Chapter 11. Application of metabolomic analysis to nutrigenetic and nutrigenomic research

Hui-Ming Lin[1] and Daryl D. Rowan[2]

[1]Cancer Research Program, Garvan Institute of Medical Research, 384 Victoria Street, Darlinghurst, Sydney, NSW 2010, Australia.

[2]The New Zealand Institute for Plant & Food Research Limited, Private Bag 11600, Palmerston North 4442, New Zealand

Introduction

Metabolomic analysis has been defined as "a comprehensive and quantitative analysis of the metabolome" (Fiehn 2001), where the metabolome is the full suite of metabolites contained within a biological system. Due to the diversity of the chemical properties of metabolites and the limitations of analytical techniques, it is presently impossible to measure the complete metabolome. Therefore the definition for a similar term, metabonomic analysis, appears more appropriate - "the quantitative measurement of the time-related multi-parametric metabolic response of living systems to pathophysiological stimuli or genetic modification" (Nicholson et al. 1999). Currently, the terms "metabolomic" and "metabonomic" are considered to be synonymous. In short, metabolomic analysis refers to the comprehensive study of the responses of small molecules (metabolites) to experimental or environmental challenge. High-throughput analytical techniques (e.g. GC-, CE- and LC-MS, and NMR) are used to measure multiple metabolites simultaneously in biological samples.

In nutrigenetic and nutrigenomic research, metabolomic analysis can be used to determine how food-derived metabolites are modified by the consumer, including by the intestinal microbiota, and how they may influence gene expression to produce a certain phenotype. Metabolomic analysis can also be used to identify metabolite biomarkers that indicate consumption of particular foods or the consumer's state of health and risk of disease. This chapter provides a brief introduction to metabolomic methodologies, and explains how metabolomic analysis can be applied in nutrigenomic and nutrigenetic research. Some examples of applications of metabolomics analysis to nutrigenetic and nutrigenomic research are provided.

Metabolomic methodologies

Analytical platforms

Metabolomic analysis requires analytical technologies that are capable of the precise quantification of numerous and diverse metabolites in biological samples. No single analytical technique can measure the complete metabolome due to the diverse chemical properties of metabolites and the limitations of the analytical technologies presently available. The use of different biological samples, sample processing methods and analytical technologies (platforms and instruments) results in the measurement of a broad range of metabolites and is required for comprehensive analysis of the metabolome.

The choice of analytical platforms and instruments depends on the classes of metabolites that may, *a priori*, be of interest, and whether a targeted or non-targeted approach to their measurement is to be taken. In a non-targeted approach, no prior selection of metabolites for precise quantification is made and the methodology focuses on the measurement of the maximum number of metabolites rather than on the type or chemical identity of these metabolites. This approach may measure metabolites of unknown chemical structure and allows for the discovery of experimental effects on novel metabolites of as yet unknown function. Thus sample preparation is usually minimal to enable the retention in the sample extract of as many metabolites as possible. This non-targeted approach is more suitable for metabolite profiling studies that do not require metabolite identification and where the pattern of metabolites signals, rather than biochemical processes, is of interest or will be used to discriminate between classes of samples. In contrast, a targeted approach involves the precise measurement of specific metabolites or classes of metabolites (e.g. B-vitamins and tryptophan metabolites (Midttun et al.

2009)). Sample preparation is usually more extensive to preserve and enrich for these metabolites, and instrument settings are optimized for enhanced and selective detection to aid accurate quantification of the metabolites.

The major analytical platforms used to measure metabolites in metabolomic analysis are mass spectrometry (usually coupled with chromatographic instruments, e.g. the hyphenated techniques of gas chromatography (GC-), liquid chromatography (LC-) and capillary electrophoresis (CE-) mass spectrometry), and Nuclear Magnetic Resonance (NMR) spectroscopy, described as follows.

Mass spectrometry

Mass spectrometry refers to the measurement of the apparent masses (technically the mass/charge ratios) of metabolites or the products of their fragmentation. Metabolites are introduced into the high vacuum of the mass spectrometer where they are ionized (converted into charged ions) or are broken into smaller ionized fragments which can be manipulated and measured by the instrument. The fragmentation of a metabolite is generally dependent on its chemical structure, thus unique mass spectra (graphs of mass fragment ions and their abundance) can be generated for most metabolites and used for compound identification.

For metabolomic analysis, mass spectrometers are usually coupled to chromatographic instruments to give a separation of the metabolites in a sample prior to mass spectrometric detection. This increases the number of individual metabolites that can be detected and measured. The type of chromatographic instrument interfaced to the mass spectrometer depends

on the metabolites of interest, but is typically a gas or liquid chromatograph or uses capillary electrophoresis.

