Review Title: Recent Advances in the Application of Metabolomics for Nutrition and Health.

Authors: Diana González-Peña and Lorraine Brennan

Affiliation:

UCD School of Agriculture and Food Science, Institute of Food and Health, UCD, Dublin 4, Ireland

Emails:

Diana González-Peña: diana.gonzalezpena@ucd.ie

Lorraine Brennan: lorraine.brennan@ucd.ie

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Metabolomics in nutrition research

Corresponding Author: Lorraine Brennan

Corresponding Author contact information

UCD Institute of Food and Health, UCD School of Agriculture and Food Science, UCD, Belfield, Dublin 4, Ireland.

lorraine.brennan@ucd.ie

00353 1 7162811

ABSTRACT

Metabolomics is the study of small molecules called metabolites in biological samples. Application of metabolomics to nutrition research has expanded in recent years with emerging literature supporting multiple applications. Examples of key examples include the application of metabolomics in the identification and development of objective biomarkers of dietary intake, the role of metabolomics in developing personalised nutrition strategies and application in large scale epidemiology studies to understand the link between diet and health. In this review, we provide an overview of the current applications and identify key challenges that need to be addressed for further development of the field. Successful development of metabolomics for nutrition research has the potential to improve dietary assessment, help deliver personalised nutrition and enhance our understanding of the link between diet and health.

KEYWORDS: Metabolomics, Dietary Biomarkers, Personalised Nutrition, Metabotypes

INTRODUCTION

Since its emergence, metabolomics has enhanced prospects in the field of nutrition and food science. Since the term "metabolomics" was coined in late 90s, the continuous improvement of high-throughput analytical tools such as chromatography, nuclear magnetic resonance (NMR) and mass spectrometry (MS) has allowed advancement in applications. As the field developed, different terms have emerged in the literature essentially referring to the use of the same analytical approaches to measure metabolites. For instance, metabolomics, metabonomics or nutrimetabolomics have all been used as exchangeable terms. Likewise, metabolomic fingerprinting, metabolic or nutritional phenotyping and metabolic profiling have often been used to refer either untargeted approaches or targeted approaches. For the sake of clarity, we will use the term metabolomics throughout this review, but intend to encompass all aspect of measurement of metabolites in biological samples. Furthermore, we will use the term metabolome to refer to the full complement of metabolites.

The main analytical approaches selected for screening and generation of metabolomic data in nutrition are NMR and mass spectrometry coupled to a chromatography technique. NMR-based metabolomics is a robust and reliable technique which requires minimal sample preparation (Gowda and Raftery 2017, Markley, et al. 2017). One-dimensional (1D) ^{1}H NMR has been the most widely used NMR approach in nutritional metabolomics. However, two dimensional (2D) NMR methods such as TOCSY, ^{1}H J-RES and ^{1}H - ^{13}C HSQC can also be useful and in particular can aid the identification of metabolites (Brennan 2014, van Duynhoven and Jacobs 2016). Alternatively, mass spectrometry is based on the acquisition of spectral data in the form of a mass-to-charge ratio (m/z). Each molecule is defined by a different peak pattern and reflects a relative intensity. Direct mass spectrometry-based platforms such as direct injection/infusion

(DIMS) allow for high-throughput and fast metabolite screening. This is as a result of the minimum sample treatment without the need of previous chromatographic or electrophoretic separation, which permits reducing the times of analysis (Gonzalez-Dominguez, et al. 2017, Khamis, et al. 2017). Nevertheless, most MS-based approaches require coupling with separation techniques prior to MS analysis. Although, DIMS analysis provides good metabolomic coverage, chemical isomers and small differences in monoisotopic masses can only be detected by using ultrahigh resolution instruments such as Orbitrap-MS. Liquid chromatography (LC), gas chromatography (GC) and capillary electrophoresis (CE) are often coupled with MS (Scalbert, et al. 2009). The use of such approaches reduces the high complexity of the biological sample and optimises the MS analysis of different sets of molecules by reaching the detector at different times. Within these platforms a wide range of ionization and mass selection methods is available. However, electrospray ionization (ESI), electronic impact (EI) and atmospheric pressure chemical ionization (APCI) ion sources are the most employed techniques in nutrition and food MS analysis. A common concern for both hyphenated techniques and DIMS are the occurrence of matrix effects and ion suppression, especially when using ESI sources.

The potential and usefulness of metabolomics approaches have been widely demonstrated in the nutrition field. For example, a recent study carried out in the UK has been able to classify a free-living population into groups by dietary patterns using an NMR approach (Garcia-Perez, et al. 2017). NMR spectroscopy has also been successfully applied to discover food biomarkers and estimate intake of foods. Tartaric acid was used as a dose responsive urinary biomarker to quantify the intake of grape in a dietary intervention study (Garcia-Perez, et al. 2016); the quantification of proline betaine in a cross sectional study also allowed estimation of citrus intake (Garcia-Perez, et al. 2016). Illustrating the usefulness of MS approaches, four biomarkers of milk intake were identified in a twin cohort and validated in independent populations (Pallister, et al. 2017). Metabolomics of urine and faecal samples has helped to ascertain the effects of breast-, formula feeding and bifidobacteria supplementation on neonates and infants (Bazanella, et al. 2017, Dessi, et al. 2016).

It is worth noting that the selection of the appropriate platform is dependent on the research question and the types of metabolites to be measured. Each analytical platform has its own strengths and limitations. For example, NMR-based metabolomics provides high reproducibility and structural information which can be extremely useful in the identification of unknown metabolites. However, MS-based approaches offer greater sensitivity (Emwas 2015). Moreover, the broad nature of metabolites and their differences in dynamic range (from pico- up to millimolar) requires the use of different metabolomic platforms to ensure optimal coverage of the metabolome.

In short, metabolomics studies can be achieved with 2 broad strategies: I) Untargeted approaches that aim for the simultaneous measurement of hundreds/thousands of small molecules, whose profile constitutes a unique and specific hallmark of health/nutritional status; and II) Targeted approaches which aim to detect and measure a predefined set of metabolites (Cajka and Fiehn 2016). Importantly, both metabolomic strategies are able to reflect the response to a diversity of stimuli such as diets or foods in the composition of biofluids and tissues. However, in recent years the trend has been the application of these strategies simultaneously or in sequence since the results are complementary (Figure 1). Whereas untargeted approaches can help uncover new metabolites and new hypotheses, targeted approaches support well-defined hypotheses and allow the accurate detection and quantification/semi-quantification of predefined metabolites. The data analysis and processing steps in each approach allow for multiple options and may differ substantially among platforms (*e.g.* Fiehn 2016, Gorrochategui, et al. 2016, Misra, et al. 2017, Pontes, et al. 2017). Although significant progress has been achieved in computational techniques, advances in the processing of metabolomics raw data, development of databases and repositories for identification of metabolites are a permanent hot topic in the field (Barupal, et al. 2018, Tsugawa 2018). A brief description of the workflow for each strategy is summarized in Figure 1.

