

*A Meeting of the Nutrition Society, hosted by the Irish Section, was held at the O'Reilly Hall, University College Dublin, Dublin, Republic of Ireland on 18–20 June 2008*

## Symposium on 'The challenge of translating nutrition research into public health nutrition'

### Session 2: Personalised nutrition Metabolomic applications in nutritional research

Lorraine Brennan

*UCD Conway Institute, UCD School of Agriculture, Food Science and Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Republic of Ireland*

Metabolomics aims to profile all small molecules that are present in biological samples such as biofluids, tissue extracts and culture media. Combining the data obtained with multivariate data analysis tools allows the exploration of changes induced by a biological treatment or changes resulting from phenotype. Recently, there has been a large increase in interest in using metabolomics in nutritional research and because of the intimate relationship between nutrients and metabolism there exists great potential for the use of metabolomics within nutritional research. However, for metabolomics to reach its full potential within this field it is also important to be realistic about the challenges that are faced. Examples of such challenges include the necessity to have a clear understanding of the causes of variation in human metabolomic profiles, the effects of the gut microflora on the metabolomic profile and the interaction of the gut microflora with the host's metabolism. A further challenge that is particularly relevant for human nutritional research is the difficulty associated with biological interpretation of the data. Notwithstanding these and other challenges, several examples of successful applications to nutritional research exist. The link between the human metabolic phenotype, as characterised by metabolomic profiles, and dietary preferences proposes the potential role of metabolomics in personalised nutrition.

#### Metabolomics: Analytical methods: Human metabolic profiling

Metabolomics is the term used to describe the study of small molecules or metabolites present in biological samples such as biofluids, tissues and cellular extracts. The aim of metabolomics is to profile all the metabolites present in the samples to enhance the understanding of the effect of a particular stimulus on metabolic pathways. To date, the main technologies used to achieve this objective are NMR and MS. Each platform has its own advantages and disadvantages, which have been detailed in previous reviews<sup>(1–3)</sup>. These reviews include detailed arguments for and against particular platforms, and while such a detailed discussion is beyond the remit of the present article, it should be recognised that no single platform will measure all metabolites present in a biological sample.

<sup>1</sup>H NMR spectroscopy allows the simultaneous measurement of proton-containing small-molecular-weight molecules in complex biological samples. Little or no pretreatment is required and the technique is high throughput. Additionally, the technique is extremely robust and studies have shown high cross-laboratory and cross-platform reproducibility<sup>(4,5)</sup>. Recent advances in MS techniques have led to the use of hyphenated techniques such as liquid chromatography–MS and GC–MS in metabolomics. One of the main advantages of these techniques is the associated high sensitivity; however, a drawback is the necessity for sample preparation before analysis. In GC–MS metabolites are first vapourised into the gas phase and then separated by passage through a GC column. The metabolites are then ionised and the masses recorded.

**Abbreviations:** PLS-DA, partial least-square discriminant analysis.

**Corresponding author:** Dr Lorraine Brennan, fax +353 1283 7211, email [lorraine.brennan@ucd.ie](mailto:lorraine.brennan@ucd.ie)

The metabolites can be identified by comparison with databases. In liquid chromatography–MS-based metabolomics the metabolites are separated by liquid chromatography and detected using MS. Again, identification relies on comparison with databases. Advances in column technology in combination with operation at high pressures have resulted in an increase in capacity, resolution and sensitivity.

It is unfortunate for the development of the field that two descriptive terms, ‘metabolomics’ and ‘metabonomics’, appeared simultaneously in the literature. Many subsequent papers have attempted to distinguish the fields represented by these terms, often adding to the confusion. However, here, no such attempt is made and for consistency the field is referred to as ‘metabolomics’; however, any relevant literature search should also include ‘metabonomics’.

