and differences between samples; the loadings plot is a similar summary of the variables (i.e., signal intensities). The loadings can be viewed as a means to interpret the pattern seen in the scores plots, as the two plots are complementary. Thus, PCA can facilitate the simultaneous comparison of a large number of complex objects such as biofluid spectra and provide information on biochemical (metabolite) changes with relation to physiological variations. PCA can then be seen as a knowledge-building step of the investigator within the data analysis. This technique is particularly efficient for the identification of outliers. The analysis of the variance of outliers can provide important information on metabolic changes, i.e., strong responders to a specific stimulus, and on the quality of data acquisition, i.e., poor water suppression in NMR spectroscopy or matrix effect in MS. One has then to decide the removal of outliers from statistical analysis considering the possible loss of metabolic information and the robustness of the obtained models. A careful analysis of outliers is essential if the investigation requires the application of supervised techniques (requiring *a priori* knowledge as to the class of the sample) that are frequently employed in nutritional metabonomics. Among the numerous available supervised techniques, partial least-square discriminant analysis<sup>29</sup> (PLS-DA) and its variant combining a data filtering step such as orthogonal signal correction (OSC) and orthogonal partial least-square discriminant analysis<sup>51,52</sup> (O-PLS-DA) are at present the most popular

in metabonomics research. PLS-DA provides a way to filter out metabolic information which is not correlated to the predefined classes whereas the PLS-DA loadings, similarly to PCA loadings, yield information on which spectral signals are associated with the observed clustering giving then a means for metabolic interpretation. Further refinement of PLS-DA models can be achieved using the O-PLS-DA technique, where the variation in the X matrix (variables) and the Y matrix (observations or classes) is separated into three parts. The first part contains the variation common to X and Y, the second part contains the specific variation for X, so-called structured noise, and the last part contains the residual variance. The O-PLS-DA method provides a prediction similar to that of PLS-DA, but the interpretation of the models is improved because the structured noise is modeled separately from the variation common to the X and Y matrices. Therefore, the O-PLS-DA loadings and regression coefficients allow a more straightforward and accurate interpretation than PLS-DA, which models the structured noise together with the correlated variation between the X and Y matrices. To test the validity of the model against overfitting, the cross-validation parameter  $Q^2$  is computed as in PLS-DA. The O-PLS-DA loadings plot can then be processed according to the method described by Cloarec et al.<sup>27,28</sup> This consists of combining the back-scaled O-PLS-DA loadings from a model where the data had been autoscaled (each parameter has a mean of zero and a variance of one) with the variable weights of the same model in the same plot. For this purpose, each O-PLS loading is first multiplied by the standard deviation (back scaling) of its corresponding variable and then plotted as a function of its related chemical shift but with a color code linked to the weights of the selected latent variable. In this way, the common resonances from metabolites involved in the discrimination, i.e., treatment versus placebo, are highlighted. The interpretation of the loadings is therefore straightforward for the spectroscopist because the resulting plot provides roughly the same shape as that of a real spectrum.

### Metabonomics Applications in Nutrition Research

Metabonomics has been identified as a very promising approach to assess functionalities of foods and nutrients via the simultaneous measurement of multiple metabolic end-points in complex organisms. Over the last 10 years, numerous scientists have discussed the high potential to generate new insights for the understanding of the complex relationships between human or animal metabolism and foods. Nutrition might then benefit from new major advances in the knowledge of metabolism to decipher the mechanisms of action of foods and diets to promote health. From a technical point of view, one can distinguish the so-called targeted approaches where the measures are focused on the quantitative measurements of specific metabolites or the sub-profiling of molecular families, i.e., lipids, amino acids, bile acids, organic acids, from the holistic approach where no *a priori* selection of any metabolites is made.

#### Analysis of Metabome Subsets by Targeted Approaches.

The complete review of different applications of targeted analyses in nutrition goes beyond the scope of this article. We will thus report some applications that we consider as representative of targeted profiling within the frame of nutritional metabonomics. The statistical processing of amino acid profiling has been reported by Noguchi et al.<sup>53</sup> In this study, the potential of the cluster analysis of multivariate correlations (CAMC) method for the processing of metabolic subsets is

described and evaluated for deciphering the relationships between amino acid concentrations, as the result of net intakes from diet, catabolic, anabolic, and transport rates, and metabolic adaptation that takes place after the ingestion of different levels of protein and amino acids. They have underlined the utility of correlation-based analyses to assess the range of adequacy of amino acid intakes.

Matsuzaki et al. have investigated the metabolic effect of dietary leucine excess in male Fisher rats.<sup>54</sup> Rats were fed with leucine-enriched diets before urine, blood, and liver samples were collected. They used the CAMC method to combine physiological and toxicological variables, plasma and urine amino acid analysis and plasma metabolite profiling acquired on a gas chromatography–mass spectrometry (GC-MS) platform. In addition, the DNA microarray data from liver samples were also obtained to relate gene expression with metabolic changes in relation with dietary leucine excess. The whole data were reported as being consistent with excess leucine exerting effects through the overloading of nitrogen metabolism and that urea or  $\alpha$ -ketoisocaproate could be an early marker for the upper limit of adequate leucine intake.

Another important metabolic subset for nutrition research is represented by the whole set of cellular lipids. Watson has reviewed analytical techniques allowing the extraction and the analysis of biological lipids, i.e., lipidomics.<sup>55</sup> As a branch of metabolomics, lipidomics is a systems-based approach for the comprehensive understanding of the influence of all lipids on a biological system with respect to cell signalling, membrane architecture, transcriptional and translational modulation, cell–cell and cell–protein interactions, and response to environmental changes over time, the molecules with which they interact, and their functions within the cell.