In gas chromatography-mass spectrometry (GC-MS) analysis, liquid samples are vaporized in the injector and carried in a flow of helium gas through a capillary column which is coated internally with an adsorbent film. The temperature of the column is varied during the chromatographic run. Thus the separation of chemical compounds is mainly influenced by their boiling points, as well as their chemical affinity with the internal coating in the capillary. This technique is ideal for analyzing volatile compounds such as flavor volatiles and fatty acids that are sufficiently thermally stable to be easily vaporized for entry into the GC column. For non-volatile polar metabolites such as simple sugars and amino acids, a chemical derivatization (e.g. conversion to their trimethylsilyl ethers) is required to increase their thermal stability and volatility before they can be analyzed in this way (Halket et al. 2005).

In liquid chromatography-mass spectrometry (LC-MS), a liquid mobile phase (solvent) is used to carry compounds through a column packed with fine particles coated with an adsorbent surface. Separation of metabolites is achieved through the different affinities of metabolites for the chemical surface of the particles in the column and by progressively changing the composition of the liquid solvent phase. A wide range of both polar and non-polar metabolites, including structural and stereoisomers, can be separated by using different combinations of chromatographic LC columns and mobile phases.

The third mass spectrometry-coupled chromatographic technique with increasing use and

significance in metabolomic analysis is capillary electrophoresis-mass spectrometry (CE-MS). CE-MS is generally used for the measurement of charged compounds (Monton and Soga 2007) where the metabolites are separated according to charge and size in the electro-osmotic flow generated by the application of a high voltage to a buffered solution in a silica capillary.

Further information about mass spectrometric metabolomic analysis and the types of mass spectrometers can be found in other more extensive reviews (Dettmer et al. 2007; Kind and Fiehn 2010, Want et al. 2010).

NMR spectroscopy

Atoms whose nuclei possess appropriate magnetic properties, such as the stable isotopes ^{1}H and ^{13}C, absorb radiofrequency energy when subjected to a magnetic field. This energy absorption, which occurs at particular radiofrequencies, is referred to as Nuclear Magnetic Resonance (NMR) and is reported as the chemical shift. Energy absorption by the various nuclei in a metabolite depends on the type of nuclei and the structural arrangement of the atoms (the chemical environment). Metabolites can therefore be identified according to the pattern of their chemical shifts. Where these patterns of chemical shifts do not totally overlap, prior separation of the metabolites in the sample is not required for quantification. This enables multiple metabolites to be measured with minimal sample processing in complex samples such as urine or serum. NMR procedures for metabolomic analysis of biological samples are described in detail by Beckonert et al. 2007.

Mass spectrometry compared to NMR

The relative advantages and limitations of the various hyphenated mass spectrometry and NMR spectroscopy techniques provide different opportunities for surveying the complexity of the metabolome (Table 11.1). All these techniques are widely used in metabolomic analysis. In terms of reproducibility and ease of quantification, NMR performs better than mass spectrometry. However, NMR is a relatively insensitive technique that generally provides information about only the more highly abundant metabolites. The measurement and identification of low concentration metabolites are hindered by the presence of metabolites of higher concentrations in the sample, as the NMR resonances of the less abundant metabolites are frequently overshadowed by those of the more abundant metabolites. Since NMR is non-destructive to samples, these less abundant metabolites can however be recovered for analysis by more sensitive and selective mass spectrometric methods.

The coupling of mass spectrometers to chromatographic instruments for prior separation of metabolites in a complex sample increases the number of detectable metabolites. In addition, mass spectrometry is better suited to detecting low abundant metabolites, provided that suitable operating parameters (e.g. chromatographic separation from interfering metabolites and appropriate ionization conditions) are found. This is especially in the case of LC-MS where efficient ionization of metabolites is required for high sensitivity. When mixtures of metabolites are analyzed by LC-MS, selective ionization of the more easily ionized metabolites commonly occurs (ion suppression) resulting in poor quantitation or even the failure to detect particular metabolites. This problem is usually overcome by chromatographic separation of metabolites.

Chemical derivatization (e.g. formation of trimethylsilyl ether derivatives) is required before non-volatile metabolites such as sugars, and organic and amino acids, can be analyzed by GC-MS. Such derivatizations are not generally used in LC-MS analysis although they can be used to enhance the detection of specific classes of metabolites. Chemical derivatization can complicate the analysis through formation of multiple chemical derivatives and the production of chemical artifacts. Thus LC-MS analysis offers faster sample throughput and greater flexibility compared to GC-MS analysis that involves chemical derivatization.

However, metabolite identification for GC-MS is greatly aided using computer-based matching to published mass spectral libraries and databases, as the use of electron ionization at a standard 70 electron volts ionization energy results in highly reproducible mass spectra. In comparison, the reproducibility of LC-MS mass spectra is poor due to the use of different ionization techniques, energies and the effects of the sample matrix (Halket et al. 2005).