### Data analysis

As with other ‘omic’ techniques metabolomics generates large amounts of data and an important part of any metabolomics study is the analysis of the data using multivariate statistics. There are many statistical methods available for analysis of metabolomics data; the most commonly-used methods include principal component analysis, partial least-square discriminant analysis (PLS-DA) and orthogonal PLS-DA. Principal component analysis is an unsupervised technique (i.e. no information about class membership is used in the analysis) that allows visualisation of the data with the aim of identifying inherent grouping of samples as a result of the similarity of the metabolic composition. It is essentially a statistical method that reduces a large number of variables into a smaller number of principal components. The data is then visualised on a scores plot that plots the data according to the principal components. Samples with similar metabolic profiles will appear close together on the scores plot. However, using only a scores plot does not provide information on metabolites that may be causing any trend in the plot. To assess this factor a loadings plot must be used in conjunction with the scores plot and the loadings plot can be viewed as a means of examining the scores plot.

Supervised techniques (i.e. class information is used in the analysis), such as PLS-DA and orthogonal PLS-DA, require assumed knowledge of the class membership and are used to identify spectral signals that are contributing to clustering of the data. A drawback of this type of data analysis is the risk of overfitting, and models built using these approaches should always be validated with independent data. Recently, other methods for analysis of metabolomic data have appeared in the literature, including genetic algorithms, machine learning approaches, Bayesian modelling and artificial neural networks<sup>(6,7)</sup>.

### Factors affecting the human metabolic profile

The human metabolic profile or the metabolome is influenced by a number of phenotypic, physiological and external factors. Numerous studies have been carried out to assess these factors in human subjects. An investigation

of the effects of gender, age and BMI on the metabolic profiles of urine and plasma from sixty-six males and eighty-four females has found increased plasma levels of choline, LDL, HDL and unsaturated lipids in females compared with the males and higher levels of VLDL, creatinine, valine and isoleucine in males compared with females<sup>(8)</sup>. In the urine samples higher levels of taurine and creatinine were reported for males whereas citrate was higher for females. Clear age-related metabolic changes were also observed for both males and females, with distinct profiles for the young (18–29 years) and old (>46 years) subjects. In addition to gender- and age-related differences, changes related to BMI were also reported. Lean subjects (BMI <21 kg/m<sup>2</sup>) plasma levels of choline and citrate were found to be elevated, whereas for obese subjects (BMI >25 kg/m<sup>2</sup>) levels of tyrosine, isoleucine and glycoproteins were increased. Metabolic changes related to BMI were also observed in urine samples. Interpreting their results, the authors conclude that young females with a low BMI synthesise more lipids and have a lower protein turnover than young males. With higher BMI females show an increase in protein turnover while lipid synthesis remains unchanged, but for males lipid synthesis increases.

In another study investigating the effects of gender on the metabolic profiles of urine showed that the tricarboxylic acid cycle intermediates citrate and fumarate as well as creatine were higher for females than for males<sup>(9)</sup>. However, for males metabolites associated with fatty acid oxidation (carnitine, acetylcarnitine and acetone) were higher compared with females.

Population studies have suggested that diet plays an important role in determining the metabolic profiles<sup>(5,10)</sup>. An investigation of the effects of standardising the diet for 24 h before biofluid collection<sup>(11)</sup> has shown that following the standardisation the inter-subject variability of the urinary metabolic profiles was reduced whereas there were no effects on plasma. From this and other studies it is clear that dietary intake has an impact on the metabolic profiles of healthy human subjects. Deciphering the exact relationship between diet and the influence on the metabolic profiles will be an important step in the future.

Diurnal variation has been documented in urine samples taken from healthy volunteers<sup>(11,12)</sup>. Morning and night urine samples from thirty healthy volunteers have shown a clear separation between the metabolic profiles, with creatinine being the most discriminating metabolite<sup>(11)</sup>. Furthermore, in 146 morning and 196 afternoon urine samples some of the major differences have been reported to be related to dietary factors (mannitol and xylose) and the gut microflora (dimethylamine), with other differences including higher concentrations of creatinine in the morning samples<sup>(9)</sup>.