Applications of metabolomics in nutrition related research are continuing to grow and it is without a doubt making a positive impact on the field. Consequently, the objective of this review is to present current trends in the application of metabolomics in nutrition research and identify a number of key challenges that need attention for the further progression of the field. A detailed review of the analytical techniques and data analysis strategies is beyond the scope of this review and the readers are referred to a number of recent reviews on these topics (Barupal, et al. 2018, Brennan 2014, Covington, et al. 2017, Emwas, et al. 2018, Fan and Lane 2016, German, et al. 2007, Gorrochategui, et al. 2016, Guo, et al. 2012, Markley, et al. 2017, Rangel-Huerta and Gil 2016).

APPLICATION OF METABOLOMICS IN NUTRITION INTERVENTION STUDIES

The application of metabolomic studies or the inclusion of metabolomic approaches as part of nutrition intervention studies have increased over recent years. In general terms, nutrition intervention studies provide insights into the link between nutrition and health/disease. A dietary intervention can involve whole diets, specific foods or extracted substances such as micronutrient or phytochemicals. The evaluation of the effects of nutrition interventions are complex due to the fact that many of the perturbations are subtle and often difficult to detect. Nevertheless, application of metabolomics can aid our understanding of the potential effects of a dietary intervention.

After a thorough literature review on nutrition studies applying metabolomics, 46 key studies representative of the broad spectrum of possibilities in the nutrition field were selected and grouped in 8 categories according to the type of foods or nutrient source evaluated (Table 1). Scientific papers where the main objective was the identification of dietary biomarkers or dietary pattern biomarkers are included in *section 3.*

The evaluation of the effects of acute, medium and long term dietary interventions has become one of the most widespread application of metabolomics in nutrition research. These studies included exploration of effects in healthy populations, effects in disease conditions and effects through different stages of life. An overview of key studies is given in Table 1. In an effort to highlight the different applications some pertinent examples are described below.

A number of studies focused on early nutrition, including newborn and infants which have employed metabolomics have emerged in the literature in recent years. Cesare Marincola, et al. (2016) reported the use of NMR based metabolomics for examining the influence of milk feeding types on newborns. The urinary profiles stimulated by two types of formula milk, either or not enriched with functional ingredients, were explored over four months of life and compared with the effect of breast-feeding. The results demonstrated similar characteristics for the growth with the three milk types, while prominent quantitative differences were detected for specific metabolites such as *e.g.* pantothenic acid, choline, threonate, tartrate, cis-acotinate, and lactate, between formula feeding and the human lactation. The usefulness of these studies lie in the possibilities of optimising infant formulas by minimizing the gap in composition and outcomes with human milk. Bazanella, et al. (2017) designed a double-blind, randomized, placebo-controlled study to evaluate the impact of bifidobacteria supplementation on the microbiome during the first years of life. The study included metabolic profiling and analysis of short-chain fatty acids in faecal samples by LC-MS approaches, in addition to 16S ribosomal RNA gene amplicon sequencing to explore the diversity of faecal microbiota. The combination of metabolite and microbiota data showed differences between breast-fed and formula-fed infants at month 1, showing a decreased in Bacteroides and Blautia spp. associated with changes in lipids and unknown metabolites. However, colonization of the supplemented Bifidobacterium strains was not detected in long term (24 months) identifying the need to perform further work to see the long term impact.

Examination of the publications relating to fruit and vegetable consumption (Table 1) reveals that the majority of the studies selected are focused on the characterization of the metabolic response to different types of diets. An example of such a study by Larmo and colleagues (Larmo, et al. 2013) addressed the effects of consumption of berries and their fractions on the serum metabolome of overweight women. The intervention demonstrated significant modifications on NMR profiles of the four berry diets ($P<0.001$-0.003). As example, dried sea buckthorn berries (SBs), modified the levels of triglycerides in small HDL particles as well as in serum creatinine and phenylalanine, whereas sea buckthorn oil (SBo) produced a decrease in serum-free cholesterol, albumin, and lactate concentrations, among other modifications. Changes induced by berries differed between women who had higher and lower cardiometabolic risk baseline, being favourable pronounced for individuals at higher cardiometabolic risk.

Application of metabolomics to study the effect of fibre and grain sources on glycemic and weight loss management has advanced our knowledge of their potential health benefits. For example, Rasmussen and colleagues examined the influence of low-calorie diets assigned to a high-GI or low-GI in a long-term dietary study (Rasmussen, et al. 2012). The study was performed as a parallel intervention trial with five different diets. After following an 8-week low-calorie diet, the overweight subjects defined their diets following the supermarket model for food consumption. The urine was collected after 1, 3 and 6 months and the NMR metabolite profiling was performed. Changes in the metabolites formate and hippurate were identified and linked to the intervention. Lankinen et al. (2011), focused on the modification induced in plasma following intake of high-fiber rye bread (RB). Lipidomics (UPLC-MS) and GCxGC-TOF/MS analysis were performed in postmenopausal women with elevated total cholesterol and BMI (20–33 kg/m2). Ribitol, ribonic acid and indoleacetic acid ($P<0.001$) were found increased, while ribonic acid and tryptophan were positively correlated ($r = 0.40$; $P = 0.003$). The results suggested a positive effect of rye bread on satiety and weight maintenance. Other key-studies included examining the effects of proteins from fish, meat or supplements in different populations and their influence on human metabotypes; key results are highlighted in Table 1. Metabolomics has also played a key role in progressing our understanding of the metabolic effects of dairy, probiotics and different lipid loads (Table 1).

Examination of these studies together has revealed that blood and urine were used for the majority of studies. The use of urine was predominantly used when examining the metabolism or transformation of food/diets, while blood was employed for studying alterations in the metabolism of endogenous compounds. Metabolomic analysis of faecal samples appears as a current trend to study the implication and modifications of gut microbiota. Examination of the metabolites can reveal important functional information and has played a role in linking altered microbiota to various metabolic conditions. Overall, application of metabolomics to nutrition intervention studies has enhanced our understanding of the role of various diets and dietary components in health promotion.