Watkins et al. have used quantitative lipid profiling to characterize lipid metabolism in phosphatidylethanolamine-*N*-methyltransferase (PEMT)-deficient mice fed diets containing varying concentrations of choline.<sup>56</sup> The study design consisted of three groups of six mice: a choline-deficient group, a control group, and supplemented groups receiving a mean of 3 and 13 mg of choline/day, respectively. Lipid extracts from liver and plasma were subjected to a class fractionation using thin layer chromatography followed by the analyses of fatty acid methyl esters with gas chromatography. The analysis of the quantitative lipid profiles showed that choline supplementation restored liver but not plasma phosphatidylcholine (PC) concentrations of PEMT-deficient mice to levels commensurate with control mice. In addition, choline supplementation also restored plasma triglyceride concentrations to normal levels but did not restore plasma cholesterol ester concentrations in the PEMT-deficient mice. Thanks to this lipid profiling, they observed that PEMT-deficient mice also had substantially diminished concentrations of docosahexaenoic and arachidonic acids in plasma, independent of choline status. These results indicated that choline supplementation rescued some but not all of the phenotypes induced by the knockout. PMET implications in physiological processes other than the established role as a compensatory pathway for PC biosynthesis were discussed.

Another level of specificity in targeted profiling is exemplified by the tracer-based metabolomics approach reported by Lee et al. in a recent review.<sup>57</sup> Tracer-based metabolomics focuses on the simultaneous determination of metabolite distribution and flux determination using tracers, i.e., stable isotopes, in a single experiment. The tracer distribution within a set of metabolites accurately defines a metabolic phenotypic feature

that depends on the functional state of the studied biological system and its response to genetic and nutrient environment changes. This approach then provides a collection of metabolites linked by the relationship of shared metabolic pathways, common substrates and cofactors. They discussed the potentialities of the tracer-based metabolomics approach for addressing the complex issue of the relationship between nutrient intake and cancer risk.

**Applications Using Holistic Analyses.** The literature reports several investigations on the application of the holistic metabolomics approach for the assessment of metabolic changes in relation with diet, specific bioactive ingredients and supplements, as well as ethnical and lifestyle components in general population. Besides, there is a growing interest in understanding the metabolic and physiological contribution of the gut microflora co-metabolome in complex organisms.

**(a) Metabolic Assessment of Nutritional and Dietary Interventions.** Bales et al. published one of the first studies attempting to measure the metabolic effects in relation with dietary changes in 1984. In this work, they applied  $^1\text{H}$  NMR spectroscopic analysis of human urine after a fasting period and exercise.<sup>12</sup> The  $^1\text{H}$  NMR spectrum of urine collected immediately after a hard exercise showed increased level of lactate whereas fasting resulted in ketonuria as seen by urinary excretion of acetoacetate, acetone, and 3-D-hydroxybutyrate.

One should recognize that over the last 20 years, the metabolomics concept has been mainly developed in toxicology and pharmacology oriented investigations. However, the fundamental role of diet in the overall metabolism was already mentioned in toxicological studies as exemplified by the work published by Gray et al. in 1990 on the relationship between acute testicular damage and urinary and plasma creatine concentration in the rat.<sup>58</sup> In this study where metabolic effects of cadmium chloride were investigated, they observed using NMR analysis that food restriction resulted in increased levels of plasma creatine.

Very recently, Selman et al. have examined whole-genome transcriptional changes together with the plasma metabolic changes induced by acute caloric restriction using the C57BL/6 mouse model.<sup>59</sup> The study involved 20 male mice, which were assigned at fourteen weeks of age to the caloric restriction (CR) or *ad libitum* (AD) feeding regime. CR mice underwent a step-down regime with daily food intake reduced to 90% of AD mice at 14 weeks, 80% at 15 weeks, and 70% at 16 weeks of age, i.e., 30% CR relative to AD controls. The whole-genome RNA transcript profiles in liver, skeletal muscle, colon, and hypothalamus and plasma metabolite profiling using NMR were performed. Among the transcriptional changes, acute CR switched gene expression away from lipid biosynthesis and toward fatty acid catabolism,  $\beta$ -oxidation, and gluconeogenesis. The NMR analysis of plasma revealed increases in lactate, 3-hydroxybutyrate, cholesterol, and associated low-density lipoprotein levels under acute CR, in addition to increased plasma creatine and several glucogenic amino acids (methionine, glutamine, alanine, and valine), sustaining a metabolic switch toward energy conservation and gluconeogenesis. Also, plasma levels of glucose and lipid signals including very low-density lipoproteins (VLDL) were decreased in the CR group. Liver and muscle switched their gene expression away from energetically expensive biosynthetic processes toward energy conservation and utilization processes, including fatty acid metabolism and gluconeogenesis. Both muscle and colon switched gene expression away from cellular proliferation. They

concluded that mice undergoing acute CR rapidly take up many transcriptional and metabolic changes of long-term CR, suggesting that the beneficial effects of CR may require only a short-term reduction in caloric intake.