Overall, the respective chromatographic-coupled mass spectrometry techniques each have their own advantages and limitations. Ultimately, the choice of analytical platforms and instruments depends on their availability and the particular classes of metabolites which may be of interest. Ideally more than one platform or instrument should be used for a more comprehensive coverage of the metabolome.

Data analysis

The data generated from metabolomic analysis of biological samples consist of measurements of the absolute and relative concentrations of many metabolites. These measurements collectively

represent the metabolite profile of the sample. Depending on the type of instrument, data processing is required to convert the information about metabolites from the raw data files into a form suitable for statistical analysis. For example, relevant chemical signals such as peak areas of ions arising from individual metabolites must be extracted from GC-MS or LC-MS mass spectrum. The peak areas from the spectrum of several samples have to be aligned (corrected for differences in chromatographic retention times between samples) and collated into a single data file for statistical analysis. Data processing software is usually provided with the instrument but may be instrument dependent or limited in function. Open source software that is instrument independent is also available, such as XCMS and MzMine (Tohge and Fernie 2009, Smith et al. 2006).

Normalization is an essential data processing step that is required to correct for factors that could bias the interpretation of the data, such as variation arising from analytical processing (e.g. batch differences) or the biological condition of the samples (e.g. urine concentration). Various data normalization techniques are available (Craig et al. 2006; Dieterle et al. 2006). The inclusion of quality control samples throughout the process of sample analysis assists in monitoring and correcting for any changes in analytical performance. Recommended quality control samples are pooled aliquots of all the samples in an experiment, as every metabolite present in any sample will have its corresponding reference in the pooled quality control samples (Sangster et al. 2006).

Metabolomic data consists of measurements of multiple metabolites together with values for relatively few other experimental parameters (treatments, time points). Such data can be analyzed using multivariate statistical techniques, which consist of unsupervised and supervised

classification methods (Trygg et al. 2007). Unsupervised classification methods, such as Principal Components Analysis (PCA) and hierarchical clustering, analyze the variation in the data without prior designation of samples into their classes (Manly 2005, Ringner 2008). Such methods are useful for identifying unexpected variations and trends in the data. Supervised classification methods, such as multiple T-tests and partial-least squares discriminant analysis (PLS-DA), compare the variation of samples according to their designated classes. Such methods are used for identifying the variables (metabolites) that contribute to the differences between the sample classes. Free web-based metabolomic data analysis software incorporating these statistical analyses, such as MetaboAnalyst, are now available (Xia et al. 2009).

Public mass spectral databases of metabolites, such as Golm, METLIN and MassBank, are available to assist in structure identification (Kopka et al. 2005; Smith et al. 2005; Horai et al. 2010). Public websites with information on metabolites and metabolic pathways are also available to assist in the interpretation of the function of metabolites of interest (Tohge and Fernie 2009). Examples include the Human Metabolome Database (HMDB)(Wishart et al. 2009) and the KEGG Pathway database (www.genome.jp/kegg).

Metabolomic applications in nutrigenomics/nutrigenetics

Nutrigenetic and nutrigenomic research seeks to increase our understanding of the interaction between foods, genes and nutrition by studying the effects of diet on gene expression and by studying genetic differences in dietary response. Knowledge gained from nutrigenetic and nutrigenomic research can be used to develop personalized nutrition strategies for optimum health. To achieve all these aims, an understanding of the metabolism of the food of interest and

the identification of the bioactive food compound is necessary. Quantification of exposure to the bioactive compound is required to study its molecular and physiological effects. Biomarkers to assess health response to bioactive food components are also required to evaluate beneficial effects. These requirements can be achieved using metabolomic analysis, as the technology enables multiple metabolites to be screened in parallel in biofluids, tissues or food (Figure 11.1).

Identification of bioactive food components

The use of metabolomic techniques to profile food metabolites is not a novel concept as chromatography-coupled mass spectrometry techniques and high-performance liquid chromatography (HPLC) have been used to characterize the composition of plant-based foods long before the term "metabolomic" was coined. Over the years, instrument technologies are becoming more powerful with increasing sensitivity to identify bioactive compounds in foods. The process of identifying bioactive compounds in a food may involve separating the components of the food into fractions according to solvent solubility or chromatography, followed by testing of the fractions for bioactivity in *in vitro* cell-based assays or *in vivo* animal feeding studies (bioassay-guided fractionation). Metabolomic analysis can then be performed on the bioactive fractions to identify the metabolites that are present. For example, fractionation of extracts from raspberry fruit by preparative HPLC by Mullen et al. 2002, and gel filtration by Ross et al. 2007, led to a partial discrimination of metabolites responsible for the antioxidant and cellular anti-proliferative properties of these extracts.

Isolation or synthesis of the tentatively identified bioactive compounds may need to be carried out to confirm chemical identity. These samples can then be tested using the *in vitro* or *in vivo*

models to confirm their bioactivity. Where the bioactivity of a food is ill-defined or is not related in a simple way to one or a few food constituents, the bioassay-guided approach fails. In this case, bioactivity may have to be represented by the metabolite profile of the food, which may be all the metabolites measured, or a subset of metabolites that best discriminates the food according to its bioactivity.