In recent years, there has been considerable interest in the interactions between gut microflora and host metabolism. Numerous animal studies have suggested a relationship between gut microflora and the appearance of certain metabolites in the urine<sup>(13–15)</sup>. While it is reasonable to assume that such a relationship exists in human subjects, the direct evidence is still lacking for many urinary metabolites. Nevertheless, a connection between the appearance of equol in human urine and certain gut bacteria is well

established<sup>(16,17)</sup> and supports the hypothesis. The application of metabolomics to metabolic profiling of faeces offers the potential to investigate gut microflora metabolism and its interaction with host metabolism. Detailed metabolic profiling of faeces samples from thirty-nine healthy subjects at three different time points has shown inter- and intra-individual variability<sup>(18)</sup>. However, the inter-individual variability was found to be associated with variability in metabolite concentrations rather than composition, leading to the suggestion that different colonic flora ultimately metabolise substrates to the same metabolic end points. It is known that for human subjects the gut bacterial profiles do not vary greatly over time, making the observed intra-individual variability intriguing. One possible explanation could be the influence of diet, and in particular polyphenol compounds, on the metabolic profiles. While metabolic profiling of faeces from healthy volunteers offers real potential there is still a need to establish the influence of gut microflora on such profiles and on profiles of other biofluids such as urine.

In recent years important progress has been made that has enhanced the understanding of the factors that influence the metabolic profiles of human subjects; however, there are still many factors to be investigated, including the influence of genetic background, environment and lifestyle.

#### Examples of applications of metabolomics to human nutrition

While it is not the intention to give an exhaustive review of the literature relating to the application of metabolomics to nutritional research, it is useful to highlight some of the examples, thus showing the potential applications. An NMR-based metabolomics approach has been used to investigate the effects of vegetarian, low-meat and high-meat diets on metabolic pathways in human subjects<sup>(19)</sup>. Twelve healthy men enrolled into the study were provided with the diets for 15 d in a cross-over design and urine samples were collected after 10 d on each diet. It was found that the principal component plot was dominated by inter-individual variation and analysis by orthogonal PLS-DA showed clustering of the diets. Further analysis was focused on the vegetarian and high-meat diets, which contained equal amounts of protein (143–149 g protein/d). The high-meat diet was found to be associated with increased levels of creatinine, creatine, trimethylamine-N-oxide, taurine and 1- and 3-methylhistidine. Increased levels of p-hydroxyphenylacetate and decreased levels of N,N,N-trimethyllysine were found to be associated with the vegetarian diet. Overall, this study shows the potential use of the metabolomics to examine the metabolic effects of dietary modulations in human subjects and identify markers and pathways associated with certain diets.

To probe the effects of food constituents in shaping the metabolic profile the influence of modulating the intake of phytochemicals on metabolic profiles has been investigated<sup>(20)</sup>. Twenty-one healthy volunteers underwent a 6 d dietary intervention that comprised, following their normal diet, a low-phytochemical diet and subsequently a standard-phytochemical diet. The statistical analysis of the

NMR and liquid chromatography–MS data indicates that the urinary metabolic profiles were changed following the standard-phytochemical diet. Overall, the results show that recent dietary intake modulates the metabolic profiles and that a full understanding of the effects of dietary constituents on the profiles is necessary if metabolomics is to advance in the area of human nutrition.