Griffin et al. employed the metabolomics approach for studying the metabolic effect of vitamin E supplementation in a mouse model of motor neuron degeneration (*mnd*), which comprises the most common neurodegenerative disorders of childhood.<sup>60</sup> The metabolic phenotype of brain and plasma of this model exhibiting a profound vitamin E deficiency was determined using solid- and solution-state  $^1\text{H}$  NMR spectroscopy. The blood plasma biochemical abnormalities were characterized by decreased concentrations of glucose, lactate, and methyl signals from lipids accompanied by an increase of lipidic methylene signal. The cerebral extract profiles of *mnd* were marked by increases in  $\beta$ -hydroxybutyrate, taurine, glutamate, and phenylalanine and decreases in creatine, aspartate, glutamine, and  $\gamma$ -aminobutyric acid. The intact cerebral cortical tissue analyzed by high-resolution magic angle spinning NMR showed increased lipid content, lipid methylene signal, and a slower degradation rate of *N*-acetyl-L-aspartate to acetate. The vitamin E dietary supplementation induced significant decreases in diallylic protons of unsaturated lipids in plasma and in  $\beta$ -hydroxybutyrate and phenylalanine in cerebral extracts. However, the supplementation did not fully normalize the concentrations of other low-molecular-weight metabolites including glutamate, glutamine, aspartate, and creatine within cerebral tissue from *mnd* mice. These results were discussed in terms of faulty production of mitochondrial-associated membranes, thought to be central to the deficits in the *mnd* mouse model. This study demonstrated the efficacy of the metabolomics approach for characterizing metabolic phenotypes to explore the functional genomics of a given gene modification.

Solanky et al. have investigated metabolic changes following dietary intervention with soy isoflavones in five healthy premenopausal women.<sup>61</sup> In this study, the subjects were maintained under strictly controlled dietary conditions to minimize the effect of confounding dietary factors. After 1 month, covering the first complete menstrual cycle, an amount of 60 g of soy protein corresponding to 45 mg of isoflavones was added to the basal diet. Fasting plasma samples were collected every 3 days during each diet period and were submitted to  $^1\text{H}$  NMR and multivariate statistical analysis. They observed decreases in plasma sugars and increase in lactate in relation to soy intake. Increases in lipoprotein together with subject-specific variations in glucogenic amino acids (isoleucine and valine), triglycerols, and choline were also reported. This study allowed them to suggest a soy-induced alteration in energy metabolism. Two years later, they conducted a similar study on the biochemical effects of conjugated (glucosides) and unconjugated (aglycones) soy isoflavones on the metabolism of healthy premenopausal women using  $^1\text{H}$  NMR-based metabolomics of urine.<sup>62</sup> The results showed that the intake of both conjugated and unconjugated soy isoflavones resulted in an increase of TMAO in the 24 h urine. Other metabolic changes were noted such as increases in *N*-acetylglutamate/glutamine/glutamate, citrate, methylamine, dimethylamine, and creatine and decreases in creatinine and hippurate. Comparative analysis of the metabolic effects of conjugated and unconjugated soy isoflavones suggest similar biological activities but with different magnitudes, indicating that conjugation has a significant effect that may relate to bioavailability and

metabolism. They proposed that these results obtained on plasma and urine suggest an inhibitory effect of isoflavones on glycolysis, resulting in a general shift in energy metabolism, from carbohydrate metabolism to lipid metabolism.<sup>61,62</sup>

The biochemical effects of epicatechin, a bioactive flavonoid widespread in many food products like green tea, cocoa, and chocolate products, have been studied in Sprague–Dawley rats using <sup>1</sup>H NMR analysis of urine samples in combination with PCA.<sup>63</sup> Urine samples were collected twice daily (0–8 and 8–24 h) from 10 male animals prior to dosing and after 2 days of 22 mg epicatechin intake via drinking water. Biochemical effects were evident on urinary profiles, showing the bioavailability and biological activity of epicatechin. Decreased urinary concentrations of taurine, citrate, dimethylamine, and 2-oxoglutarate were observed during the first 8 h after dosing together with epicatechin-related metabolites. They interpreted these metabolic changes as possibly being linked to the proposed effects of dietary polyphenols on kidney function and a shift in energy metabolism from carbohydrate metabolism to lipid/amino acid metabolism. This study has emphasized the potential of the metabonomic approach for studying bioactivity of polyphenols naturally occurring in many foods such as tea, for instance.

Wang et al. have applied a NMR-based metabonomic approach to evaluate the systemic plasma metabolic consequences of rats in response to exposure to a maternal separation stress in isolation or followed by a secondary stressor (water avoidance) later in life.<sup>64</sup> They have evaluated the effects of dietary supplementation with long chain polyunsaturated fatty acids (LC-PUFAs), which can putatively ameliorate the effects of stress. In this study, rats were fed with a standard diet and with a n-3 LC-PUFA-enriched diet. The animals were submitted to single and combined stresses including an early life stress in which rat pups were separated from their mothers daily for 12 days with a duration of 180 min after birth and an acute water avoidance stress at 3–4 months. The statistical processing of plasma profiles by O-PLS-DA revealed that both stresses caused depletion in the levels of total lipoproteins and triglycerides and elevated levels of amino acids, glucose, lactate, creatine, and citrate when compared to samples obtained from the control rats. Additional increases of acetyl-glycoproteins, 3-D-hydroxybutyrate and choline were observed in plasma of rats subjected to water avoidance stress. Furthermore, both stresses had the effect of modification of the lipoprotein compositions by subtle changes in high density lipoprotein (HDL) and VLDL fractions that were putatively linked to elevated levels of stress hormones. The LC-PUFAs dietary supplementation was found to be unable to reverse the metabolic changes induced by stress.