Metabolomic analysis may also be used to probe the relationship between food composition or bioactivity with the genetic variation of the plants or animals that the food was derived from (Heuberger et al. 2010, Stewart et al. 2007). For example, LC-MS metabolite profiles of cooked rice from diverse rice varieties, performed by Heuberger et al. 2010, could differentiate the rice varieties according to their subspecies classification. The total phenolics and vitamin E levels were linked to single nucleotide polymorphisms (SNPs) of genes in the biosynthesis pathways of these compounds. In another example, LC-MS profiling of polyphenolic levels of raspberry fruits, performed by Stewart et al. 2007, showed that the metabolites levels were influenced by either environmental conditions or genetic variety, indicating that the levels of some polyphenols were regulated by rigorous genetic control. Overall, these studies demonstrate that metabolomic analysis in conjunction with genomic analysis can uncover genetic influences on the regulation of the levels of bioactive or nutritional metabolites in the food. This knowledge can be used to optimize food composition through environmental or genetic manipulation of the plant or animal.

Quantification of dietary exposure

Accurate quantification of both food composition and of bioactive food components is required

to study the molecular or physiological effects of foods. Conventional methods of measuring dietary exposure such as self-reporting of dietary intakes by study participants and the use of food composition databases are inadequate and widely acknowledged to be flawed (Macdiarmid and Blundell 1998). Self-reporting depends on the individual's memory, self awareness, honesty and dietary response to the data collection process. Food composition databases provide only a general or standardized value for the concentration of a food component which may vary according to the food source (e.g. the effect of natural plant variation or the use of different cultivars), food preparation (e.g. processing, cooking) and the analytical methods used to determine the composition. Additionally, the amount of the food component consumed does not necessarily reflect the actual amount absorbed by the body due to differences between individuals in selective absorption by intestinal transporters or metabolism by the intestinal microbiota. Measurement of food-derived metabolites that appear in biofluids such as urine and plasma will give a better indication of the actual amount absorbed by the body. An understanding of the metabolism of the food component, and time-response relationships between consumption of the food component and the appearance of metabolites in biofluids is thus required to select a suitable metabolite biomarker of exposure to the food component.

The ability of metabolomic analysis to screen multiple metabolites in parallel makes the technique suitable for analyzing biofluids and tissues for metabolites produced by the metabolism of food components (Fardet et al. 2008; Stalmach et al. 2009; Urpi-Sarda et al. 2009; Winning et al. 2009; Llorach et al. 2010; O'Sullivan et al. 2011) or by the action of the intestinal microbiota on non-digestible food components (Jacobs et al. 2009). Fardet et al. 2008 used LC-MS to analyze urine collected from rats fed with diets supplemented with lignins or phenolic

acids. Data analysis using PLS-DA showed that the metabolic fingerprints of the urine samples were similar between lignin-supplemented diets and control diets, confirming that lignins are largely inert to digestion and are not absorbed into the body. In contrast, various metabolites arising from the metabolism of the phenolic acids were identified.

In a further example, Stalmach et al. 2009 studied the metabolism of coffee polyphenols in humans by using LC-MS to analyze urine and plasma collected at several time points within 24 hours after drinking coffee. Coffee components that were absorbed by the small intestine or large intestine could be differentiated according to their presence in urine and plasma samples at different time points. Metabolites that were quickly excreted were also identified according to their absence in plasma and presence in urine. Urinary dihydrocaffeic acid-3-O-sulfate and feruloylglycine were proposed as potential and sensitive biomarkers for coffee consumption. Overall, these studies demonstrate that metabolomic analysis of biofluids can be used to understand the metabolism of food components and to identify biomarkers of food intake.

Assessment of dietary intervention

The goal of nutrigenetics and nutrigenomic research is to develop personalized dietary strategies for the optimal health of human individuals. Biomarkers of health status and of responsiveness to dietary components are therefore required to evaluate the efficacy of nutrigenomic and nutrigenetic dietary regimes. The metabolite profile of an organism is closely associated with its genotype (Fiehn et al. 2000; Assfalg et al. 2008), as genes code for proteins that participate in metabolic pathways. The influence of external factors on gene expression and biochemical pathways, such as diet and pathogens, will lead to changes in the metabolite profile. Therefore

the levels of metabolites in biofluids can provide information about the health status of an organism and their response to diet. Metabolite biomarkers of diseases are already successfully used in clinical practice – elevated glucose levels in blood after fasting as an indicator of diabetes, and creatinine levels in blood as an indicator of renal function. Nevertheless, new and more accurate biomarkers of diseases and therapeutic response are always sought after.

