

Metabolomics in nutrition research – a powerful window into nutritional metabolism

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Metabolomics is the study of small molecules present in biological samples. In recent years it has become evident that such small molecules, called metabolites, play a key role in the development of disease states. Furthermore, metabolomic applications can reveal information about alterations in certain metabolic pathways under different conditions. Data acquisition in metabolomics is usually performed using nuclear magnetic resonance (NMR)-based approaches or mass spectrometry (MS)-based approaches with a more recent trend including the application of multiple platforms in order to maximise the coverage in terms of metabolites measured. The application of metabolomics is rapidly increasing and the present review will highlight applications in nutrition research.

Introduction

Metabolomics refers to the comprehensive measurement of small molecules, called metabolites, present in a biological sample. The assessment of these metabolites gives detailed information on metabolic pathways and biological processes. Additionally, a number of metabolites have been implicated in disease states and as a result can reveal information on disease phenotypes [1]. Moreover, metabolites reflect the downstream activity of genes and proteins and hence assessment of their levels often reveals more about the specific phenotype; as a result, metabolomics holds great promise for the development of disease biomarkers and monitoring disease progression. Furthermore, metabolite levels are influenced by environmental factors such as diet, lifestyle factors and environmental agents meaning that assessment of metabolic profiles can play a significant role in a range of disciplines. With an ever-increasing number of scientists collaborating with metabolomics experts, the application of metabolomics is having a significant impact on many fields of research. A clear example is the emerging field of immunometabolism, where metabolic reprogramming identified through metabolomics is changing the fundamental understanding of immunity and infection [2–4].

The measurement of metabolites in biological samples is primarily achieved using NMR- and MS-based techniques. Data can be acquired as a full profile, where as many metabolites as possible are measured, or in a targeted mode, where a list of predefined metabolites are measured. Each technology has a number of advantages and disadvantages which have been previously detailed [5]. Briefly, NMR-based metabolomics is a robust and reproducible technique requiring minimal sample preparation, thus allowing relatively high-throughput analysis [6]. This reproducibility is extremely important when analysing samples from large epidemiology studies. Furthermore, NMR is non-destructive and thus preserves the biofluid and allows further analysis to be performed. NMR-based metabolomics simultaneously provides structural and quantitative information, which can be exploited for the identification of unknown metabolites, a major bottleneck in metabolomics. The major disadvantage of the NMR-based approach is the lack of sensitivity, meaning that this approach results in the measurement of only the most abundant metabolites.

MS-based techniques are extremely sensitive and enable a large coverage of the metabolome [5]. A drawback of the MS-based approaches is the reduced reproducibility in comparison with NMR-based metabolomics, making the acquisition of samples from large epidemiological studies challenging.

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MS-based techniques commonly combine MS with chromatography and the most common approaches are gas-chromatography-MS (GC-MS) and liquid-chromatography-MS (LC-MS). GC-MS is commonly used for the detection of fatty acids and a range of polar metabolites such as amino acids, and tricarboxylic acid (TCA) cycle and glycolysis intermediates. For GC-MS, the metabolites must be volatile or modified to become volatile; however, there are a number of compounds for which this will not work. For LC-MS, metabolites are first separated by LC and there are a range of columns available depending on the type of metabolites to be measured. With respect to MS-based techniques, the analysis can be performed in a profiling mode, where there is no prior selection of compound class, or in a targeted mode, where specific metabolite classes are measured. If a researcher has a particular hypothesis with respect to a certain metabolite class, a targeted analysis may be more appropriate. Furthermore, external standards and labelled internal standards are necessary for absolute quantification. However, for relative quantification the inclusion of labelled standards is not necessary and is often the starting point for many metabolomics studies. As a result of the chemical diversity and range of metabolites present in biological samples, no one technique will measure all metabolites present and the current trend is to apply multiple technology platforms in order to achieve maximum coverage of the metabolome. The field of metabolomics is rapidly growing and applications are expanding across a range of disciplines. The present review will focus on applications in nutrition research.

Metabolomics workflow

The metabolomics workflow generally consists of the following steps: (1) sample collection and preparation, (2) data acquisition and (3) data analysis and interpretation. The options for data acquisition have been dealt with in the introduction.