Van Dorsten et al. have compared the effects of black and green tea consumption on human metabolism.<sup>65</sup> In this study, 17 healthy male volunteers consumed black tea, green tea, or caffeine taken as placebo in a randomized crossover study. Participants were asked to follow a low-polyphenol diet during the study and received a daily intake of 1 g of tea solids and 360 mg of caffeine for the control group. Twenty-four-hour urine and blood plasma samples were analyzed by NMR-based metabonomics. Green and black tea consumption resulted in similar increases in urinary excretion of hippuric acid and 1,3-dihydroxyphenyl-2-O-sulfate, both being reported as end products of tea flavonoid metabolism by colonic bacteria. Several unidentified aromatic metabolites were detected in urine specifically after green tea intake. Interestingly, green and black

tea intake also had differential impacts on endogenous metabolites in urine and plasma. Green tea intake caused a stronger increase in urinary excretion of several citric acid cycle intermediates such as pyruvate, oxaloacetate, and citrate. Plasma analysis revealed that black tea consumption caused a shift in the lipoprotein distribution and a reduction of glucose and acetate. Similar results were reported for green tea concerning plasma glucose and specific lipoproteins. Furthermore, even if the metabolic effects in plasma of both teas could not be statistically differentiated in a robust fashion, green tea intake was associated with lower levels of lactate and alanine and higher concentrations of acetate and  $\beta$ -hydroxybutyrate. This study demonstrated the strong potential of the NMR-based metabonomics approach to detect subtle metabolic changes in relation with black and green tea consumption in comparison to the caffeine placebo. From these results, they reported that tea intake might have an effect on human oxidative energy metabolism and biosynthetic pathways.

Recently, Wang et al. have applied metabonomics to the study of human biological responses to chamomile tea ingestion.<sup>66</sup> Fourteen male and female subjects participated in the study, which consisted in three phases of a two-week duration: a control period, a dosing period with a daily ingestion of 200 mL of chamomile, and a post-dosing period. In this experimental design, no food restrictions were applied and dietary information from the evening and morning preceding urine collection was recorded to establish eventual confounding dietary effects. Urine samples were submitted to <sup>1</sup>H NMR analysis, and the resulting profiles were processed with multivariate statistics. A first PCA analysis underlined the occurrence of a metabolic confounding factor due to diet heterogeneity within the trial. Indeed, some outlying samples differed by a higher content in TMAO, which could be related to fish consumption before urine collection. With the outliers removed, the PCA scores plot has then highlighted a gender-based confounding factor, urinary profiles from females being characterized by a relatively higher content of citrate and glycine. To establish metabolic responses in relation to chamomile tea intake, a statistical filtering method (OSC) was applied to the dataset, removing effects from confounding factors and inter-subject variability. The consequent PLS-DA analysis showed a clear clustering of the subjects as a function of chamomile intake. The chamomile intake was then associated with a decrease in urinary excretion of creatinine and increases in glycine, hippurate, and an unidentified aromatic metabolite. The post-dosing samples were characterized by elevated urinary hippurate, testifying of a prolongation of the metabolic effect of chamomile tea ingestion during the two-week post-dosing period. The observed metabolic modulations were ascribed to possible chamomile-induced changes in gut microflora metabolism.

The use of metabonomics-generated data in nutrition faces the challenge that changes in the metabolic profiles of biologically complex organisms like humans in response to special diets or foods may be difficult to distinguish from normal physiological variation. Walsh et al. have examined the effect of acute dietary standardization on the metabolic profiles of several biological fluids of healthy humans.<sup>67</sup> Thirty female and male participants were enrolled in this study aiming at evaluating intra- and inter-individual metabolic variation. Urine, blood plasma, and saliva were analyzed by <sup>1</sup>H NMR in combination with multivariate statistics. Intra-individual variation was investigated, allowing people to follow their normal diet and then

to repeat exactly their previous diet and their physical activity. The inter-individual biochemical variability was assessed by providing the subjects with a standardized diet and by avoiding any vigorous activity. In addition, diurnal variation of urine and saliva was also studied. Important biochemical variability was observed for all biofluids at both intra- and inter-individual levels. The standardization of the diet resulted in a reduction of the inter-individual variability in urine but not in plasma and saliva. Significant diurnal variations were noted mainly caused by creatinine and acetate for urine and saliva respectively. They reported urine as a sensitive metabolic profile that reflects acute dietary intake as opposed to plasma and saliva.