Metabolomic analysis of biofluids and tissues has the potential for biomarker discovery. Ideally, large well-characterized cohorts with longitudinal samples should be used for biomarker discovery. The accuracy of potential biomarkers must be validated in independent cohorts. A few noteworthy examples of biomarker discovery by metabolomic analysis are described as follows:

Wang T. J. et al. 2011 identified a metabolite profile that predicted future diabetes, by performing LC-MS metabolomic analysis on plasma samples of 189 non-diabetic individuals who later developed diabetes within 12 years, and 189 matched controls. Approximately 60 metabolites were measured using a targeted approach. Elevated levels of five amino acids, isoleucine, leucine, valine, tyrosine and phenylalanine, predicted future diabetes. This result was validated in a further study comprising 163 cases and 163 controls. The authors cited other studies that supported the role of amino acids in pathogenesis of diabetes, such as the development of insulin resistance in animals and humans supplemented with branched-chain amino acids, and correlation of fasting concentrations of amino acid with obesity and insulin levels.

Holmes et al. 2008 identified potential urinary biomarkers of cardiovascular disease risk by

performing NMR metabolomic analysis of urine samples from 4630 individuals from East Asian and Western populations. The urinary metabolite profiles discriminated populations according to geographical regions, diets and blood pressure. Formate, alanine and hippuric acid were associated with blood pressure and were identified as potential biomarkers of cardiovascular disease risk.

The identification of accurate biomarkers for disease is challenging due, in part, to the genetic variation that occurs within human populations. Distinct metabolite profiles, associated with specific genetic variants, can be determined by combined genetic genome-wide association studies and metabolomic analysis as demonstrated by Gieger et al. 2008, Illig et al. 2010 and Suhre et al. 2011. The cumulative work of these three studies revealed associations between blood metabolite levels and genetic loci related to disease and of pharmaceutical relevance. Overall, the levels of over 250 metabolites from 60 biochemical pathways were measured in serum samples from 2820 individuals in two large population-based European cohorts (German KORA and British TwinsUK studies), using liquid chromatography-tandem mass spectrometry (LC-MS-MS). Geiger et al. 2008 established that the ratios of metabolites as a surrogate for enzymatic activity explained a higher proportion of genetic variance than the use of single metabolite concentrations alone. Illig et al. 2010 showed that for 8 of 9 genetic loci, the genetic variant is located in or near genes encoding enzymes or solute carriers with biochemical relevance to the metabolites. Finally, Suhre et al. 2011 identified 37 genetic loci that were associated with metabolite concentrations, of which 25 of the loci account for 10-60% of the differences in metabolite levels per allele copy. Cross-reference of these genetic loci to databases of disease-related and pharmaceutically-relevant genetic associations provided new insights into

the functions of these loci and confirmed previous associations. For example, SLC2A9 gene variants were associated with uric acid concentrations and self-reported gout (Doring et al. 2008).

Even when genetic variants are not known, metabolite profiles can identify subgroups that are likely to respond differently to a diet. Clayton et al. 2006 showed that the urinary metabolite profiles of rats prior to drug treatment could predict their response towards paracetamol. The urinary profiles were generated by NMR analysis. In another example, Rezzi et al. 2007 showed that metabolites profiles could be linked to dietary preference, where chocolate-loving and chocolate-indifferent individuals have metabolite differences which are present even in the absent of chocolate stimulation. The metabolite profiles of the individuals were measured by NMR analysis. Some of the differential metabolites were derived from intestinal microbiota metabolism, suggesting that dietary habits may influence intestinal microbiota profile. Dietary preference can affect health, and is influenced by taste response which is genetically mediated (Rezzi et al. 2007).

Overall, these studies demonstrate the potential of metabolomic analysis in identifying biomarkers of disease that may be used to assess the efficacy of dietary interventions. The advantage of using metabolites biomarkers in biofluids to assess health status is that these fluids can be sampled repetitively without dire consequences to the animal or human subject. This is useful in both animal and human studies, as the effect of dietary intervention can be monitored over time and the outcome of the experiment may be quickly determined through early changes in metabolite biomarkers. Repetitive sampling of biofluids is also required for monitoring human patients and provides a form of biological replication to confirm findings.