Sample collection

Metabolomics has been applied to a range of biofluids, including serum, plasma, urine, sweat, saliva and cerebrospinal fluid. In order to minimise unwanted variation and artefacts in the datasets, attention should be given to sample collection procedures [7–9]. For example, when collecting urine samples, storage on ice immediately following collection is recommended to prevent metabolite degradation [7]. Moreover, samples should be stored at -80°C for long-term storage. For blood samples, it is important to pay attention to the collection procedure. With respect to blood samples, it is imperative that attention is given to collection procedures in order to standardise the quenching of metabolism: failure to do so will introduce unwanted variation into the data. Studies have demonstrated that collection procedures including processing, storage and transport have the potential to affect the metabolic profile [10,11]. When collecting serum samples, it is essential that samples are all allowed to clot for the same time period and processed in a uniform fashion. When using plasma samples, one needs to consider the anti-coagulant used: ethylenediaminetetraacetic acid (EDTA) is not suited to NMR analysis as it gives extra resonances and indeed some reports have also mentioned issues with MS-based analysis. The method of choice is to use lithium heparin tubes [12]. Following sample collection, storage at the lowest possible temperature is recommended, to reduce metabolite decay and freeze-thaw cycles should be avoided [13,14]. Overall, to reduce unwanted variation and bias in the data it is essential that standard operating procedures are in place for sample collection.

Statistical analysis

A range of techniques exist for metabolomics data analysis and the readers are referred to the following reviews for detailed descriptions of the various techniques used [15–17]. The present review will focus on giving an overview of some options available to the user with some guidelines on key issues to consider. Data analysis often commences with unsupervised data analysis to enable the researcher to gain an overview of the data and to identify any underlying trends in the dataset. Principal Component Analysis (PCA) and Hierarchical Clustering Analysis (HCA) are examples of unsupervised data analysis approaches. PCA is one of the most popular data analysis techniques for exploring metabolite datasets and is particularly useful for identifying outlier samples. PCA allows the reduction of a large number of variables to a smaller number of principal components which are linear combinations of the original variables. Visualisation of the principal components in a scores plot enables the user to identify outlier samples and trends within the data. An example of a PCA plot is depicted in Figure 1. When performing PCA one should always check the variance explained by the model and the number of principal components obtained; care needs to be taken with models that explain a low amount of the variance. When reporting PCA models it is imperative the variance explained by each component is reported. A further important point to realise when

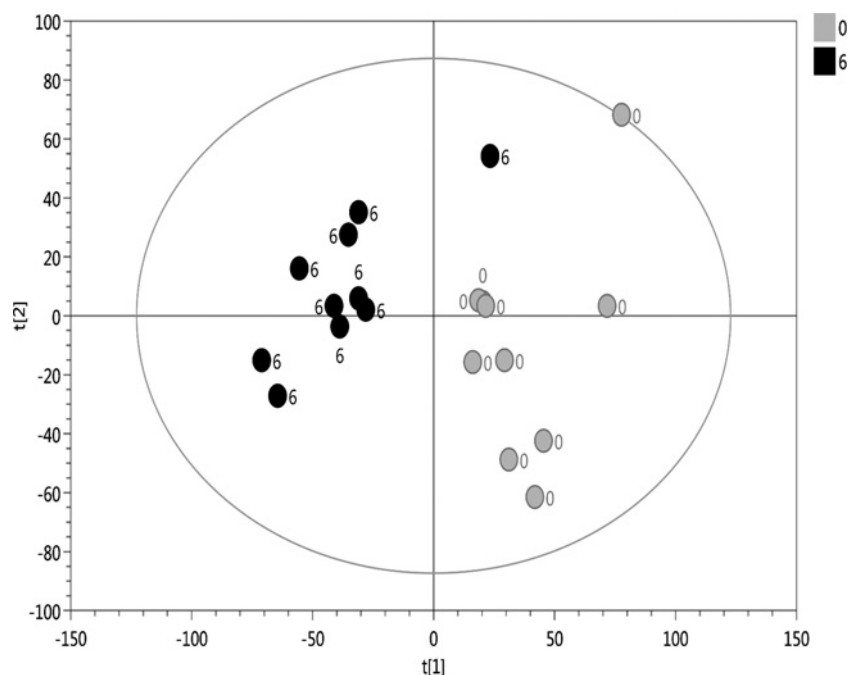


Figure 1. PCA plot obtained from the analysis of human urine.