#### Challenges faced in applying metabolomics to nutritional research

While the potential for metabolomics in nutritional research is great, there are realistic challenges that are faced in all metabolomics applications and in applications specific to nutrition research. The first challenge relates to the technology, and from the outset of a metabolomics project it should be recognised that no technology will measure all metabolites present in the biological sample. Each of the three main technology platforms have their advantages and disadvantages, and rather than discuss these in detail a particular aspect of their application that is often overlooked will be highlighted, i.e. each of the technology platforms has an inherent bias. NMR spectroscopy is insensitive and as a result will detect metabolites that are present in high concentrations and as a consequence there is the risk of detecting ‘the usual suspects’ as the discriminating metabolites. When using MS-based techniques ion suppression introduces a bias into the type of metabolites that can be measured. With GC–MS the majority of metabolites that are measured tend to be related to amino acid pathways, glycolysis, the tricarboxylic acid cycle and  $\beta$ -oxidation<sup>(21)</sup>. As a result these pathways often appear as being important. The most comprehensive approach to metabolomics is to use a combination of platforms in an attempt to gain a broad coverage of the metabolites present; however, this approach will have cost implications for the studies.

In relation to human nutrition another challenge results from the biological variation within the human population. While recent efforts have progressed the understanding of phenotypic influences such as gender, age and BMI, there is still a lot to understand about the influence of genetic background, host–microbial interactions and other phenotypic characteristics such as body composition on metabolic profiles. Another issue that should not be understated is the influence of food and all its constituents on metabolic profiles. Progress here is essential if metabolomics is to make important advancements in the area of nutritional research.

Perhaps the biggest challenge facing the advancement of metabolomics in human nutritional research is the interpretation of the identified relevant metabolites in a biologically meaningful manner. The response to food is multifactorial, as food delivers thousands of nutrients and non-nutrients that will elicit multiple organ responses<sup>(22,23)</sup>. For many human-based metabolomics studies urine is the biofluid of choice, and relating metabolic changes observed in the urine to organ-specific changes and to relevant metabolic pathways is not trivial. Although conceptually plasma may be easier to interpret, as it gives metabolic

information at that current time and gives information about the metabolic flow between organs, it is still not easy to relate observed changes to changes occurring at the organ level. The area of animal toxicology has advanced in biological interpretation largely through research efforts such as the Consortium for Metabonomic Toxicology (known as COMET)<sup>(24,25)</sup>, a consortium of academics and industries that has built a database of biomarkers associated with a series of liver and kidney toxins. As a result of this and other efforts distinct biomarkers are now known to be associated with certain organ toxicity, and in certain cases the specific target region of toxicity can be associated with the appearance of certain metabolites in the urine<sup>(25,26)</sup>.

Ultimately, advances in biological interpretation in the future will decide whether or not metabolomics will deliver its full promise in nutritional research. In considering how to proceed in this area it is clear that an alternative approach to that used in toxicology is needed. Some of the approaches that should expand the ability to interpret metabolomics data are the combination of such data with detailed phenotypic data, the use of flux studies to determine changes in metabolic fluxes and correlation of these studies with the static or time-averaged metabolomic measurements. In addition, the use of metabolic challenge tests to disturb certain pathways and observe the metabolomic response should enhance the ability to interpret in a biological meaningful way changes observed through metabolomic studies. Compared with other 'omic' technologies metabolomics has the advantage that a large wealth of information exists in the literature on metabolic pathways and metabolic biochemistry. In the advancement of the area, the endeavour must be to build on this information rather than re-discovering it.

### Potential role of metabolomics in personalised nutrition

Although being able to give nutritional advice on the personal level is still a long way from being realised, it is possible to envisage a potential role for metabolomics in the future development of personalised nutrition. The concept of a metabolic phenotype or metabotype has been previously introduced<sup>(19,27)</sup>, and it is proposed to be influenced by a combination of genetic and environmental factors such as diet. The identification of different metabolic phenotypes in certain human populations and combination of this information with other phenotypic data to give dietary advice could be envisaged. A recent publication in the area of drug toxicology highlights the potential use of metabolomics in this area. It reports the use of a combination of predose metabolic profiling and statistical analysis to predict the response of individual animals to drug treatment<sup>(28)</sup>. Translating this result to nutritional research, it can be envisaged that metabolomics may be used to predict whether an individual will respond to a certain dietary treatment. Indeed, a recent publication in the nutrition field also supports the idea that this approach could be feasible. Twenty-two healthy male volunteers selected from seventy-five volunteers based on their chocolate preferences (chocolate loving or chocolate hating)