USE OF METABOLOMICS IN THE IDENTIFICATION OF DIETARY BIOMARKERS

Traditional methods for assessment of dietary intake rely on self-reported tools such as 24-h recalls, food diaries, and food-frequency questionnaires. There are a number of well-defined limitations associated with such methods (Gibbons, et al. 2015, Kipnis, et al. 2002). Recall errors, energy underreporting and difficulties in the estimation of portion size are inherent issues that affect the results and their interpretations. These errors can be the origin of misclassification of subjects, reduction of statistical significance and subsequently attenuation of the potential diet-disease relationships (Jenab, et al. 2009, Prentice, et al. 2011).

In recent years, the concept of dietary biomarkers as objective measures of food intake has emerged. Metabolomics has played a key role in the identification of potential new dietary biomarkers and a number of pioneering studies have emerged which clearly demonstrate the potential of such biomarkers in the quantification of food intake. García-Perez et al (2016) described an analytical approach to identify and further quantify dietary biomarkers. The metabolite tartaric acid was initially identified using NMR spectroscopy, as a potential biomarker of grape intake in an acute grape challenge dietary intervention study. The grape marker was subsequently validated by demonstrating the possibility to estimate the amount of grape consumed in a dose-response randomized controlled trial. In this case, the excretion of tartaric acid in urine had a strong relationship with the amount of grape consumed in the controlled environment (r^2 = 0.90 after 24 h). Recent studies led by our group have also shown successful results. Gibbons et al. (2017), measured proline betaine in urine samples following various amounts of citrus intake. A clear dose response was observed and calibration curves were constructed to allow estimation of intake from the biomarker level. Of particular note, a correlation of 0.92 was reported between actual intake and predicted intake highlighting excellent agreement between biomarker level and actual intake. Importantly, the ability of the biomarker to estimate intake was examined in an independent cross sectional study of 560 individuals. The results demonstrated that there was excellent agreement between the self-reported intake (estimated from a 4 day semi-weighed food diary) and the estimated intake from the biomarker. Together these examples demonstrate clearly the potential of urinary biomarkers to estimate intake and lay the foundations for future studies.

Examination of the literature revealed that there are a number of putative biomarkers for various foods which are summarised in Table 2. A full review of each food is beyond the scope of this review, however, some key examples are highlighted here. A number of studies have examined investigated biomarkers of meat intake. Stella et al. (2016) found marked differences in the metabolic signature of volunteers consuming a high-meat diet *vs* a vegetarian and low meat diets. Pattern recognition analysis performed in urine samples analysed by ^{1}H NMR spectroscopy revealed increased levels of the urinary creatinine, creatine, TMAO, taurine, and 1- and 3-methylhistidine in the group consuming a higher amount of meat. More recent studies have confirmed that 3-methylhistidine is more specific for white meat intake (Cheung, et al. 2017).

In recent years a number of studies have identified biomarkers of coffee intake. An intervention study applying NMR analysis suggested 2-furoylglycine as a novel candidate for the consumption of coffee (Heinzmann, et al. 2015). 2-furoylglycine was found, among other previously reported potential biomarkers such as *N*-methylpyridinium, in the urine of coffee drinkers after a 6-day controlled study. Its excretion profile was characterized in a further coffee challenge with 5 volunteers whose diet, except for coffee consumption, was not restricted. The maximal excretion of 2-furoylglycine was registered after 2 h consumption (p=0.0002) returning to the baseline after 24h. The authors proposed this new marker as highly specific to coffee consumption since its formation is produced during the roasting of coffee beans (Heinzmann, et al. 2015).

Despite the number of putative biomarkers of foods described in the literature it is also worth noting that are still very few well documented and validated dietary intake biomarkers. In the case of dietary biomarkers a recent publication has highlighted guidelines for evaluation of the quality of candidate food intake biomarkers (Dragsted, et al. 2018). The scheme includes assessment of the plausibility, dose-response, time-response, robustness, reliability, stability, analytical performance, and inter-laboratory reproducibility. It is evident that further work is needed to validate these putative biomarkers in order to advance the field.

Furthermore, interactions with the broader Food Science community and in particular application of metabolomics directly to the foods has great potential. The area of FoodOmics has expanded in recent years and detailed analysis of the metabolite composition of foods has the potential to inform the development of new dietary biomarkers. Biological plausibility was identified as one of the key criteria for biomarker assessment and application of metabolomic profiling of the foods can aid in this aspect by demonstrating the presence of certain metabolites or precursors in the foods of interest. Future collaboration between both fields of research should yield significant advancements in the nutrition field.

Increasingly, evidence is emerging that the overall pattern of dietary intake is more important to understand relationships with health and disease (Corella, et al. 2018, Cunha, et al. 2018, Pistollato, et al. 2018). As a consequence the use of multiple biomarkers to track/monitor dietary patterns is of growing importance. Garcia-Perez and colleagues built multivariate models to classify people into a dietary pattern based on the NMR urinary metabolomics data from a controlled intervention study (Garcia-Perez, et al. 2017). The four diets used were designed to have a step variance in the WHO healthy eating guidelines. The classification model was confirmed in independent studies and revealed that individuals were classified into patterns with a higher or lower non-communicable disease risk.

Other work has demonstrated the use of metabolomics to monitor adherence to a new Nordic diet (NND) or the Average Danish Diet (ADD) (Khakimov, et al. 2016). Such examples demonstrate the potential in terms of adherence to certain diets which may play a role in intervention monitoring. Work in our own laboratory has developed a multivariate model based on urinary metabolomic data to classify subjects into either a healthy dietary pattern or an unhealthy dietary pattern (Gibbons, et al. 2017). The classification into dietary patterns was supported by assessment of dietary intake and blood nutrient parameters. Further refinement and development of the models should allow for rapid and objective classification of individuals into certain dietary patterns. This in turn could feed into the delivery of personalised dietary advice and into large epidemiological studies examining the associations between dietary patterns and health parameters.

