Metabolomics as a tool in nutritional research

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Purpose of review
Metabolomics is emerging as a powerful tool for studying metabolic processes and in recent years, the applications in the area of nutrition have risen rapidly. The present review gives an overview of the current applications in the field of nutrition and identifies areas in need of advancement.

Recent findings
Applications in nutrition research can in general be divided into three main areas: identification of dietary biomarkers, study of diet-related diseases and identification of biomarkers of disease and application to dietary intervention studies as a tool to identify molecular mechanisms.

Summary
Metabolomics has made a significant impact on all the areas identified above and is set to have a major impact on the study of diet–health relationships.

Keywords
biomarkers, dietary biomarkers, metabolomics

INTRODUCTION TO METABOLOMICS

Metabolomics is an 'omics' technology that provides a powerful approach to the comprehensive analysis of all molecules present in biological samples. This technology allows the exploration of the complex interactions between diet and the human organism and therefore allows a better understanding of the implications and subtle changes in metabolism activated by foods, nutrients and disease.

The most commonly used technologies in metabolomics are nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) techniques coupled with a chromatographic step such as gas or liquid chromatography. Each of these techniques has its own advantages and disadvantages, for example NMR-based techniques have a high reproducibility, little inter-laboratory variation, are nondestructive, but have a low sensitivity compared with MS-based techniques [1]. A general consideration for metabolomic analysis is that no one technique will provide a comprehensive overview of the whole metabolome; therefore, a combination of NMR and MS-based techniques is recommended. Metabolomic analyses can be run and analysed using either a targeted or an untargeted approach. Targeted metabolomics is a hypothesis-driven approach, focused on analysing metabolites that are selected according to the research questions being asked, whereas untargeted metabolomics is used in hypothesis-free studies in which an unbiased screening of all metabolites is required. Again, no one approach will capture the whole metabolome; therefore, a combination of both approaches is preferred.

Metabolomic technologies produce large and complex datasets and therefore require advanced statistical and bioinformatic tools to aid in their interpretation. Multivariate statistics including principal components analysis (PCA), partial least squares discriminant analysis (PLS-DA) and orthogonal PLS-DA are the most commonly used for analysis of metabolomics data. The PLS-based techniques are supervised methods, which require prior knowledge of the classes and can result in overfitting of the data. Careful validation is therefore essential following this analysis. For a comprehensive review of techniques used in metabolomics and statistical analyses approaches, the reader is referred to the following reviews [1–3]. The focus of the present review is on metabolomic applications in nutrition research and we are using a broad

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KEY POINTS

- Metabolomics is the study of small molecules called metabolites and their alterations under certain conditions.
- Applications in nutrition research include identification of new biomarkers of dietary intake, the study of diet-related diseases and application to intervention studies to elucidate molecular mechanisms.

definition of metabolomics to include lipid-derived metabolites.

In general, the application of metabolomics in the area of nutrition can be divided into three main areas: dietary biomarker discovery studies, studies of diet-related diseases and dietary intervention studies [4], an overview of which can be seen in Fig. 1. In this review, we firstly focus on the potential of metabolomics as a tool in the identification of biomarkers of diet-related diseases and how this may elucidate the molecular pathways by which nutrients affect health and disease. We will then discuss the role of metabolomics in the discovery of novel dietary biomarkers that will help enhance and validate current dietary assessment methods. Finally, the applications of metabolomics to dietary intervention studies and their impact on the understanding of the effects of certain diets or food items on metabolic pathways will be discussed.

METABOLOMICS IN THE STUDY OF DIET-RELATED DISEASES

Epidemiological and clinical studies have provided clear evidence that many diseases with high rates of morbidity and mortality are associated with diet including diabetes, cardiovascular disease and a range of cancers [5▪]. Many current studies use metabolomics to measure disturbances in metabolic pathways [6] to increase the understanding of how nutrition influences metabolism and homeostatic control and how this regulation is disturbed in the early phase of diet-related diseases [7]. Therefore, metabolomics can enable the identification of biomarkers of disease and progression and elucidate the underlying mechanisms involved in the development of the disease status. This in turn will improve early diagnosis, accurate prognosis and aid adequate monitoring [5▪].