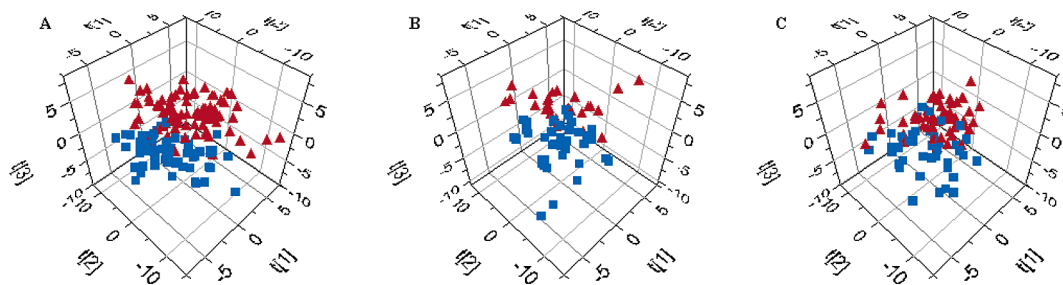
Recently, Stella et al. have applied an NMR-based metabonomic approach for the characterization of the metabolic effects of vegetarian, low meat, and high meat diets in human.<sup>68</sup> Twelve healthy men were provided the three diets in a crossover design. Each dietary period lasted 15 days with a washout period between each diet of 7 days. Three 24-h urine collections were made after the volunteers had consumed each diet for 10 days. The PCA analysis of NMR-generated urine profiles showed that individual variation was dominant, some participants showing a much greater variation than others. Despite individual variability, the loadings examination indicated that the high meat diet was associated with elevated urinary levels of creatinine, creatine, carnitine, TMAO, taurine, and 1- and 3-methylhistidine. The two remaining diets were marked by more subtle metabolic effects and samples partially co-mapped using PCA. The analysis was, however, focused on the differentiation between the high-meat and vegetarian diets, which contained the same amount of protein. The application of O-PLS-DA to the data matrix resulted in a clear clustering of the urinary metabolic profiles according to the diets. Indeed, higher urinary levels in creatine, creatinine, carnitine, acetyl-carnitine, taurine, TMAO, and glutamine characterized the metabolic signature of high-meat diet whereas the vegetarian diet was associated with higher urinary excretion of *p*-hydroxyphenylacetate, a microbial mammalian co-metabolite, and a decreased level in *N,N,N*-trimethyllysine. This study illustrates the efficacy of the metabonomic approach for measuring influence of dietary modulations on short-term human metabolism.

**(b) The Gut Microflora–Host Metabolic Interactions.** Complex organisms like humans have coevolved with gut microflora and have a commensal relationship with many microorganisms in the gut. Humans can be considered as “superorganisms” with an internal ecosystem of diverse symbiotic microbiota and parasites that have interactive metabolic processes. The homeostatic balance and the metabolome of an organism are then dependent upon not only the host but also the interaction between the host and its microfloral complement or co-metabolome. There is more and more evidence on the importance of interactions between gut microflora and host metabolism.<sup>8,9</sup> Diets modulate the complex internal community of gut microorganisms and by doing so can drastically modify nutrient bioavailability and metabolism. It is then of high importance for nutrition to understand and to metabolically characterize the interactive molecular processes between host and its microbiome. Metabonomics appears then as a favorable approach for assessing gut microflora metabolism and metabolic effects in the host through the profiling of biological fluids such as urine, which is particularly considered as a product of this “metabolome–metabolome” interaction.

Nicholls et al. have used <sup>1</sup>H NMR-based metabonomics to evaluate the acclimatization pathways of germ-free (axenic) rats to standard laboratory conditions concomitant with the associated development of gut microfloral communities.<sup>69</sup> In this study, three Fischer 344 male germ-free rats were bred in a sterile facility and then transferred into the normal environment. Urinary profiles obtained from samples collected at different time points over a period of three weeks following introduction to a standard laboratory environment were analyzed to visualize the changes in the host metabolic trajectory over the course of the study. A visual inspection of the spectra allowed the observation of two periods of glycosuria, coinciding with a low concentration of the TCA cycle intermediates (citrate, 2-oxoglutarate and succinate) in response to infection. Metabolic variations in relation to the microbial colonization included increased concentrations in 3-hydroxyphenylpropionate, but which, by the end of the experiment, had been replaced by an elevation in hippurate, and in phenylacetyl-glycine. In addition, an increased urinary excretion of TMAO was also noted and associated with the oxidation of TMA, which, in this case, was supposed to be derived from intestinal bacteria. This study has shown that a minimum of 21 days is required to allow a germ-free animal to acclimatize to a normal environment via the establishment of a stable gut microflora as revealed by NMR urinary profiling.

Very recently, Martin et al. have investigated the metabolic signatures of the *Trichinella spiralis* infection in a mouse model of post-infective irritable bowel syndrome (IBS) to evaluate if probiotic bacteria (*Lactobacillus paracasei*) supplementation could reverse metabolic abnormalities of the post-infective state.<sup>41</sup> Plasma samples and jejunal and longitudinal myenteric muscle samples were analyzed by NMR-based metabonomics employing magic-angle-spinning NMR device for tissue metabolic profiling. The plasma metabolic profile of *T. spiralis*-infected mice was characterized by an increased energy metabolism (lactate, citrate, alanine), fat mobilization (acetoacetate, 3-D-hydroxybutyrate, lipoproteins), and a disruption of amino acid metabolism due to increased protein breakdown, all of which were related to the intestinal hypercontractility. The jejunal metabolic profile indicated increased levels of taurine, creatine, and glycerophosphorylcholine, which were associated with the muscular hypertrophy and disrupted jejunal functions. The probiotic treatment normalized the muscular activity and the disturbed energy metabolism, as evidenced by decreased glycogenesis and elevated lipid breakdown in comparison with untreated *T. spiralis*-infected mice. This work illustrated the positive role of specific probiotic supplementations in the treatment of post-infective IBS, highlighting the considerable potential of the metabonomics approach for surveying modulation of mammalian–microbiota interactions.