Finally, organising the appearance of these new biomarkers, a consensual classification for the correct ontology and flexible grouping of biomarkers in the area of nutrition has been also published (Gao, et al. 2017). The classification proposed is based on the most likely use of the biomarker and the following subclasses were proposed: “food compound intake biomarkers (FCIBs), food or food component intake biomarkers (FIBs), dietary pattern biomarkers (DPBs), food compound status biomarkers (FCSBs), effect biomarkers, physiological or health state biomarkers”. Furthermore, as the number of identified food biomarkers expands and is expected to increase in the coming year there is a need for joint collaboration in the field. In this sense, the Food Biomarkers Alliance (FoodBAll) is directed to identify and validate food intake biomarkers gathering the expertise in food metabolomics of thirteen European countries (Brouwer-Brolsma, et al. 2017). FoodBall also contributes to the development of databases to support this work. Examples of the databases includes: FoodDB (http://foodb.ca/), FoodComeEx (http://foodcomex.org/), PhytoHub (http://phytohub.eu/) and Phenol-Explorer (http://phenol-explorer.eu/). Collectively these databases are useful in connecting metabolites to the foods but also to their metabolism in humans. Finally, more work is needed in developing joint efforts for the identification of the many unknown features that appear in the metabolomics datasets.

ROLE OF METABOLIC PHENOTYPING IN PERSONALISED NUTRITION

Metabolic phenotyping has grown as a strategy to reflect the interplay between environmental factors such as diet, physical activity and genotype. Use of metabolomics in metabolic phenotyping has opened up the possibility for the delivery of optimum individualised dietary advice and personalised healthcare solutions. In parallel to this goal, the stratification of the population for epidemiological studies according to their metabotypes (metabolic phenotypes) is an option to reach larger segments of the population (Nicholson 2006). It seems affordable that the current medical checkouts targeting usual individual markers or food habits will be replaced by more complex and informative analysis. These analysis will reveal individual metabolic signatures by means of high-throughput screening and thus, provide a more complete view of nutritional and health status.

Recently, metabolomics has emerged as a tool for determining metabotypes: this is a process where combinations of specific metabolites are used to classify individuals into groups or clusters based on a similar metabolic phenotype. From a nutrition perspective metabolic phenotyping or metabotyping offers the possibility of examining responses to dietary interventions and the potential of delivering tailored dietary advice to a specific metabotype.

Our previous work endeavoured to support the development of a metabotyping approach for the delivery of targeted or personalised nutrition. Initially, using four metabolic parameters we developed the concept and identified four metabotypes in an Irish cross-sectional population (O'Donovan, et al. 2015). For each of these clusters algorithms were developed to enable the delivery of targeted dietary advice based on cluster membership. Importantly comparison of the targeted advice with individualised dietary advice revealed good agreement: a mean match of 89.1 % was observed for a random selection of 99 individuals. Further development of this concept was performed in a pan-European study where a more expansive set of metabolites was used to perform the metabotyping. The use of algorithms based on the metabotype to deliver targeted dietary advice resulted in delivery of advice that agreed with a personalised approach (O'Donovan, et al. 2017). The results from both these studies indicate that the metabotyping framework may be a useful approach to deliver dietary advice at a population level. However, further work is need to decipher if such an approach would lead to improved dietary intake and alter disease risk parameters.

In recent years, the importance of individual responses to dietary interventions has become evident. Understanding and identifying profiles that can predict response is important for the building of an evidence base for the further development of personalised nutrition. Application of a metabotyping approach to characterise differential responses to dietary interventions is important. Work from our laboratory demonstrated the use of the metabotype approach in identifying a positive response to a vitamin D intervention. A metabotype characterised by low concentrations of vitamin D and higher concentrations of adipokines was responsive to vitamin D supplementation (O'Sullivan, et al. 2011). In a separate study using a similar concept, we identified differential responses to an oral glucose tolerance test. In total four distinct metabolic responses were identified and at "risk" metabolic group was highlighted through the approach (Morris, et al. 2013). Work from other groups has also employed a similar approach to identify groups responsive to certain dietary interventions. Vazquez-Fresno and colleagues identified four metabolic phenotypes in a population of high cardiovascular risk individuals undergoing a randomised controlled study (Vazquez-Fresno, et al. 2016). Through the metabotyping approach they identified a red wine polyphenol responsive metabotype. Wang and colleagues identified metabotypes in a carotenoid cross-over intervention (Wang, et al. 2013). Using a k-means cluster analysis approach a total of five metabotypes were identified with differential response to the dietary carotenoids.

Finally, the metabotyping approach has played a role in the identification of metabolic phenotypes in diet related diseases. For example, Amato, et al. (2016) identified two metabotypes in a type 2 diabetes population using incretin levels. Similarly, others have identified different sub types of obesity, metabolic syndrome and pre-diabetes based on statistical analysis of metabolic and phenotypic parameters (Arguelles, et al. 2015, Zak, et al. 2014).

Overall, the application of metabotying in nutrition is still in its infancy. However, the results to date demonstrate great potential and in particular offer potential for delivery of tailored dietary advice. Further work is needed to develop the concepts and to demonstrate that implementation of such an approach can improve metabolic risk parameters.

CONTRIBUTION OF METABOLOMICS DATA TOWARD SYSTEM BIOLOGY APPROACHES

Systems biology is the most complex level of integrative biological data currently available. It progresses with the aim to explain biological properties, processes and functions at a system level. Modelling at the systems level carries theoretical advances for all scientific and medical disciplines, providing also a solid framework for the nutritional research and the progression toward personalised nutrition. The nutri-(gen-protein-metabolite)–omics technologies and the study of their fluxes, have played a significant role in the development of nutrition science in the last 10 years. Through applications of such technologies we have gained insight into the role of certain diets, dietary patterns and dietary components. Furthermore, our understanding of diet-disease relationships has also been enhanced.

Metabolomics can act as an interface for the phenotype within systems biology approaches by implementing phenotypic data related to metabolic networks into biological models. More precisely, the integration of metabolomics data obtained from nutritional interventions into more complex models is extremely useful to elucidate how food impacts health, differentiate dietary responses according to groups of individuals as well as to point out nutrients or bioactive substances responsible for the modifications that could become targets in future nutritional intervention (Badimon, et al. 2017). Metabolomics can also bring a systems approach to epidemiology and can enable the study of underlying mechanisms.