Urine was collected pre- (0 h) and post- (6 h) consumption of fish. In this plot the separation of the two time points is evident. A supervised analysis would be performed in order to determine metabolite change following the consumption of food (see Figure 2).

using PCA for data analysis is that it optimises variance in the dataset, as opposed to any class distinction. Thus, if the inter-individual variation is greater than treatment variation, the first few principal components may reveal no separation due to group, thus potentially leading researchers to draw incorrect conclusions. Work in our laboratory extended the PCA approach to enable the addition of covariates to the model [18]. Probabilistic Principal Component and Covariates Analysis (PPCCA) incorporates covariates into the model, thus allowing inclusion of variation due to the covariates, such as age and body mass index (BMI), which is important when studying human metabolism.

Supervised techniques require *a priori* knowledge of the class membership and are used to identify spectral signals or metabolites that are different between groups or classes. There are many options for supervised analysis and examples include but are not limited to: Partial Least Squares Discriminant Analysis (PLS-DA), orthogonal PLS-DA (O-PLS-DA), Random Forests and Genetic algorithms. On the whole, the most common methods are PLS-DA and O-PLS-DA. PLS-DA models maximise the covariance to the class/group variable and the corresponding variable importance plot can be used to identify variables differing between the classes. When employing PLS-DA models one needs to be careful of overfitting the data and it is critical that a cross-validation is performed to validate the model. O-PLS-DA is an extension of PLS-DA which has grown in popularity in recent years. In this analysis the variance in the variables is split into two parts: the variance correlated to the response/group classification and the variance uncorrelated to the response/group classification. The advantage of this approach is the increase in ease of interpretation of the model and the important variables/metabolites contributing to the model. To achieve this the O-PLS loadings are usually visualised by back scaling the O-PLS coefficients to give the S-line plot. In the case of NMR, the fine structures of the original NMR peaks are maintained and this type of plot usually aids metabolite identification. Figure 2 illustrates an O-PLS-DA model and the corresponding S-line for the identification of metabolites contributing to the separation between the two groups. As in the case for PLS-DA models, validation is extremely important and the results of cross-validation should be reported for each model.

Univariate analysis is also possible when analysing metabolomics data. However, if using such an approach it is imperative to correct for multiple testing. Options for performing this include the Bonferroni multiple comparison method and the false discovery rate, with the latter method being the least conservative [19,20].