Another recent study on the metabolic relationship between gut microflora and host co-metabolic phenotypes was carried out by Dumas et al.<sup>70</sup> In this study, the effect of dietary changes, i.e., switching from a 5% control low-fat diet (LFD) to a 40% high-fat diet (HFD), on plasma and urine metabolic NMR profile of the mouse strain 129S6, documented for its susceptibility to insulin resistance (IR) and nonalcoholic fatty liver disease (NAFLD), were investigated. The statistical analysis of the NMR profiles indicated that the 129S6 mouse showed dyslipidemia with low plasma levels of phosphatidylcholine, increased glucose, pyruvate, and TMAO signals and high urinary excretion of di- and tri-methylamine and TMAO. They demonstrated significant association between a specific meta-



**Figure 1.** Assessment of gender, age, and BMI-specific metabolic changes based on PLS-DA models generated on  $^1\text{H}$  NMR spectra of human plasma. (A) males (■,  $n = 66$ ) and females (▲,  $n = 84$ ); (B) young (▲, 18–29 years,  $n = 30$ ) and older (■,  $>46$  years,  $n = 24$ ) subjects; (C) lean (▲, BMI  $< 21 \text{ kg/m}^2$ ,  $n = 57$ ) and obese (■, BMI  $> 25 \text{ kg/m}^2$ ,  $n = 43$ ) subjects. Reprinted with permission from *Anal. Biochem.*<sup>73</sup> Copyright 2006, Elsevier Ltd.

bolic phenotype, e.g., low plasma phosphatidylcholine and high urinary methylamines, and genetic predisposition to HFD-induced NAFLD in mice. The urinary excretion of methylamines from the precursor choline was directly related to microflora metabolism indicating significant interaction between the mammalian host and microbiota metabolism.

**(c) Intra- and Inter-Individual Metabolic Variability.** The complexity of the metabolome reflects the numerous relationships and regulation mechanisms from genetics, normal physiological and pathophysiological processes, gut microflora metabolism, environmental factors, and diet. One of the main challenges in nutritional metabonomics relies on the separation of specific metabolic signatures due to well-determined foods or diets within the metabolome composed itself of numerous signatures from the so-called confounding factors, i.e., gender, aging, physiological status, and lifestyle. Different studies have attempted to describe these metabolic signatures.

The influences of multiple physiological parameters on biofluid compositions in animal models used in pharmacology and toxicology research have been widely reviewed by Bollard et al.<sup>17</sup> They reported different metabolic signatures in relation to intrinsic (species, strain, genetically modified animals, gender, aging, inter-animal variation due to genetic variability or stress, hormonal effects) and extrinsic (diurnal effects, diet, fasting and water deprivation, temperature, sleep deprivation, stress, acclimatization, and gut microflora) factors. The metabolic changes due to diurnal variation are, for instance, well established as an intra-individual variability source. Bollard et al. applied, for the first time, the NMR-based metabonomic approach for the study of metabolic changes in urine of female rats according to diurnal and estrus cycle fluctuations.<sup>71</sup> Diurnal effects could be attributed to relatively low levels of taurine, hippurate, and creatinine in daytime compared with nighttime urine samples. Inversely, urinary excretion of glucose, succinate, dimethylglycine, glycine, creatine, and betaine were higher in the daytime. Furthermore, the separation of the different stages of the hormonal cycle was attributed to perturbations in the levels of citrate, succinate, 2-oxoglutarate, taurine, creatine, creatinine, hippurate, glucose, TMAO, and *N*-acetylglycoprotein resonances. As reported previously, the diurnal effects on metabolic profiles of urine and saliva were also observed in human subjects.<sup>67</sup>

Another important physiological parameter causing metabolic changes and hence participating in the intra-individual overall variability is aging. Williams et al. have applied  $^1\text{H}$  NMR and HPLC-MS to the study of changes of urinary endogenous metabolites associated with aging in male rats over a 5-month period.<sup>72</sup> Age-related alterations to the urinary profile were

clearly apparent, with greatest differences observed with urine collected at 4 and 6 weeks. NMR spectra showed increases in creatinine, taurine, hippurate, amino acids/fatty acids with age, whereas citrate and glucose/myoinositol were decreased. HPLC-MS allowed the observation of an increase in the urinary excretion of carnitine associated with aging. They have associated these age-related metabolic perturbations with the maturation of gut microflora, changes in kidney functions, liver metabolism, and other developmental events like growth and sexual maturation.

We have recently assessed specific metabolic differences in humans based on gender, age, and body mass index (BMI), applying  $^1\text{H}$  NMR-based metabonomic analysis of blood plasma and urine samples (Figure 1).<sup>73</sup> The application of PLS-DA revealed that gender differences were associated with relative increased plasma concentrations of choline, low-density lipoproteins (LDLs), HDLs, and unsaturated lipids in women, whereas VLDLs, creatine/creatinine, valine, and isoleucine were greater in plasma from men. The urinary excretion of taurine and creatine/creatinine was higher in men than in women, whereas citrate was increased in women urines. Plasma levels of valine, isoleucine, alanine, and tyrosine were greater in older women ( $>46$  years) than in young women (18–29 years), whereas older men were characterized by increased concentrations in total lipoproteins and unsaturated lipids. In the urinary profiles, the average amount of creatine/creatinine decreased with age for both genders, whereas dimethylamine, citrate, and glycine showed gender-specific changes. Besides, plasma concentrations in choline and citrate increased in lean participants (BMI  $< 21 \text{ kg/m}^2$ ) whereas levels in tyrosine, isoleucine, and glycoproteins were elevated in obese subjects (BMI  $> 25 \text{ kg/m}^2$ ). Altogether, these results indicated that young and lean women had higher rates of lipid biosynthesis than men who showed higher rates of protein turnover. With increasing age, metabolism favored lipid synthesis over protein turnover in men whereas overall lipid biosynthesis decreased in women as a putative effect from hormonal changes.