The latest trends in nutritional system biology use the computational fusion of omics-data obtained by genomics, transcriptomics, proteomics and metabolomics approaches into comprehensive models with diagnosis and predictive capacity. These models also permit the inclusion of data and control of confounding data related to the omics datasets but obtained from more classical approaches. Such is the case when medical parameters *e.g.* glucose, HOMA-IR, enzyme activities or blood pressure records are associated with omics approaches (Drenos 2017, Kim, et al. 2017, Sperisen, et al. 2015, Yu and Zeng 2018). Lampe et al. (2013) categorised and illustrated integrative analysis in the field of nutrition within three levels of complexity: I) concordance analysis methods in which two different omics datasets are correlated and provide information about components that interact between them *e.g.* gene expression and proteomics; II) sequential integration methods, whose models incorporate multiple omics dataset with the purpose to discover biomarkers or elucidate biological mechanism and; III) concurrent integrations methods, which are built as sequential integration methods but incorporate activity of biological pathways and emerging data. This usually evidences how a merged model improves its value compared to a single source of data. Accordingly, the complexity of the tools applied for processing and treatment of multiple data sets also increases at each level. While the first level is usually sorted out with multivariate statistical methods, the second and third levels require more complex tools such as metabolite set enrichment analysis, pathways analysis or network based methods whose outputs are not always easy to interpret (Barupal, et al. 2018, Lampe, et al. 2013).

At the moment, enrichment statistics have become a complementary, more consistent and informative tool for system biology approaches. An overview of the bioinformatics tools currently available for enrichment analysis of metabolomics data is presented in recent work by Marco-Ramell et al. (2018). Enrichment analysis tests most often reported fit with the following two types 'Hypergeometric or Fisher Exact tests' or 'Kolmogorov-Smirnov test'. Their purpose is to bridge biological insights to groups of metabolites (Barupal, et al. 2018). In a further step, pathways analysis and metabolic networks can be represented using nodal architecture and pathway map diagrams with different levels of complexity. For these functions a number of platforms is available such as *e.g.* pathway collages, MetaCyc —*https://metacyc.org/pathway-collage-info;* MetaboAnalyst —*http://www.metaboanalyst.ca* MetScape—*http://metscape.ncibi.org/;* MetExplore—*http://metscape.ncibi.org/;* CytoScape—*http://www.cytoscape.org/* (Chong, et al. 2018, Cottret, et al. 2010, Karnovsky, et al. 2012, Paley, et al. 2016). Alternatives to biochemical pathway mapping have been also proposed such as that based on chemical similarity (ChemRICH—*http://chemrich.fiehnlab.ucdavis.edu/* (Barupal and Fiehn 2017). Additionally, computational text mining approaches can help to extract literature related to the compounds of interest. For example, the NutriChem database (—*http://sbb.hku.hk/services/NutriChem-2.0/*) was developed with the aim to explore the effect of plant-based foods on human health (Ni, et al. 2017).

The number of studies in nutrition and food sciences has grown exponentially during the last years. In 2009, the *Annual Review of Nutrition* published an interesting review compiling nutritional studies at systems level (Panagiotou and Nielsen 2009). The paper highlighted the value of systems biology using as illustration the studies on yeast that link nutrition, genome and phenotype. Moreover, examples integrating the results from different approaches such as *e.g.* dietary preferences, plasma metabolites, urine metabolites and gut microbial metabolites; fusion of metabolites in plasma with hepatic fat and proteome; or transcriptome and proteome analysis were introduced. More recent trends have been also presented in two reviews; the first, by Badimon et al. (2017) emphasising the application and integration of omics technologies and focusing on the role of diet, functional foods and bioactive compounds in diseases related to oxidative damage; while the second by van Ommen et al. (2017), centred on the advances of system biology towards personalised nutrition. Other interesting works performing data fusion are the published by Lacroix et al. (2015) and Kim et al. (2017), where systems biology approaches have been used to evaluate the response to nutritional interventions such as *e.g.* caloric restriction or polyphenols on aging, and for the identification of prognostic metabolites for prediction of responses against oxidative stress and inflammation. These studies appear as illustrators for the feasibility of new avenues for the integration of nutritional metabolomics studies in system biology approaches. It opens promising new perspectives for the nutrition research.

FUTURE CHALLENGES

Although some important obstacles such as the acquisition of large datasets for the holistic approach of metabolomics have been overcome, several challenges have yet to be sorted out at multiple stages of the nutritional metabolomics workflow.

With respect to food intake biomarkers there is an urgent need to advance the field so that reliable biomarkers can be used in epidemiological studies. Examples of the work needed includes the following: I) Performing studies for the validation and confirmation of putative biomarkers; II) Developing studies to evaluate the capacity of those markers to estimate intake through dose-response studies and evaluation in ethic diverse population groups; and III) Exploring the composition of foods by novel high-throughput technologies to search for new metabolites and metabolite associations that may be particular for the food eaten and could be distinctively associated with food intake in further steps. In a boarder sense, the investigation of how nutrient and food excesses, deficiencies and specific substances modify homeostasis and affects health status is a complex challenge that metabolomics can help decipher. In the long term, this research will help to define the effects and influence of diet under pathological circumstances.

In addition to the challenges associated to each specific branch of nutrition research, there are challenges regarding analytical and computational features to be considered. From this operational viewpoint we have highlighted a few key challenges. Elucidating the chemical structure and the origin of unknown significant compounds detected by untargeted approaches, remains a bottleneck in the step for identification of metabolites. Action is needed to address the design of agile and standardized procedures to establish either data processing or analytical pipelines that clarify the nature and provenance of the unknown entities. Sharing of authentic standards is essential and cross laboratory interactions should enhance this field. The current metabolomics workflows generates large amounts of data and the handling of such data raises new questions about data analysis, treatment and their integration. There is a multiplicity of options to treat the datasets before applying statistical analysis. However, different types of filtration, transformation and imputation of missing value strategies can bring divergent results from the same data and render data incomparable. As a result there is a need for unifying the workflow criteria to treat different types of datasets by means of agile platforms allowing the communication of the latest advances to the scientific community efficiently. Moreover, differences in data structures and formats of datasets as well as differences in timescales and dynamic ranges between data from metabolomics alone or in conjunction with other data sources, are a cause of discussion. These issues can be a limitation for the use of data in further integrative analysis and thus require actions to move the field towards standardisation. In order, to maximise data sharing, the deposition of data in a uniform manner into databases is encouraged. An example of a suitable platform is the Phenotype Database: this platform was designed for the storage and sharing of nutrition related data and has modules for metabolomics data sharing (*https://dashin.eu/interventionstudies/).*

Despite the challenges the future of nutritional metabolomics is bright. It has the potential to play an important role in many aspects of nutrition science. Addressing the above challenges will help pave the way forward and enable the full potential of metabolomics in nutrition research.