underwent a 1-week double-cross-over study in which they consumed either chocolate or a bread placebo on the two test days and followed a standardised diet throughout the study<sup>(29)</sup>. NMR profiling of 24 h blood and urine samples revealed that the chocolate preference of the individual could be predicted from both biofluid samples even in the absence of the chocolate stimulus. Such a prediction of the dietary preference of the individual indicates that the metabolic profile may indeed contain a wealth of information relating to the diet of an individual and it may be possible to predict dietary response, thus proposing a role for metabolomics in personalised nutrition.

### Acknowledgements

The author gratefully acknowledges support from the Health Research Board Ireland and the Department of Agriculture Food Institutional Research Measure. There are no conflicts of interest.

### References

1. Robertson DG (2005) Metabonomics in toxicology: a review. *Toxicol Sci* **85**, 809–822.
2. Lenz EM & Wilson ID (2007) Analytical strategies in metabolomics. *J Proteome Res* **6**, 443–458.
3. Keun HC (2006) Metabonomic modeling of drug toxicity. *Pharmacol Ther* **109**, 92–106.
4. Keun HC, Ebbels TM, Antti H *et al.* (2002) Analytical reproducibility in (1)H NMR-based metabonomic urinalysis. *Chem Res Toxicol* **15**, 1380–1386.
5. Dumas ME, Maibaum EC, Teague C *et al.* (2006) Assessment of analytical reproducibility of 1H NMR spectroscopy based metabolomics for large-scale epidemiological research: the INTERMAP Study. *Anal Chem* **78**, 2199–2208.
6. Vehtari A, Makinen VP, Soinen P, Ingman P, Makela SM, Savolainen MJ, Hannuksela ML, Kaski K & Ala-Korpela M (2007) A novel Bayesian approach to quantify clinical variables and to determine their spectroscopic counterparts in 1H NMR metabonomic data. *BMC Bioinformatics* **8**, Suppl. 2, S8.
7. Stoyanova R, Nicholson JK, Lindon JC & Brown TR (2004) Sample classification based on Bayesian spectral decomposition of metabonomic NMR data sets. *Anal Chem* **76**, 3666–3674.
8. Kochhar S, Jacobs DM, Ramadan Z, Berruex F, Fuerholz A & Fay LB (2006) Probing gender-specific metabolism differences in humans by nuclear magnetic resonance-based metabolomics. *Anal Biochem* **352**, 274–281.
9. Slupsky CM, Rankin KN, Wagner J *et al.* (2007) Investigations of the effects of gender, diurnal variation, and age in human urinary metabolomic profiles. *Anal Chem* **79**, 6995–7004.
10. Lenz EM, Bright J, Wilson ID, Hughes A, Morrisson J, Lindberg H & Lockton A (2004) Metabonomics, dietary influences and cultural differences: a (1)H NMR-based study of urine samples obtained from healthy British and Swedish subjects. *J Pharm Biomed Anal* **36**, 841–849.
11. Walsh MC, Brennan L, Malthouse JP, Roche HM & Gibney MJ (2006) Effect of acute dietary standardization on the urinary, plasma, and salivary metabolomic profiles of healthy humans. *Am J Clin Nutr* **84**, 531–539.
12. Maher AD, Zirah SF, Holmes E & Nicholson JK (2007) Experimental and analytical variation in human urine in 1H