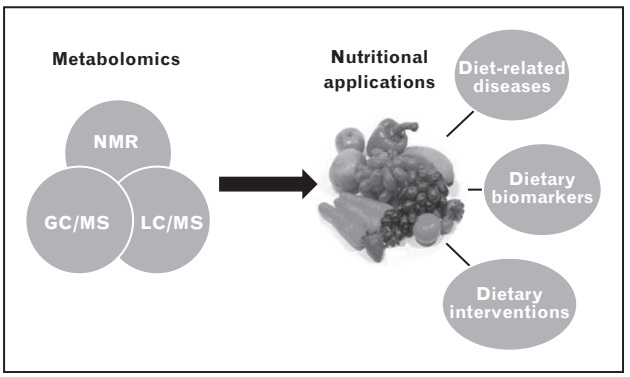

FIGURE 1. Application of metabolomics in the area of nutrition.

In recent years a number of studies have emerged in the literature that have applied metabolomics to the study of type 2 diabetes mellitus (T2DM). Through the application of metabolomics, a number of possible risk factors of T2DM have been identified and include lipid molecules such as free fatty acids, bile acids and amino acids, in particular the branched chain amino acids (BCAAs) [8–10]. Newgard and colleagues specifically identified the BCAAs as being associated with insulin resistance of obesity in humans as well as in a variety of animal models [9,10]. The elevated levels of BCAAs in obese subjects suggest increased catabolism of BCAAs or 'BCAA overload' as described by the authors. The findings were then confirmed in an animal study in which rats were fed different diets including a high-fat diet and a high-fat diet supplemented with BCAAs (high-fat/BCAA). High fat/BCAA rats were found to be equally insulin-resistant as high-fat fed rats, which indicated that in terms of a poor diet high in fat, BCAAs contribute to the development of obesity-associated insulin resistance [10]. A study by Wang *et al.* exploited the Framingham database to identify metabolites that could predict the development of future diabetes. Interestingly, a panel of five markers, which included three BCAAs (leucine, isoleucine and valine) and two aromatic amino acids (phenylalanine and tyrosine) had highly significant associations with future diabetes. To identify the most predictive biomarker combination, the authors calculated log-likelihood ratios (LHR statistic) and evaluated model discrimination; the combination of isoleucine, phenylalanine and tyrosine was found to be the most predictive and was associated with a five-fold to seven-fold higher risk of developing diabetes. These findings were replicated in an independent perspective cohort, illustrating the importance of amino acid metabolism early in the pathogenesis of diabetes [11]. Furthermore, patients who underwent gastric bypass surgery have decreased BCAA levels compared with subjects undergoing dietary intervention despite similar weight loss leading authors to suggest that the BCAAs play a role in their improved glucose homeostasis. Although these studies have consistently identified BCAA as predictors of insulin

resistance or diabetes risk, the exact mechanism by which this is mediated is unclear.

Although these studies and others highlight metabolomics as a tool for identifying biomarkers of many diet-related diseases, few have been translated into clinical diagnostics to date. In order for this to happen, current biomarkers require validation in large-scale epidemiological studies to characterize their clinical application.

METABOLOMICS AND DIETARY BIOMARKERS

Current self-reporting dietary assessment methods [food-frequency questionnaires (FFQs), 24-h recalls, weighed food records] are associated with errors including energy under-reporting, recall errors and difficulty in assessment of portion sizes [12,13]. To improve dietary assessment methods, novel biomarkers of dietary intake are required. Currently, biomarkers exist for salt, protein, sucrose and fructose intake (sodium/nitrogen/sucrose and fructose measured in 24 h urine samples) and energy expenditure (the doubly labelled water technique) [12,14]. These biomarkers provide nearly unbiased estimates of absolute intake and are therefore extremely useful for validating self-reporting instruments [15]. Metabolomics has emerged as a key tool in dietary biomarker discovery. The application of metabolomics has, in general, taken three discovery approaches: acute intervention studies, cohort studies and analysis of dietary patterns and metabolic profiles. In acute intervention studies participants consume specific food items, biofluids are collected, metabolomic techniques applied and potential biomarkers are identified. Within cohort studies, low and high consumers of a specific food are selected from food intake data. Comparison of their metabolic profiles can result in the discovery of potential biomarkers. In the third approach, analyses of dietary patterns in conjunction with metabolomic profiles are carried out to identify nutritypes and biomarkers. Validation of the biomarkers in an independent study is desirable following the discovery stage.