SUMMARY POINTS

1. Metabolomics has the potential to enhance our understanding of the link between diet and health.
2. Objective food intake biomarkers can estimate the intake of certain foods and studies have demonstrated excellent agreement with actual intake and self-reported intake.
3. Metabolomic biomarkers can aid in the classification of individuals into dietary patterns.
4. Metabotyping has great potential in the delivery of targeted nutrition to large population groups.

ACKNOWLEDGMENTS

LB acknowledges the support of the European Research Council (647783).

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FIGURE CAPTIONS

Figure 1. Overview of the metabolomic workflow

The metabolomic workflow for untargeted and targeted approaches can be summarized in five consecutive steps. Each of these steps has multiple options and each step is usually tailored to study design and research question. Application of an untargeted approach can often lead to subsequent targeted analysis of specific metabolites.

Table 1. Compilation of intervention nutritional studies applying targeted and untargeted metabolomics

TYPE OF INTERVENTION (FOOD/DIET TESTED)	MAIN OBJECTIVE	STUDY DESIGN DURATION/NUMBER OF PARTICIPANTS	SAMPLE	ANALYTICAL PLATFORM	OBSERVATION
INFANT & CHILDREN NUTRITION					
Low-protein/low caloric density formula with probiotics *vs.* high-protein formula and breast feeding *(Martin, et al. 2014)*	Metabolic response to protein content of infant formula on infants body weight gain	RCT parallel 12 m / Infants from overweight and obese mothers (n=300)	Urine Faeces	^{1}H NMR	Metabolic differences between breast and formula feeding: – Carbohydrate metabolism: ↑ lactate and milk oligosaccharides (stool) – Energy metabolism: different Krebs cycle and NAD/NADP metabolic pathways – Growth and development: ↑ IGF-1 – Protein metabolism: ↑ protein-derived SCFAs (stool), ↑ Urea cycle and nitrogen balance Lipid metabolism: ↑ β-oxidation (carnitine) ↑ ketogenesis (lipids and ketogenic AAs)
Formula milk enriched with functional ingredients *vs.* standard formula and human milk *(Cesare Marincola, et al. 2016)*	Effects of postnatal nutrition milk formulas characterising the urinary metabolome	RCT 130 d / Newborn (n=60)	Urine	^{1}H NMR	Formula milks *vs* human milk: – ↑ choline, ↑ tartrate, ↓ citrate, ↑ threonic acid , ↓ fucosyl moieties, ↓ *n*-acetylated compounds, ↑ pantothenic acid, ↑ lactate, ↓ formate
Breastfed milk *vs.* formula milk *(Dessi, et al. 2016)*	Effect of different diet regimens in urine metabolite profiles of IUGR, AGA and LGA neonates	CT 7 d / Neonates (n=35)	Urine	GC–MS	Differences in the metabolite excretion profile of neonates: – Formula milk: ↑ glucose, galactose, glycine and myo-inositol in urine – Breast milk: aconitic acid, aminomalonic acid and adipic acid – At 7 days: neonates fed with formula milk shared ↑ pseudouridine with IUGR and LGA at birth. Breastfed neonates shared ↑ pyroglutamic acid, citric acid, and homoserine, with AGA at birth
Standard whey-based formula containing Bifidobacterium bifidum, B. breve, B. longum , B. longum subspecies infantis *vs.* controls (placebo and breastfed) *(Bazanella, et al. 2017)*	Impact of infant formula supplemented with Bifidubacterium on structural and functional changes in the gut from birth through the first year of life and after 2 years	RCT 12 m / Newborn infants (n = 117)	Faeces	UHPLC–qTOF/MS	– Supplementation associated with ↓ detection of bacteroides fragilis and blautia species – Fucosylated hmos correlated with the occurrence of bifidobacteria – Faecal metabolites discriminating between b, f+, and f2: sterol lipids, glycerophospholipids and fatty acids – Exogenous bifidobacteria failed to colonize the infant gut
Skimmed milk and low-fat meat supplements *(Bertram, et al. 2007)*	Capability of NMR-metabonomics. Effects of animal proteins in prepubertal children	CT 7 d / 8-y boys (n=30)	Urine Serum	^{1}H NMR	– Milk diet: ↓ urinary excretion of hippurate —alterations in gut microflora – Meat diet: ↑ urinary excretion of creatine – Other discriminating metabolites: TMAO (meat), ↑ in intensities of lipid signals (CH3, (CH2)n, CH ¼ CH-CH2 and CH2-CO) (milk)
Functional meat product, containing 0.02% rosemary extract, 0.001% vitamin E and 0.3% PUFAs *(Balderas, et al. 2010)*	Capabilitiy of CE-UV detecting differences in urine of diabetic children. Effect of designed meat products in children	CT 12 m / 6-11-y children (n=49)	Urine	CE–UV	– Diabetic children: ↑ nitrites, citrate, phenyllactate, glutamate, creatinine and urea; ↓ glutarate, guanidine, phospho-L-serine, benzoate, urate, and glycerate – TD/H after 12 months of receiving the extract: ↑ nitrite, citrate, ketoglutarate aminoadipate, phenyllactate, glutamate, creatinine, phospho-L-serine pyroglutamate; ↓ urea and p-hydroxyphenyllactate
FRUIT AND VEGETABLE					
Normal diet (ND) and low-phytochemical diet (LPD) *vs.* standard phytochemical diet *(Walsh, et al. 2007)*	Role of dietary phytochemicals on human urinary metabolomic profiles	CT 6 d / Healthy (n=21)	Urine	LC–MS ^{1}H NMR	Discriminating metabolites between the LPD and the ND: – ^{1}H NMR: ↑ hippurate in the ND samples and ↑ creatinine and methyl histidine in LPD samples – LC-MS: m/z 180.068, 105.028 (both corresponding to hippurate), 312.217, 197.07, and 169.036 (unidentified) associated with ND samples. m/z 180.068, 105.028 (relating to hippurate), 413.045, 312.217, and 169.036 (unidentified) peak intensities associated with SPD samples
Basal, low-phytochemical diet, devoid of fruit and vegetables, *vs.* basal diet supplemented with cruciferous vegetables, soy foods, and citrus fruits *(May, et al. 2013)*	Urinary metabolomic pattern characterization in response to a high-phytochemical diet	RCT crossover 2 wk / Overweight-obese women (n=10)	Urine	LC–MS/MS (LTQ-FT)	– Proline-betaine, sulforaphane, and several isoflavones biomarkers of citrus, crucifers and soy intake, respectively – ↑ in urinary excretion of shorter-chain acylcarnitines and TCA cycle-intermediates suggesting a change in energy utilization from glucose to fat with diets low in fruit and vegetables – Comparison with 3DFR and FFQ in a cross-sectional, observational study of free-living individuals (n=60)
Berry diets: dried SBs, sea buckthorn phenolics ethanol extract mixed with Maltodextrin (SBe+MD) (1:1), SBo, and frozen bilberries *(Larmo, et al. 2013)*	Effects of berries on serum metabolome, concentrations of circulating lipids, lipoproteins, and low-molecular-weight metabolites in women with risk of cardiovascular disease and type 2 diabetes	RCT crossover 33–35 d / Women with CDV, T2D risk (n= 110)	Serum	^{1}H NMR	Positive changes observed in the baseline group of higher cardiometabolic risk – Dried SBs induced beneficial effects on serum triglycerides and VLDL subclasses – SBo ↓ the serum concentration of total and LDL cholesterol and apolipoprotein B – SBe+MD ↑ effect on serum triglycerides and VLDL