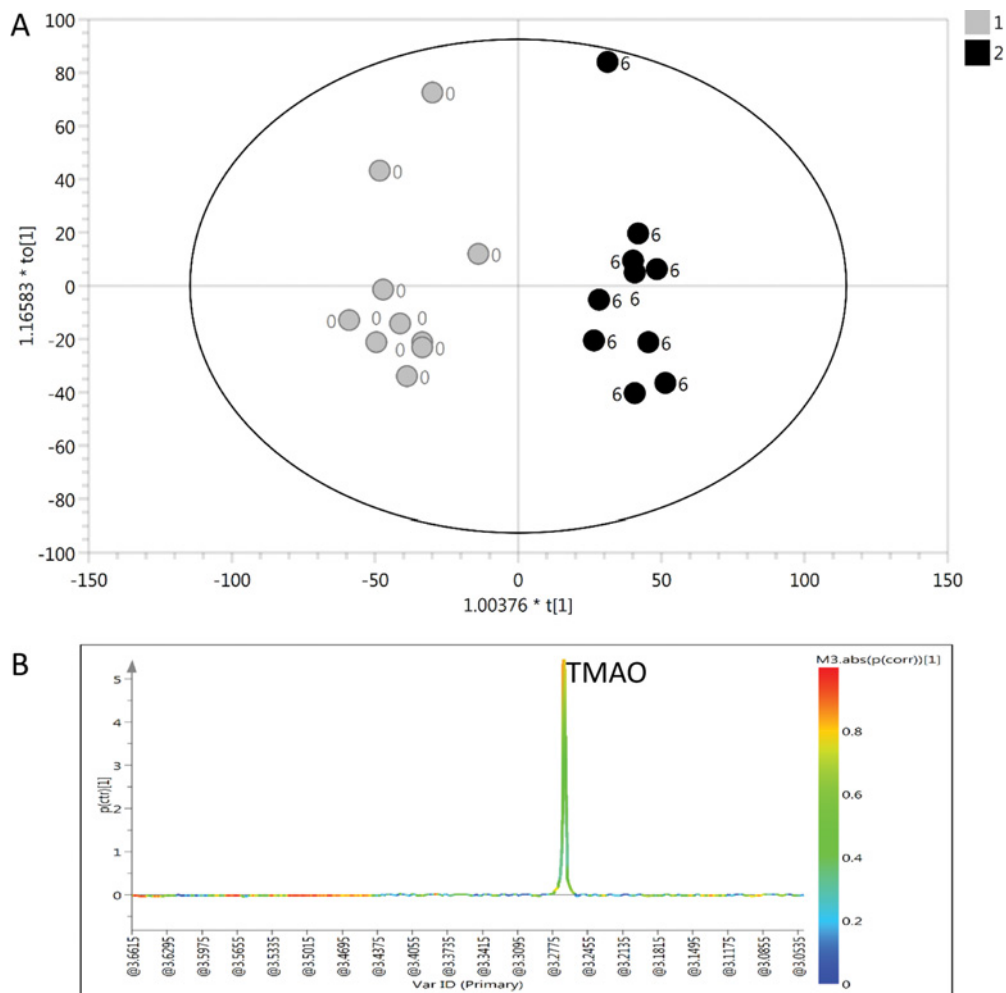


Figure 2. O-PLS-DA plot (RE-, R2Y, Q2) of human urine samples pre- and post-fish consumption (A). (B) Analysis of the corresponding S-line identifies the regions of the NMR spectrum that influence the separation of the groups in plot A. In this instance trimethylamine-N-oxide (TMAO) is higher in the samples 6 h post-consumption.

As many of the metabolomic studies currently performed aim to identify biomarkers associated with different conditions, the need to assess the performance of such biomarkers has grown. The most commonly used approach is the receiver operating characteristic (ROC) analysis which can assess the sensitivity and specificity of biomarkers or a combination of biomarkers. For a detailed tutorial on the use of ROC curves in biomarker analysis the reader is referred to an excellent review by Xia and colleagues [21]. While a number of commercial packages are available to perform the statistical analysis of metabolomics data one should also be aware of a number of freely available and easy-to-use options. Of particular note is the Metaboanlyst suite which does not require any knowledge of a programming language and is easy to use while still offering a range of modules for analysis [22].

Applications of metabolomics in nutrition

The application of metabolomics in nutrition research has expanded rapidly in recent years. Due to the intimate relationship between the food we eat and our metabolic pathways, metabolomic analysis is ideally suited to study relationships between food and health. Areas where metabolomics has played a key role to date include:

(1) identification of new dietary biomarkers, (2) application to intervention studies to understand the potential role of the diet in health promotion, (3) the study of diet-related diseases and (4) precision nutrition. With respect to dietary biomarkers the area has been previously reviewed and readers are referred to a number of key reviews in the field [23–25]. Briefly, metabolomics has been successful in identifying a number of putative biomarkers of food intake and will continue to play a key role in the validation of such markers. For the present review, the focus will be on applications in relation to intervention studies, diet-related diseases and precision nutrition.

Application of metabolomics to understand mechanisms associated with interventions

The literature is abundant with examples of where metabolomics has been used to examine the influence of certain diets or dietary components on metabolic pathways with the goal to understand the mechanisms through which they infer beneficial health effects. The present section is not an exhaustive review of the literature but rather an illustration of the potential of metabolomics in this field.

A recent example illustrating the concept is the metabolomic analysis of samples from 145 individuals who followed either the New Nordic Diet (NND) or the Average Danish Diet (ADD) for a six-month period [26]. Previous work had established that the NND resulted in weight loss and improved blood pressure in obese Danish adults [27]. The GC-MS-based metabolomics analysis revealed alterations in metabolites that reflected gluconeogenesis and ketosis leading the authors to make suggestions in relation to the mechanism through which the NND elicits its beneficial effects. Furthermore, there were alterations in metabolite levels in individuals on the NND who lost weight compared with those who did not, indicating the power of the approach for metabolic phenotyping.

Using NMR-based metabolomics to examine serum samples from 33 postmenopausal women consuming either wholegrain rye bread or a refined wheat bread revealed that consumption of rye bread decreased the branched chain amino acids leucine and isoleucine and increased NN-dimethylglycine [28]. The alterations in these metabolites and their associated pathways suggest mechanisms by which wholegrain rye bread confers beneficial health effects. The application of metabolomics to weight loss studies has revealed its potential impact on insulin resistance, enabling the identification of potential benefits of different dietary regimes. Using a targeted approach to measure amino acids in two independent studies revealed significant decreases in branched chain amino acids and aromatic amino acids, both of which have been previously associated with insulin resistance and type 2 diabetes [29]. Interestingly, a lower protein diet (15% of energy compared with 25%) demonstrated a stronger effect on reducing the branched chain amino acid valine. Overall, the study by Zheng et al. is a good example of how applying metabolomics to a pre- and post-dietary intervention can give insights into potential mechanisms of the health benefits of certain diets.