A pioneer large-scale human population screening study using metabonomics was recently published by Dumas et al.<sup>49</sup> In this epidemiological work, biochemical variability of urinary profiles from population samples in Japan (Aito Town), North America (Chicago), and China (Guangxi) were obtained by  $^1\text{H}$  NMR spectroscopy and processed with multivariate statistics. The loadings interpretation allowed visualizing population-specific metabolites such as increased excretion in TMAO in the Japanese population and  $\beta$ -aminoisobutyric acid and ethanol in Chinese specimens. The higher concentration level

in TMAO was consistent with the high dietary intake of fish in the Japanese population.

Finally, Lenz et al. have assessed the comparability of metabonomic urinary profiles from samples collected in British and Swedish populations.<sup>74</sup> The urinary profiles were clearly prone to variability due to dietary and lifestyle influences. An increased urinary excretion in TMAO was indeed observed in the Swedish population as a consequence of the fish-diet, whereas one urine specimen from the British population exhibited unusually high level of taurine because of the Atkins diet.

### Future Roles of Metabonomics in Nutrition Sciences

Metabonomics is uniquely suited to explore the complex relationship between nutrition and metabolism to investigate the role that dietary components play in health maintenance and disease development. Indeed, this powerful approach has the potential to explore mechanisms of homeostatic control and to assess how metabolic balances may be disturbed by deficiencies or excesses of dietary components. Metabolic profiles of body fluids or tissues can be then regarded as important indicators of normal phenotype, physiological, or pathological states, giving a unique opportunity to discover new biomarkers of disease for both prognostic and diagnostic applications.<sup>75,76</sup>

**Development of a New Concept for Biomarkers.** If progress in the field of nutrition has previously been limited by analytical challenges, newly developed systems biology platforms and metabonomics in particular allow the assessment of metabolic responses of complex biological system to multicomponent mixtures of dietary ingredients. The major challenge then becomes how to associate biological effects of foods with relevant biomarkers. As opposed to single conventional biomarkers, such as glucose or cholesterol, a novel biomarker concept is needed to be indicative of subtle changes in homeostasis.<sup>1,3,77</sup> The disease state can indeed be seen as the consequence of the dysregulation of communication and subsequent changes in dynamics and loss of homeostasis. Metabonomics, a methodology that quantitatively depicts in a holistic fashion the final endpoints of regulatory physiological processes, i.e., the metabolites, is most likely an outstanding approach to deliver candidate biomarkers to describe this loss of homeostasis. Metabonomics can then ensure the evolution from single biomarkers strategies toward biomarker patterns. Several encouraging applications for the diagnosis of complex multi-factorial diseases have already been reported in the field of cardiovascular disease,<sup>78</sup> type 2 diabetes,<sup>79,80</sup> hypertension,<sup>81</sup> and epithelial ovarian cancer.<sup>82</sup> Metabonomics can then generate biomarker patterns that could be used not only for prognostic or diagnostic purposes but also as multiparametric metabolic probes to assess efficacy of drugs or nutritional interventions designed to correct homeostasis dysregulations.

Using such biomarkers in nutritional metabonomics would make possible the simultaneous monitoring of both efficacy and safety aspects of foods. Single nutrients may have multiple known and unknown biochemical targets and physiological actions, which may not be easily addressed with conventional single biomarkers. The health assessment of nutritional components is even further complicated by the fact that single dietary constituents are almost never consumed as separate entities but as part of a dietary mixture. The overall metabolic response of the subject will be the result of complex interactions with the food mixture constituents, possibly acting synergistically, as well as extrinsic factors such as environmen-

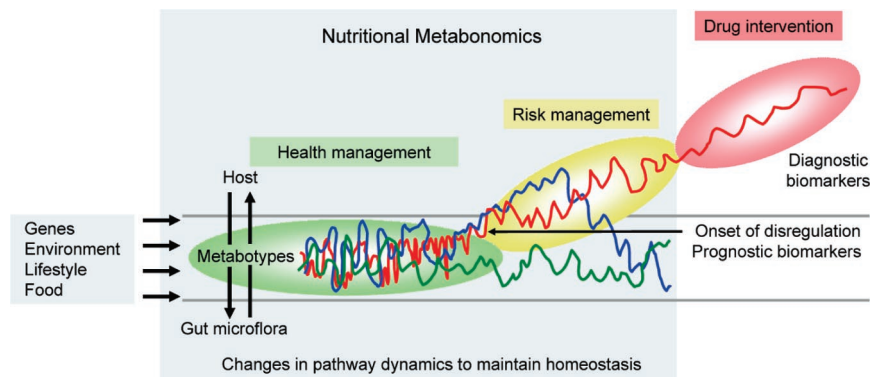
tal stressors and lifestyle factors. Indeed, evidence exists showing that important interactions occur by combining foods or food components. For example, the combination of soy and tea as part of a complete diet appears more effective than either alone in inhibiting prostate cancer growth and metastasis in a mouse model of human prostate cancer.<sup>83</sup> Considering also the inter-individual variability of people at various levels of biological organizations, i.e., genetic polymorphism, metabolic phenotype, it becomes of high scientific relevance to develop and validate biomarker patterns able to assess global homeostasis changes. These biomarkers are useful because they can act both as diagnostic or prognostic parameters and also as metrics of efficacy of dietary interventions for maintaining or improving health at the individual level.