Discovery of food and gene interactions

The identification of metabolite biomarkers of dietary exposure or disease risk can provide insights into the pathways of the disease process and their modulation by food components. Specific genotypes associated with these pathways that may benefit from dietary modulation can be determined. These points are demonstrated by a noteworthy study by Wang Z. et al. 2011 who performed non-targeted metabolomic analysis of plasma samples to identify metabolite profiles that could predict the risk of cardiovascular disease. The plasma of 50 stable patients who later suffered a heart attack, stroke or death within three years, and of 50 matched controls were analyzed by LC-MS. A further cohort of 25 cases and 25 controls was used to validate the results and to narrow down the number of metabolites to 18 metabolites associated with cardiovascular risk. Three of these 18 metabolites were strongly correlated to each other. Further structural elucidation analysis using NMR, mass spectrometry, LC-MS-MS and GC-MS-MS, identified these three metabolites as trimethylamine N-oxide (TMAO), choline and betaine. Further validation using an independent cohort of 1876 subjects confirmed that fasting plasma levels of these three metabolites were associated with risk of cardiovascular disease. Animal feeding studies with isotope-labeled compounds or antibiotics showed that TMAO is derived from trimethylamine, which is produced by intestinal microbial metabolism of betaine and choline, where the latter is derived from dietary phosphatidylcholine (lecithin). TMAO is produced from trimethylamine through the action of hepatic flavin mono-oxygenases. The authors found that TMAO plasma levels correlated with the size of artherosclerotic plaques in artherosclerotic-prone mice supplemented with choline. Furthermore, TMAO plasma levels, aortic lesion formation and HDL cholesterol concentrations correlated with the expression of hepatic flavin

monooxygenases in mice. A genetic locus containing the flavin mono-oxygenase gene cluster had a strong effect on atherosclerosis in the mice. Examination of plasma TMAO levels in humans showed correlations to hepatic expression levels of flavin mono-oxygenase. Overall, these findings by Wang Z. et al. 2011 indicate that the cardiovascular risk of individuals with certain flavin mono-oxygenase genetic variants may be modulated by their dietary choline, and that plasma level of TMAO may be a potential biomarker of cardiovascular health status.

Metabolomic analysis can also be used to understand the differential effects of specific genotypes on the absorption and metabolism of foods. For example, Lin et al. 2011 performed non-targeted GCMS analysis of urine samples from interleukin-10-deficient ($IL10^{-/-}$) mice fed with extracts of the fruits of yellow- and green-fleshed kiwifruits. The $IL10^{-/-}$ mouse is a model of inflammatory bowel disease, and develops intestinal inflammation in the presence of intestinal microbiota. The authors found that $IL10^{-/-}$ mice had a higher excretion of metabolites associated with the kiwifruit diet compared with the wildtype mice. These differences in the excretion of diet-associated metabolites possibly arise through differences in intestinal microbial metabolism or through an increase in intestinal permeability, which are both known consequences of IL10 gene deficiency and of inflammatory bowel disease (Bibiloni et al. 2005, Kennedy et al. 2000). In this example, the effect of the gene variant on food metabolites was not through the direct interaction of the gene with the food metabolites, demonstrating the usefulness of metabolomic analysis in identifying unexpected effects of gene variants on food metabolism.

Summary

Metabolomic analysis is the comprehensive study of small molecule metabolites using

technologies such as mass spectrometry and NMR which enable high-throughput parallel measurements of the many metabolites in a complex biological sample. Applications of metabolomic analysis in nutrigenetic and nutrigenomic research include the identification of bioactive food components and food-derived metabolites, the quantification of dietary exposure to metabolites, the identification of biomarkers to assess the efficacy of dietary interventions, and the discovery of novel food-gene interactions.

References

Assfalg, M., I. Bertini, D. Colangiuli et al. 2008. Evidence of different metabolic phenotypes in humans. Proc Natl Acad Sci U S A 105: 1420-1424.

Beckonert, O., H. C. Keun, T. M. Ebbels et al. 2007. Metabolic profiling, metabolomic and metabonomic procedures for NMR spectroscopy of urine, plasma, serum and tissue extracts. Nat Protoc 2: 2692-2703.

Bibiloni, R., M. A. Simon, C. Albright, B. Sartor and G. W. Tannock 2005. Analysis of the large bowel microbiota of colitic mice using PCR/DGGE. Lett Appl Microbiol 41: 45-51.

Clayton, T. A., J. C. Lindon, O. Cloarec et al. 2006. Pharmaco-metabonomic phenotyping and personalizd drug treatment. Nature 440: 1073-1077.

Craig, A., O. Cloarec, E. Holmes, J. K. Nicholson and J. C. Lindon 2006. Scaling and normalization effects in NMR spectroscopic metabonomic data sets. Anal Chem 78: 2262-2267.

Dettmer, K., P. A. Aronov and B. D. Hammock 2007. Mass spectrometry-based metabolomics. Mass Spectrom Rev 26: 51-78.

Dieterle, F., A. Ross, G. Schlotterbeck and H. Senn 2006. Probabilistic quotient normalization as robust method to account for dilution of complex biological mixtures. Application in 1H NMR metabonomics. Anal Chem 78: 4281-4290.

Doring, A., C. Gieger, D. Mehta et al. 2008. SLC2A9 influences uric acid concentrations with pronounced sex-specific effects. Nat Genet 40: 430-436.

Fardet, A., R. Llorach, A. Orsoni et al. 2008. Metabolomics provide new insight on the metabolism of dietary phytochemicals in rats. J Nutr 138: 1282-1287.