- NMR spectroscopy-based metabolic phenotyping studies. *Anal Chem* **79**, 5204–5211.
13. Nicholls AW, Mortishire-Smith RJ & Nicholson JK (2003) NMR spectroscopic-based metabonomic studies of urinary metabolite variation in acclimatizing germ-free rats. *Chem Res Toxicol* **16**, 1395–1404.
  14. Martin FP, Wang Y, Sprenger N *et al.* (2008) Probiotic modulation of symbiotic gut microbial-host metabolic interactions in a humanized microbiome mouse model. *Mol Syst Biol* **4**, 157.
  15. Martin FP, Verdu EF, Wang Y *et al.* (2006) Transgenomic metabolic interactions in a mouse disease model: interactions of *Trichinella spiralis* infection with dietary *Lactobacillus paracasei* supplementation. *J Proteome Res* **5**, 2185–2193.
  16. Setchell KDR, Brown NM & Lydeking-Olsen E (2002) The clinical importance of the metabolite equol – A clue to the effectiveness of soy and its isoflavones. *J Nutr* **132**, 3577–3584.
  17. Bolca S, Possemiers S, Herregat A *et al.* (2007) Microbial and dietary factors are associated with the equol producer phenotype in healthy postmenopausal women. *J Nutr* **137**, 2242–2246.
  18. Jacobs DM, Deltimple N, van Velzen E, van Dorsten FA, Bingham M, Vaughan EE & van Duynhoven J (2008) (1)H NMR metabolite profiling of feces as a tool to assess the impact of nutrition on the human microbiome. *NMR Biomed* **21**, 615–626.
  19. Stella C, Beckwith-Hall B, Cloarec O *et al.* (2006) Susceptibility of human metabolic phenotypes to dietary modulation. *J Proteome Res* **5**, 2780–2788.
  20. Walsh MC, Brennan L, Pujos-Guillot E, Sebedio JL, Scalbert A, Fagan A, Higgins DG & Gibney MJ (2007) Influence of acute phytochemical intake on human urinary metabolomic profiles. *Am J Clin Nutr* **86**, 1687–1693.
  21. Griffin JL & Vidal-Puig A (2008) Current challenges in metabolomics for diabetes research: a vital functional genomic tool or just a ploy for gaining funding? *Physiol Genomics* **34**, 1–5.
  22. Rezzi S, Ramadan Z, Fay LB & Kochhar S (2007) Nutritional metabolomics: applications and perspectives. *J Proteome Res* **6**, 513–525.
  23. Gibney MJ, Walsh M, Brennan L, Roche HM, German B & van Ommen B (2005) Metabolomics in human nutrition: opportunities and challenges. *Am J Clin Nutr* **82**, 497–503.
  24. Lindon JC, Nicholson JK, Holmes E *et al.* (2003) Contemporary issues in toxicology the role of metabolomics in toxicology and its evaluation by the COMET project. *Toxicol Appl Pharmacol* **187**, 137–146.
  25. Lindon JC, Keun HC, Ebbels TM, Pearce JM, Holmes E & Nicholson JK (2005) The Consortium for Metabonomic Toxicology (COMET): aims, activities and achievements. *Pharmacogenomics* **6**, 691–699.
  26. Nicholson JK, Connelly J, Lindon JC & Holmes E (2002) Metabonomics: a platform for studying drug toxicity and gene function. *Nat Rev Drug Discov* **1**, 153–161.
  27. Dumas ME, Barton RH, Toyé A *et al.* (2006) Metabolic profiling reveals a contribution of gut microbiota to fatty liver phenotype in insulin-resistant mice. *Proc Natl Acad Sci USA* **103**, 12511–12516.
  28. Clayton TA, Lindon JC, Cloarec O *et al.* (2006) Pharmacometabonomic phenotyping and personalized drug treatment. *Nature* **440**, 1073–1077.
  29. Rezzi S, Ramadan Z, Martin FP, Fay LB, van Bladeren P, Lindon JC, Nicholson JK & Kochhar S (2007) Human metabolic phenotypes link directly to specific dietary preferences in healthy individuals. *J Proteome Res* **6**, 4469–4477.