As is evident from the above examples, metabolomics is a powerful tool in the assessment of metabolic changes following dietary interventions. One of the main challenges in the field lies in the mapping of observed changes in metabolite levels to metabolic pathways. This, without a doubt, requires knowledge of basic biochemistry and an inherent understanding of metabolic pathways. The collaboration between scientists from different disciplines is important to obtain meaningful interpretation of the data.

Diet-related disease studies

In recent years, the applications of metabolomics to diet-related diseases such as type 2 diabetes and cardiovascular disease has increased dramatically [30–34]. Of particular note is the emergence of evidence from metabolomic studies with respect to the branched chain amino acids and the risk of development of insulin resistance and type 2 diabetes. A number of studies have demonstrated elevated levels of branched chain amino acids are associated with insulin resistance [35–37]. Moreover, a combination of branched chain amino acids and aromatic amino acids have been shown to be predictive of a future risk of diabetes [38]. While the majority of these studies have used MS-based techniques for the measurement of the amino acids there are also some noteworthy examples where NMR has been used. In a study of 1,680 young adults, NMR was used to quantify amino acids and data analysis revealed that the circulating levels were predictive of insulin resistance [39]. Overall, the emergent role of branched chain amino acids in insulin resistance and type 2 diabetes demonstrates the ability of metabolomics to open up new avenues of research and to challenge the current *status quo* in the understanding of the development of diet-related diseases.

Finally, the role of the gut microbiota is emerging as an important potential player in the development of a number of diet-related diseases and metabolomics is contributing significantly to the field. Metabolomics can readily identify metabolites that originate from mammalian-gut microbial co-metabolism and thus offers a unique method to follow

alterations in gut microbial metabolism and add some functional meaning to alterations in bacterial levels [40–42]. In essence, it enables a functional readout that helps interpretation of the potential alterations in the microbiota.

Precision nutrition

Precision nutrition or personalised nutrition can be defined as the tailoring of dietary advice to the individual. A recent study demonstrated, using a randomised control trial, that personalising dietary advice improved the dietary habits of individuals compared with receiving general healthy eating guidelines [43]. Metabolomics has the potential to play a significant role in the development and delivery of precision nutrition. Metabolic phenotyping through the use of metabolomics can identify individuals who will respond to different interventions. Such a predictive approach could enable researchers to tailor dietary advice to the individual. Examples of where metabolomics has identified differential responses to dietary interventions includes choline and vitamin D interventions. Analysis of the baseline metabolomic profiles in a choline depletion study resulted in a prediction of which subjects developed liver dysfunction when deprived of dietary choline [44]. Fifty-three subjects received a choline-sufficient diet for 10 days (550 mg choline/70 kg/d) followed by a choline-depleted diet (<50 mg choline/70 kg/d) for up to 42 days. Statistical analysis of the data identified metabolomic profiles that could predict whether subjects developed liver disease or not.

In a three-month vitamin D and calcium intervention, metabolomics analysis of baseline samples identified profiles predictive of response to the intervention [45]. In this instance, response to the intervention was defined by changes in parathyroid hormone (PTH). The use of such a profile to tailor or personalise the nutritional strategy given to individuals would enhance success.

Conclusions

Metabolomics is a powerful emerging technology that is set to significantly affect the field in the coming years. The application of metabolomics in nutrition research has flourished recently and the examples illustrated above clearly depict its ability to help in our understanding of mechanisms underlying dietary interventions. Furthermore, the application of metabolomics in the study of diet-related diseases has the potential to challenge our current thinking and thus develop better prevention strategies in the long term. An area not covered by the review is the field of dietary biomarkers; metabolomics has enormous potential in this field and is set to make significant progress in the coming years. Finally, an exciting emerging application of metabolomics is the field of precision nutrition. The use of a metabolomic profile to tailor dietary advice to an individual, based on a broad set of biomarkers that reflect disease risk and dietary habits, has great potential within the context of precision nutrition and medicine.