Fiehn, O. 2001. Combining genomics, metabolome analysis, and biochemical modelling to understand metabolic networks. Comp Funct Genomics 2: 155-168.

Fiehn, O., J. Kopka, P. Dormann et al. 2000. Metabolite profiling for plant functional genomics. Nat Biotechnol 18: 1157-1161.

Gieger, C., L. Geistlinger, E. Altmaier et al. 2008. Genetics meets metabolomics: a genome-wide association study of metabolite profiles in human serum. PLoS Genet 4: e1000282.

Halket, J. M., D. Waterman, A. M. Przyborowska et al. 2005. Chemical derivatization and mass spectral libraries in metabolic profiling by GC/MS and LC/MS/MS. J Exp Bot 56: 219-243.

Heuberger, A. L., M. R. Lewis, M. H. Chen et al. 2010. Metabolomic and functional genomic analyses reveal varietal differences in bioactive compounds of cooked rice. PLoS One 5: e12915.

Holmes, E., R. L. Loo, J. Stamler et al. 2008. Human metabolic phenotype diversity and its association with diet and blood pressure. Nature 453: 396-400.

Horai, H., M. Arita, S. Kanaya et al. 2010. MassBank: a public repository for sharing mass spectral data for life sciences. J Mass Spectrom 45: 703-714.

Illig, T., C. Gieger, G. Zhai et al. 2010. A genome-wide perspective of genetic variation in human metabolism. Nat Genet 42: 137-141.

Jacobs, D. M., E. Gaudier, J. van Duynhoven and E. E. Vaughan 2009. Non-digestible food ingredients, colonic microbiota and the impact on gut health and immunity: a role for metabolomics. Curr Drug Metab 10: 41-54.

Kennedy, R. J., M. Hoper, K. Deodhar et al. 2000. Interleukin 10-deficient colitis: new similarities to human inflammatory bowel disease. Br J Surg 87: 1346-1351.

Kind, T. and O. Fiehn 2010. Advances in structure elucidation of small molecules using mass spectrometry. Bioanal Rev 2: 23-60.

Kopka, J., N. Schauer, S. Krueger et al. 2005. GMD@CSB.DB: the Golm Metabolome Database. Bioinformatics 21: 1635-1638.

Lin, H. M., S. J. Edmunds, S. Zhu, L. R. Ferguson and D. D. Rowan 2011. Metabolomic analysis reveals differences in urinary excretion of kiwifruit-derived metabolites in a mouse model of inflammatory bowel disease. Molecular Nutrition and Food Research in press.

Llorach, R., I. Garrido, M. Monagas et al. 2010. Metabolomics study of human urinary metabolome modifications after intake of almond (Prunus dulcis (Mill.) D.A. Webb) skin polyphenols. J Proteome Res 9: 5859-5867.

Macdiarmid, J. and J. Blundell 1998. Assessing dietary intake: Who, what and why of under-reporting. Nutr Res Rev 11: 231-253.

Manly, B. F. J. 2005. Multivariate statistical methods: a primer. Boca Raton, Chapman & Hall/CRC Press.

Midttun, O., S. Hustad and P. M. Ueland 2009. Quantitative profiling of biomarkers related to B-vitamin status, tryptophan metabolism and inflammation in human plasma by liquid chromatography/tandem mass spectrometry. Rapid Commun Mass Spectrom 23: 1371-1379.

Monton, M. R. and T. Soga 2007. Metabolome analysis by capillary electrophoresis-mass spectrometry. J Chromatogr A 1168: 237-246.

Mullen, W., J. McGinn, M. E. Lean et al. 2002. Ellagitannins, flavonoids, and other phenolics in red raspberries and their contribution to antioxidant capacity and vasorelaxation properties. J Agric Food Chem 50: 5191-5196.

Nicholson, J. K., J. C. Lindon and E. Holmes 1999. 'Metabonomics': understanding the metabolic responses of living systems to pathophysiological stimuli via multivariate statistical analysis of biological NMR spectroscopic data. Xenobiotica 29: 1181-1189.

O'Sullivan, A., M. J. Gibney and L. Brennan 2011. Dietary intake patterns are reflected in metabolomic profiles: potential role in dietary assessment studies. Am J Clin Nutr 93: 314-321.

Rezzi, S., Z. Ramadan, F. P. Martin et al. 2007. Human metabolic phenotypes link directly to specific dietary preferences in healthy individuals. J Proteome Res 6: 4469-4477.

Ringner, M. 2008. What is principal component analysis? Nat Biotechnol 26: 303-304.

Ross, H. A., G. J. McDougall and D. Stewart 2007. Antiproliferative activity is predominantly associated with ellagitannins in raspberry extracts. Phytochemistry 68: 218-228.