Paleolithic-type (PD) diet *vs.* Nordic Nutrition Recommendations (NNR) diet *(Chorell, et al. 2016)*	Plasma metabolic response in relation to insulin sensitivity after weight loss induced by a diet intervention	CTs 5 wk / Women (n=10) 6 m / Postmenopausal women (n=70)	Plasma	GC–TOF/MS	PD improved insulin sensitivity compared to NNR *via* ↓ DGLA and ↑ MI and b-HB – 6 months intervention: PD group ↓ 1,5-AG, DGLA (20:3, n-6), lauric acid (12:0), glycine, tryptophan and tyrosine; and ↑ MI, DHA (22:6, n-3), ascorbic acid, β-HB, serine and oxalic acid compared to NNR group. Changes in amino acids; concomitant ↓ in SFA and *n*6-PUFAs
Orange juice *(Moreira, et al. 2018)*	Effect of two-week orange juice consumption by a mass-spectrometry based metabolomics approach	Single arm trial 15 d / Healthy (n=15)	DBS Plasma	FIA–MS GC–MS	– ↑ of short-chain acylcarnitines and ↓ of medium and long-chain acylcarnitines – ↑ C3:1, C5-DC(C6-OH), C5-M-DC, C5:1-DC, C8, C12-DC, lysopc18:3, myristic acid, pentadecanoic acid, palmitoleic and palmitic acid and ↓in nervonic acid, C0, C2, C10, C10:1, C16:1, C16-OH, C16:1-OH, C18-OH, PC aa C40:4, PC ae C38:4, PC ae C42:3, PC ae C42:4 and cholesterol levels
GRAIN & FIBER					
Diet rich in whole grain rye and rye bran products *vs.* a diet of refined whole grain products with added cellulose as control *(Moazzami, et al. 2011)*	Effects of a diet rich in whole grain rye products on the plasma profile of prostate cancer patients	RCT crossover 6 wk / Early-stage prostate cancer men (n=24)	Plasma	^{1}H NMR	Shift in energy metabolism toward catabolic status – ↑ 3-hydroxybutyric acid, acetone, betaine, N,N-dimethylglycine, and dimethyl sulfone, after RP intake – Fasting plasma homocysteine and leptin ↓ after RP intake compared to WP intake
Rye breads *vs.* white-wheat breads (≤20% of total energy intake) *(Lankinen, et al. 2011)*	Changes in the metabolic profile produced by high-fiber rye bread to study the mechanisms underlying the health effects of rye bread	RCT crossover 8 wk / Postmenopausal women (n= 39)	Plasma	UPLC–qTOF/MS GCxGC–TOF/MS	– SM (d18:1/25:1) and SM(d18:1/25:3) ↑ at the end of the RB period compared with WB – Ribitol, ribonic acid, and 1H-indole-3-acetic acid (indoleacetic acid) ↑ during RB period – Ribonic acid and tryptophan concentrations positively correlated – Myristoleic and oleic acid concentrations ↓ during the RB period
Low GI diet, low protein diet, high GI diet and low GI high protein diet *(Rasmussen, et al. 2012)*	Effects of high vs low protein and low- *vs.* high-GI diets maintaining weight loss in families with history of obesity	CRT parallel 6 m / Healthy overweight (n=109)	Urine	^{1}H NMR	– ↑ formate in the HGI diet groups – Hippurate associated with dietary fibre intake
High fiber (HF) diet *vs.* low fiber (LF) diet (Ready meals: Pasta Bolognese, Chicken Tikka Masala, and Fish with spinach and mashed root vegetables) *(Johansson-Persson, et al. 2013)*	Alterations of plasma metabolome profiles to identify exposure and effect markers of dietary fiber intake	RCT crossover 5-wk / men (30-70 y) and women (50-70 y) with BMI>30 kg/m2 and total cholesterol 5.5–7.0 mmol/L (n=30)	Plasma	LC–qTOF/MS	– 6 features in ESI+ and 14 features in ESI– differed after HF compared to LF diet – 2-aminophenol sulfate ↑ during HF diet – m/z 153.0186 (γ-resorcylic acid) identified as a marker for a high dietary fiber intake – Nuatigenin identified at level II, but requires validation as a biomarker of oat intake
Meal with refined wheat, whole-meal rye, and refined rye breads *(Moazzami, et al. 2014)*	Postprandial metabolic responses between rye breads using NMR and targeted LC–MS metabolomics. Association with postprandial insulin responses	RCT crossover Test meal / Healthy postmenopausal women (n=20)	Serum	^{1}H NMR LC–MS	– RWB ↑ postprandial concentrations of leucine and isoleucine compared with RRB and WRB – Women with ↑ fasting leucine and isoleucine and ↓ SMs and PCs had ↑ insulin responses after all kinds of bread – Circulating ↑ BCAAs associated with ↑ risk of diabetes
Low glycemic load (GL) diet *vs.* high GL diet *(Barton, et al. 2015)*	Protective benefits of low GL diets. Modifications of plasma metabolome using a targeted metabolomics approach.	RCT crossover 4 wk / (n=20)	Plasma	LC–QTrap/MS	– Kynureate was significantly altered following Low GL
Meal consisted of commercial refined wheat bread (1177 kJ), 40 g cucumber and 300 mL non-caloric orange drink *(Shrestha, et al. 2017)*	Impact of a single meal on human metabolism. Changes in the metabolic profile of postmenopausal healthy women after ingestion of a wheat bread meal containing carbohydrates, proteins and fats	Post-prandial study / Postmenopausal women (n=19)	Urine	^{1}H NMR LC–MS/MS	The metabolic profile reflected the shift from catabolic to anabolic status – ↓ Acylcarnitines and ketone bodies reflected adaptive physiological responses to food (switch from β-oxidation to glycolysis and fatty acid synthesis). ↑ in lactate and pyruvate – Diacyl, alkyl acyl, PCs and lyso-PCs changed postprandially – All PCs ↓ at 180 min, and lyso-PCs (except for C18:2) ↓ at 45 min. Isoleucine, leucine and phenylalanine ↑ at 60 min and methionine ↑ at 45 min. Alanine and proline ↑ at 90 min
MEAT/FISH					
Low meat diet (60 g/day), high red meat diet (420 g/day) and (vegetarian diet (420 g/day from nonmeat sources) *(Stella, et al. 2006)*	Effects of three diets on the metabotype signature of humans	RCT crossover 15 d / Healthy Caucasian men (n=12)	Urine	^{1}H NMR	– High-meat diet ↑ urinary levels of creatinine, creatine, acetylcarnitine, TMAO, taurine, and 1- and 3- methylhistidine – Vegetarian diet: ↑ p-hydroxyphenylacetate – Low-meat diet and vegetarian diet signatures characterized
Fatty fish and lean fish (100–150 g/meal at least four times a week) *vs.* control group (meals made with lean meat) *(Lankinen, et al. 2009)*	Effect of fatty fish or lean fish on serum lipidomic profiles in subjects with coronary heart disease	CT parallel 8 wk / Subjects with myocardial infarction or unstable ischemic attack (n=33)	Plasma	UPLC–qTOF/MS	Protective effects of fatty fish on the progression of CHD or insulin resistance – Fatty fish group (plasma): ↓ oleic acid (18:1n-9) and dihomo-clinolenic acid (20:3n-6), ↑ a-linolenic (18:3n3), arachidonic (20:4n-6), EPA (20:5n-3), docosapentaenoic (22:5n3) and DHA (22:6n-3). ↓ Ceramides, lysophosphatidylcholines (lysoPC), DGs, phosphatidylcholines and lysophosphatidylethanolamines – Lean fish group (plasma): ↑ cis-vaccenic acid (18:1n-7), cholesterol esters and specific long-chain triacylglycerols