Summary

- Metabolomics is the study of small molecules.
- NMR and MS-based technologies are the main technologies used in metabolomic studies.
- The metabolomics workflow consists of a series of steps including samples collection, samples preparation, data acquisition, data analysis and interpretation.
- Metabolomics is a powerful tool in the assessment of metabolic changes following nutrition intervention studies.
- Metabolomic profiling has the potential to play an important role in the delivery of Precision Nutrition by identifying profiles predictive of response to interventions.

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Abbreviations

ADD, Average Danish Diet; GC-MS, gas chromatography mass spectrometry; HCA, hierarchical cluster analysis; LC-MS, liquid chromatography mass spectrometry; MS, mass spectrometry; NMR, nuclear magnetic resonance; NND, New Nordic Diet;

O-PLS-DA, orthogonal-PLS-DA; PCA, principal component analysis; PLS-DA, partial least squares discriminant analysis; PPCA, probabilistic principal component analysis; PTH, parathyroid hormone; TMAO, trimethylamine-N-oxide.

Competing Interests

The Author declare that there are no competing interests associated with the manuscript.

References

- 1 Wishart, D.S. (2016) Emerging applications of metabolomics in drug discovery and precision medicine. *Nat. Rev. Drug. Discov.* **15**, 473–484 [CrossRef](#)
- 2 Mills, E.L. and O'Neill, L.A. (2016) Reprogramming mitochondrial metabolism in macrophages as an anti-inflammatory signal. *Eur. J. Immunol.* **46**, 13–21 [CrossRef](#)
- 3 O'Neill, L.A. and Pearce, E.J. (2016) Immunometabolism governs dendritic cell and macrophage function. *J. Exp. Med.* **213**, 15–23 [CrossRef](#)
- 4 Wang, A., Huen, S.C., Luan, H.H., Yu, S., Zhang, C., Gallezot, J.D. et al. (2016) Opposing effects of fasting metabolism on tissue tolerance in bacterial and viral inflammation. *Cell.* **166**, 1512–1525, e12 [CrossRef](#)
- 5 Griffin, J.L., Atherton, H., Shockcor, J. and Atzori, L. (2011) Metabolomics as a tool for cardiac research. *Nat. Rev. Cardiol.* **8**, 630–643 [CrossRef](#)
- 6 Dumas, M.E., Maibaum, E.C., Teague, C., Ueshima, H., Zhou, B., Lindon, J.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 [CrossRef](#)
- 7 Scalbert, A., Brennan, L., Fiehn, O., Hankemeier, T., Kristal, B.S., van Ommen, B. et al. (2009) Mass-spectrometry-based metabolomics: limitations and recommendations for future progress with particular focus on nutrition research. *Metabolomics* **5**, 435–458 [CrossRef](#)
- 8 Barnes, S., Benton, H.P., Casazza, K., Cooper, S.J., Cui, X., Du, X. et al. (2016) Training in metabolomics research. I. Designing the experiment, collecting and extracting samples and generating metabolomics data. *J. Mass. Spectrom.* **51**, ii–iii
- 9 Emwas, A.H., Luchinat, C., Turano, P., Tenori, L., Roy, R., Salek, R.M. et al. (2015) Standardizing the experimental conditions for using urine in NMR-based metabolomic studies with a particular focus on diagnostic studies: a review. *Metabolomics.* **11**, 872–894 [CrossRef](#)
- 10 Dunn, W.B., Broadhurst, D., Ellis, D.I., Brown, M., Halsall, A., O'Hagan, S. et al. (2008) A GC-TOF-MS study of the stability of serum and urine metabolomes during the UK Biobank sample collection and preparation protocols. *Int. J. Epidemiol.* **37** (Suppl 1), i23–i30 [CrossRef](#)