Sangster, T., H. Major, R. Plumb, A. J. Wilson and I. D. Wilson 2006. A pragmatic and readily implemented quality control strategy for HPLC-MS and GC-MS-based metabonomic analysis. Analyst 131: 1075-1078.

Smith, C. A., G. O'Maille, E. J. Want et al. 2005. METLIN: a metabolite mass spectral database. Ther Drug Monit 27: 747-751.

Smith, C. A., E. J. Want, G. O'Maille, R. Abagyan and G. Siuzdak 2006. XCMS: processing mass spectrometry data for metabolite profiling using nonlinear peak alignment, matching, and identification. Anal Chem 78: 779-787.

Stalmach, A., W. Mullen, D. Barron et al. 2009. Metabolite profiling of hydroxycinnamate derivatives in plasma and urine after the ingestion of coffee by humans: identification of biomarkers of coffee consumption. Drug Metab Dispos 37: 1749-1758.

Stewart, D., G. J. McDougall, J. Sungurtas et al. 2007. Metabolomic approach to identifying bioactive compounds in berries: advances toward fruit nutritional enhancement. Mol Nutr Food Res 51: 645-651.

Suhre, K., S.-Y. Shin, A.-K. Petersen et al. 2011. Human metabolic individuality in biomedical and pharmaceutical research. Nature 477: 54-60.

Tohge, T. and A. R. Fernie 2009. Web-based resources for mass-spectrometry-based metabolomics: A user's guide. Phytochemistry.

Trygg, J., E. Holmes and T. Lundstedt 2007. Chemometrics in metabonomics. J Proteome Res 6: 469-479.

Urpi-Sarda, M., M. Monagas, N. Khan et al. 2009. Targeted metabolic profiling of phenolics in urine and plasma after regular consumption of cocoa by liquid chromatography-tandem mass spectrometry. J Chromatogr A 1216: 7258-7267.

Wang, T. J., M. G. Larson, R. S. Vasan et al. 2011. Metabolite profiles and the risk of developing diabetes. Nat Med 17: 448-453.

Wang, Z., E. Klipfell, B. J. Bennett et al. 2011. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Nature 472: 57-63.

Want, E. J., I. D. Wilson, H. Gika et al. 2010. Global metabolic profiling procedures for urine using UPLC-MS. Nat Protoc 5: 1005-1018.

Winning, H., E. Roldan-Marin, L. O. Dragsted et al. 2009. An exploratory NMR nutri-metabonomic investigation reveals dimethyl sulfone as a dietary biomarker for onion intake. Analyst 134: 2344-2351.

Wishart, D. S., C. Knox, A. C. Guo et al. 2009. HMDB: a knowledgebase for the human metabolome. Nucleic Acids Res 37: D603-610.

Xia, J., N. Psychogios, N. Young and D. S. Wishart 2009. MetaboAnalyst: a web server for metabolomic data analysis and interpretation. Nucleic Acids Res.

Table 11.1. Comparison of different technologies for performing metabolomic analysis.

<table>
<tr><td></td><td colspan="3">Mass spectrometry</td><td>NMR</td></tr>
<tr><td rowspan="3"></td><td colspan="3">Measures mass of ionised molecules or molecular fragments resulting from fragmentation of metabolites in a vacuum</td><td rowspan="3">Measures energy absorption of atomic nuclei in metabolites at a certain radiofrequency in a magnetic field</td></tr>
<tr><td>Gas chromatography</td><td>Liquid chromatography</td><td>Capillary electrophoresis</td></tr>
<tr><td>Separation of metabolites using a gas mobile phase and a stationary phase in a capillary column</td><td>Separation of metabolites using a liquid mobile phase and a stationary phase in a packed column</td><td>Separation of metabolites in an electrically-charged liquid phase in a silica capillary</td></tr>
<tr><td>Type of metabolites detected</td><td>Volatile compounds</td><td>Various</td><td>Charged compounds</td><td>Various</td></tr>
<tr><td>Quantification</td><td colspan="3">Optimisation of chromatographic separation and ionisation conditions required for precise quantification of a wide range of metabolites</td><td>Easy and accurate for abundant metabolites</td></tr>
<tr><td>Reproducibility</td><td>Highly reproducible mass spectra that can be used to identify compounds by matching to mass spectral libraries</td><td>Mass spectra influenced by ionisation technique and sample matrix</td><td></td><td>High reproducibility for abundant metabolites</td></tr>
<tr><td>Sensitivity</td><td colspan="3">High sensitivity for metabolites that can be ionised</td><td>Low sensitivity</td></tr>
<tr><td>Sample treatment</td><td>Chemical derivatization required for non-volatile compounds. Small amounts required.</td><td>Minimal preparation. Small amounts required.</td><td>Minimal preparation. Small amounts required.</td><td>Minimal preparation. Non-destructive, sample can be reused.</td></tr>
</table>

Figure 11.1. Contributions of metabolomic analysis to nutrigenomic and nutrigenomic research.