**Individual Health and Personalized Nutrition.** Diseases of modern civilization, such as diabetes, heart disease, and cancer are known to be influenced by dietary patterns. The mandate of nutrition goes beyond ameliorating or curing diseases to encompass a clear overall objective to preventing disease and improving health of entire populations. Key linked scientific objectives then become the understanding of the relationships between diet and disease and the understanding the relationships between diet and health. The comprehensive analysis of human and animal metabolomes will serve as the information base for modern nutrition. As discussed before, the biomarker patterns, i.e., metabonomics-generated biomarkers, will have to be used for health assessment (Figure 2). The main goal will be to predict the likelihood of future diseases within the context of an individual's overall health and identify the causal basis of risk, leading to recommendation of appropriate means, i.e., dietary changes, to avoid homeostasis loss and maintain metabolic health.

Genetic variations, environmental conditions, and dietary habits linked to cultures and lifestyle dictate individual predispositions to disease and health potential. It is also true that consumers respond differently to specific diets because of this biochemical variability and a diet that might be optimal for one individual could predispose another to disease. Human and animal biochemical variability is encoded in complex metabolic profiles generated by metabonomics, which makes it a powerful means to determine phenotypes in general populations.<sup>8,49,74</sup>

The concept of metabolic phenotype or "metabotype" defined as "the probabilistic multiparametric description of an organism in a given physiological state based on analysis of its cell types, biofluids or tissues", has been introduced.<sup>84</sup> Human metabotypes are determined by a combination of genetic and environmental factors, with diet and lifestyle representing significant sources of diversity. The determination of metabotypes in human populations would be of great value in relating quantitative physiological and biochemical data to phenotypic, genetic, and environmental variations. It would provide a unique means of characterizing the boundaries of the metabolic "normality" for health management. The measurement of diet influence on metabotypes could provide valuable information in demographic and population studies on dietary variation in man and the degree of response to dietary modulation shown by individuals. This may ultimately give insight into the role of diet and nutrition in the development of human diseases and provide crucial information for personalized nutrition.

Personalization of nutrition is the outcome for individuals who will adapt their diet and lifestyle according to knowledge



**Figure 2.** Conceptualization of nutritional metabonomics for health and risk management. The metabotypes of individuals result from gene, environment, lifestyle, food, and gut microflora interactions. Different metabotypes (represented by blue, red, and green lines) are under homeostatic regulations that aim to maintain metabolic fluctuations within a healthy range (green line). Metabonomics-generated prognostic biomarkers can be used to assess homeostasis loss and likelihood for future diseases. Nutritional metabonomics aims at optimizing nutrition for health maintenance and to restore homeostasis as illustrated by the blue line. Adapted from van der Greef et al.<sup>77</sup>

about their current and future health status, and their subsequent nutritional requirements. This knowledge could be built around the characterization of different metabotypes in human populations. Recently, Clayton et al. have described a conceptually new “pharmaco-metabonomic” approach to personalizing drug treatment.<sup>85</sup> This approach uses a combination of predose metabolite profiling and chemometrics to model and predict the responses of individual subjects. This concept of “pharmaco-metabonomics” is sensitive to both genetic and environmental influences and addresses the metabolic response at the individual level. Such a concept could be directly applied to nutrition research as a means of assessing individual responses to foods and diets. In a future scenario and as goals for future health, the dietetics professional could use such metabolic profiles to measure, predict, and optimize metabolic responses of individuals to nutritional modulations. In cases of homeostasis deviation, he/she would thus develop a coordinated approach to re-establish a metabolic trajectory for the individual consistent with his metabotype and with his existing lifestyle and health aspirations.

A last but not least issue is linked to the need of defining standard methods for sample handling, data acquisition and processing by statistical and bioinformatics tools and also for biomarker interpretation and validation as partially initiated by the SMRS group.<sup>33</sup> On this basis and similarly to the human genome project, comprehensive and integrated databases of metabolomes, i.e., collection of metabotypes, could be built in the future for public health management.

### Conclusion

With the advent of the post-genomic era, nutrition research has witnessed an explosion in strategies that could be used for understanding the complex relationship between consumer and foods. The application of metabonomics in nutrition sciences, i.e., nutritional metabonomics, has already depicted the potentialities of this approach for the quantitative measurement of the dynamic multiparametric metabolic response of living systems to dietary interventions. The profiling of biological fluids by NMR or MS ensures a simultaneous analysis of a wide range of metabolites that are the endpoints of molecular regulatory processes, diet, gut microflora metabolism and environmental factors as evidenced by the recent literature. By opening a direct biochemical window into the metabolome in

a holistic fashion, metabonomics is uniquely suited to developing new generations of biomarkers that are capable of providing better understanding of complex metabolic phenomena as well as assessing intra- and inter-individual differences. This property makes metabonomics very efficient for the generation of biomarker patterns for the comprehensive characterization of metabolic health, the prognostics and the diagnostics of diseases, and the generation of new insights in the understanding of the interactions between diet and metabolism

Defining the metabolic phenotype or “metabotype” of human populations will offer a great opportunity to evaluate the metabolic response and the degree of this response to specific dietary modulations at the individual level. Similar to the “pharmaco-metabonomic” concept, predietary intervention metabolite profiling could be used to model and predict the responses of individual subjects to special foods. Nutritional metabonomics can then be foreseen as a promising approach towards personalized nutrition.

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