- 11 Teahan, O., Gamble, S., Holmes, E., Waxman, J., Nicholson, J.K., Bevan, C. et al. (2006) Impact of analytical bias in metabolomic studies of human blood serum and plasma. *Anal. Chem.* **78**, 4307–4318 [CrossRef](#)
- 12 Pinto, J., Domingues, M.R., Galhano, E., Pita, C., Almeida Mdo, C., Carreira, I.M. et al. (2014) Human plasma stability during handling and storage: impact on NMR metabolomics. *Analyst* **139**, 1168–1177 [CrossRef](#)
- 13 Zhang, A., Sun, H., Wang, P., Han, Y. and Wang, X. (2012) Recent and potential developments of biofluid analyses in metabolomics. *J. Proteomics.* **75**, 1079–1088 [CrossRef](#)
- 14 Lauridsen, M., Hansen, S.H., Jaroszewski, J.W. and Cornett, C. (2007) Human urine as test material in 1H NMR-based metabolomics: recommendations for sample preparation and storage. *Anal. Chem.* **79**, 1181–1186 [CrossRef](#)
- 15 Bartel, J., Krumsiek, J. and Theis, F.J. (2013) Statistical methods for the analysis of high-throughput metabolomics data. *Comput. Struct. Biotechnol. J.* **4**, e201301009 [CrossRef](#)
- 16 Korman, A., Oh, A., Raskind, A. and Banks, D. (2012) Statistical methods in metabolomics. *Methods Mol. Biol.* **856**, 381–413 [CrossRef](#)
- 17 Szymanska, E., Saccenti, E., Smilde, A.K. and Westerhuis, J.A. (2012) Double-check: validation of diagnostic statistics for PLS-DA models in metabolomics studies. *Metabolomics.* **8** (Suppl 1), 3–16 [CrossRef](#)
- 18 Nyamundanda, G., Brennan, L. and Gormley, I.C. (2010) Probabilistic principal component analysis for metabolomic data. *BMC Bioinformatics.* **11**, 571 [CrossRef](#)
- 19 Storey, J.D. (2002) A direct approach to false discovery rates. *Journal of the Royal Statistical Society Series B-Statistical Methodology.* **64**, 479–498 [CrossRef](#)
- 20 Benjamini, Y. and Hochberg, Y. (1995) Controlling the false discovery rate – a practical and powerful approach to multiple testing. *J. Royal Statist. Soc. Series B Methodol.* **57**, 289–300
- 21 Xia, J., Broadhurst, D.I., Wilson, M. and Wishart, D.S. (2013) Translational biomarker discovery in clinical metabolomics: an introductory tutorial. *Metabolomics.* **9**, 280–299 [CrossRef](#)
- 22 Xia, J., Sinelnikov, I.V., Han, B. and Wishart, D.S. (2015) MetaboAnalyst 3.0—making metabolomics more meaningful. *Nucleic Acids Res.* **43** (W1), W251–W257 [CrossRef](#)
- 23 Scalbert, A., Brennan, L., Manach, C., Andres-Lacueva, C., Dragsted, L.O., Draper, J. et al. (2014) The food metabolome: a window over dietary exposure. *Am. J. Clin. Nutr.* **99**, 1286–1308 [CrossRef](#)
- 24 O'Gorman, A., Gibbons, H., Brennan, L., Poulsen, S.K., Savorani, F., Acar, E., Gurdeniz, G. and Larsen, T.M. (2013) Metabolomics in the identification of biomarkers of dietary intake. *Comput. Struct. Biotechnol. J.* **4**, e201301004 [CrossRef](#)
- 25 Gibbons, H. and Brennan, L. (2016) Metabolomics as a tool in the identification of dietary biomarkers. *Proc. Nutr. Soc.* 1–12 [CrossRef](#)
- 26 Khakimov, B., Poulsen, S.K., Savorani, F., Acar, E., Gurdeniz, G., Larsen, T.M. et al. (2016) New Nordic Diet versus average Danish diet: a randomized controlled trial revealed healthy long-term effects of the new Nordic Diet by GC-MS blood plasma metabolomics. *J. Proteome Res.* **15**, 1939–1954 [CrossRef](#)