Successful identification of putative biomarkers for many foods including citrus fruits [16▪,17–19], salmon [20], red meat [21,22], cruciferous vegetables [23▪,24] and coffee [25▪,26] have occurred. The food metabolome and these dietary biomarkers have been presented in more detail in a recent review [27▪]. As an example of a food biomarker, we highlight proline betaine, which following original identification by Atkinson *et al.* [28] has been successfully identified as a maker of citrus fruit intake by two research groups [18,19]. Proline betaine was identified using a nutritional intervention study and validated using an additional cohort [18]. Within the acute intervention study eight participants consumed a mixed-fruit meal and urine was collected. ^{1}H NMR-based urinary profiling and PLS-DA analysis was employed to identify urinary proline betaine as a biomarker of citrus fruit intake. This biomarker was validated using data from participants in the INTERMAP UK cohort demonstrating a high sensitivity and specificity for citrus fruit consumption (90.6 and 86.3% respectively) [18]. Proline betaine has also been identified as a marker of citrus intake by Lloyd *et al.* [19] after acute exposure of volunteers to orange juice.

In recent years the concept of panels of biomarkers has emerged. Work from our laboratory assessed the relationship between lipidomic profiles using serum and dietary data, measured by FFQs, in 34 participants. This study identified six novel lipid patterns. Lipid pattern 1 was highly predictive of dietary fat intake [area under the curve (AUC) = 0.82] and also classified low and high consumers with very good accuracy. Lipid pattern 4 was highly predictive of alcohol intake (AUC = 0.81) with lysophosphatidylcholine alkyl C18 : 0 (LPCeC18 : 0) identified as a potential biomarker of alcohol consumption. Lipid pattern 6 had a relatively good ability to predict dietary fish intake (AUC = 0.76), with lysophosphatidylethanolamine acyl C18 : 2 (LPEaC18 : 2) and phoshatidylethanolamine diacyl C38 : 4 (PEaaC38 : 4) identified as potential biomarkers [29].

The identification of novel biomarkers of dietary intake, through the application of metabolomics, offers the potential of a more objective measure of dietary intake compared with traditional dietary assessment methods. Biomarkers will not replace these traditional dietary methods, but will instead enhance and validate findings. Currently dietary biomarker discovery has focused on single markers to represent food groups. To advance this area, combinations or panels of biomarkers may provide more accurate measures of dietary exposures. Validation of novel biomarkers discovered in acute studies is also imperative.

APPLICATION OF METABOLOMICS TO INTERVENTION STUDIES

Applications of metabolomics to dietary intervention studies enable researchers to study the mechanistic effects of the interventions and therefore establish how the diets/food items impact on metabolic pathways. Applications of this type are currently increasing yearly and a full overview of examples in this area is beyond the scope of this review. As a key illustration some recent examples are highlighted. Recently, a lipidomic approach was

applied to the investigation of four isoenergetic diets differing in n-3 fatty acids and polyphenol content [30] in subjects with a high cardiovascular risk. The high n-3 fatty acid diet resulted in increased phospholipids and triacylglycerols of long-chain polyunsaturated fatty acids in plasma. Furthermore, five lipids which were previously associated with a decreased risk of diabetes were found to be increased in the high n-3 fatty acid diet groups reinforcing the potential positive effects of these fatty acids on health. Dependency network analysis was employed to identify the relationships between the lipids and key clinical measurements illustrating the potential power of this approach to link certain diets to health parameters.

In a randomized crossover trial of 8 weeks of rye bread or 8 weeks of refined wheat bread metabolomics analysis revealed lower serum levels of leucine and isoleucine and higher betaine and *N,N*-dimethylglycine levels after rye bread intake [31]. These metabolite changes allowed the authors to conclude that changes in BCAA metabolism and one carbon metabolism may be potential mechanisms through which consumption of rye bread exerts its beneficial effects.

As advances are made in metabolomics technologies the applications to intervention studies will increase. For these to make a real impact on our understanding of interventions it is imperative that mapping to metabolic pathways improves. Currently, a real limitation exists in widely available tools to achieve this.

CONCLUSION

As technology advances are made in metabolomics and lipidomics the applications across many disciplines increase. Nutrition research is set to benefit greatly from the use of these technologies. Applications of metabolomics to the discovery of dietary biomarkers have the potential to enhance greatly our ability to assess dietary intake. In the context of diet-related diseases and intervention studies, we need to advance our ability to map metabolites to biological pathways and processes. This will aid interpretation of results and thus enhance our understanding of diet and disease relationships.

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Conflicts of interest

None.

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