- 27 Poulsen, S.K., Due, A., Jordy, A.B., Kiens, B., Stark, K.D., Stender, S. et al. (2014) Health effect of the New Nordic Diet in adults with increased waist circumference: a 6-mo randomized controlled trial. *Am. J. Clin. Nutr.* **99**, 35–45 [CrossRef](#)
- 28 Moazzami, A.A., Bondia-Pons, I., Hanhineva, K., Juntunen, K., Antl, N., Poutanen, K. et al. (2012) Metabolomics reveals the metabolic shifts following an intervention with rye bread in postmenopausal women—a randomized control trial. *Nutr. J.* **11**, 88 [CrossRef](#)
- 29 Zheng, Y., Ceglarek, U., Huang, T., Li, L., Rood, J., Ryan, D.H. et al. (2016) Weight-loss diets and 2-y changes in circulating amino acids in 2 randomized intervention trials. *Am. J. Clin. Nutr.* **103**, 505–511 [CrossRef](#)
- 30 Wurtz, P., Soininen, P., Kangas, A.J., Mäkinen, V.P., Groop, P.H., Savolainen, M.J. et al. (2011) Characterization of systemic metabolic phenotypes associated with subclinical atherosclerosis. *Mol. Biosyst.* **7**, 385–393 [CrossRef](#)
- 31 Kang, S.M., Park, J.C., Shin, M.J., Lee, H., Oh, J., Ryu, D.H. et al. (2011) ¹H nuclear magnetic resonance based metabolic urinary profiling of patients with ischemic heart failure. *Clin. Biochem.* **44**, 293–299 [CrossRef](#)
- 32 Wang, Z., Klipfell, E., Bennett, B.J., Koeth, R., Levison, B.S., Dugar, B. et al. (2011) Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature* **472**, 57–63 [CrossRef](#)
- 33 Qiu, G., Zheng, Y., Wang, H., Sun, J., Ma, H., Xiao, Y. et al. (2016) Plasma metabolomics identified novel metabolites associated with risk of type 2 diabetes in two prospective cohorts of Chinese adults. *Int. J. Epidemiol.* [CrossRef](#)
- 34 Carter, T.C., Rein, D., Padberg, I., Peter, E., Rennefahrt, U., David, D.E. et al. (2016) Validation of a metabolite panel for early diagnosis of type 2 diabetes. *Metabolism – Clin. Exp.* **65**, 1399–1408 [CrossRef](#)
- 35 Shah, S.H., Crosslin, D.R., Haynes, C.S., Nelson, S., Turer, C.B., Stevens, R.D. et al. (2012) Branched-chain amino acid levels are associated with improvement in insulin resistance with weight loss. *Diabetologia*. **55**, 321–330 [CrossRef](#)
- 36 Newgard, C.B. (2012) Interplay between lipids and branched-chain amino acids in development of insulin resistance. *Cell Metab.* **15**, 606–614 [CrossRef](#)
- 37 Newgard, C.B., An, J., Bain, J.R., Muehlbauer, M.J., Stevens, R.D., Lien, L.F. et al. (2009) A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. *Cell Metab.* **9**, 311–326 [CrossRef](#)
- 38 Wang, T.J., Larson, M.G., Vasan, R.S., Cheng, S., Rhee, E.P., McCabe, E. et al. (2011) Metabolite profiles and the risk of developing diabetes. *Nat. Med.* **17**, 448–453 [CrossRef](#)
- 39 Wurtz, P., Soininen, P., Kangas, A.J., Ronnema, T., Lehtimäki, T., Kahonen, M. et al. (2013) Branched-chain and aromatic amino acids are predictors of insulin resistance in young adults. *Diabetes Care* **36**, 648–655 [CrossRef](#)
- 40 Nicholson, J.K., Holmes, E., Kinross, J., Burcelin, R., Gibson, G., Jia, W. et al. (2012) Host-gut microbiota metabolic interactions. *Science*. **336**, 1262–1267 [CrossRef](#)
- 41 Smirnov, K.S., Maier, T.V., Walker, A., Heinzmann, S.S., Forcisi, S., Martinez, I. et al. (2016) Challenges of metabolomics in human gut microbiota research. *Int. J. Med. Microbiol.* **306**, 266–279 [CrossRef](#)
- 42 Yen, S., McDonald, J.A., Schroeter, K., Oliphant, K., Sokolenko, S., Blondeel, E.J. et al. (2015) Metabolomic analysis of human fecal microbiota: a comparison of feces-derived communities and defined mixed communities. *J. Proteome Res.* **14**, 1472–1482 [CrossRef](#)
- 43 Celis-Morales, C., Livingstone, K.M., Marsaux, C.F., Macready, A.L., Fallaize, R., O'Donovan, C.B. et al. (2016) Effect of personalized nutrition on health-related behaviour change: evidence from the Food4me European randomized controlled trial. *Int. J. Epidemiol.* [CrossRef](#)
- 44 Sha, W., da Costa, K.A., Fischer, L.M., Milburn, M.V., Lawton, K.A., Berger, A. et al. (2010) Metabolomic profiling can predict which humans will develop liver dysfunction when deprived of dietary choline. *FASEB J.* **24**, 2962–2975 [CrossRef](#)
- 45 Elnenaei, M.O., Chandra, R., Mangion, T. and Moniz, C. (2011) Genomic and metabolomic patterns segregate with responses to calcium and vitamin D supplementation. *Br. J. Nutr.* **105**, 71–79 [CrossRef](#)