Balanced diets with lean-seafood *vs.* non-seafood proteins *(Schmedes et al. 2018)*	Effect of different protein sources in fasting and postprandial serum metabolites and lipid species	RCT crossover 4 wk / Healthy (n = 27)	Serum	^{1}H NMR UPLC–qTOF/MS	– Lean-seafood diet ↓ serum isoleucine and valine in fasting state; ↓ lactate and ↑ citrate and trimethylamine N-oxide during postprandial state – Non-seafood diet ↑ 26 lipid species in fasting state, *e.g,*. ceramides 18:1/14:0 and 18:1/23:0 and lysophosphatidylcholines 20:4 and 22:5
DAIRY PRODUCTS & FATS					
Low-fat dairy diet *vs.* two full-fat dairy diets (fermented and non-fermented) *(Nestel, et al. 2013)*	Effects of different dairy product-rich diets on potential biomarkers of CHD including a lipidomic analysis of plasma	RCT crossover 3 wk / Overweight/ obese subjects (n=12)	Plasma	LC–QTrap/MS GC–MS	– Non-fermented dairy diet: ↑ sphingomyelin and ↓ plasmalogen species phosphatidylcholine plasmalogen and phosphatidylethanolamine plasmalogen – Full-fat dairy diets: ↑ phosphatidylcholine containing 15:0 and 17:0
Diet increased in energy intake (1,250 kcal/day reached with high-energy, high-fat snacks and a liquid-oil-based supplement mixed in a dessert) *(Heilbronn, et al. 2013)*	Effect of overfeeding on lipids in men and women	Single arm trial 28-d / Healthy (n=41)	Serum	LC–QTrap	– Overfeeding: ↑ Alkenylphospahtidylethanolamine (PE(P)) and their precursor aklylphosphatidylethanolamine (PE(O)); ↑ total ceramide and ↓ Lysoalkylphosphatidylcholine (LPC(O)) and diacylglycerol – ↑ HDL, PE(P) and PE(O) suggest a change in HDL lipid composition with overfeeding
Breakfast meals containing dairy fat or vegetable (soy) oil *(Meikle, et al. 2015)*	Effect of dairy fat and soy oil on the postprandial lipidome in men	RCT crossover Test meal / Males (n= 21)	Plasma	LC–QTrap/MS	↑ in lipids with potential antioxidant capacity in postprandial period after dairy meals – Dairy meal: ↑ plasma phospholipids. ↑ alkenylphosphatidylethanolamine postprandially Soy: ↓ sphingomyelin, ether-linked, lysophospholipids, alkenylphosphatidylcholine, alkylphosphatidylethanolamine, alkenylphosphatidylethanolamine, lysoalkylphophatidylcholine, and lysophosphatidylethanolamine.
Carbohydrate-rich, low fat diet (30E% fat, 55E% carbohydrates, 15E% protein) *vs* a low carbohydrate, high fat diet (45E% fat, 40E% carbohydrates, 15E% protein) *(Frahnow, et al. 2017)*	Characterization of the metabolic adaptation after switching from a low fat to a high fat Western-style diet in mono- and dizygotic twins	Crossover trial 6 wk / Non-obese, healthy, twin pairs (n=46)	Plasma	DIMS (QExactive)	High heritability of basal concentrations of specific lipid species with strong dependence on sex, BMI and age – Finding of 5 different reactions
PROBIOTICS & PREBIOTICS					
Probiotic L. rhamnosus GG *vs.* placebo *(Lahti, et al. 2013)*	Impact of a probiotic on the composition and stability of the intestinal microbiota and serum lipid profiles	RCT 3 wk / Healthy Finnish adults (n = 25)	Serum	UPLC–qTOF/MS	The intestinal commensals are implicated in the metabolism of various lipid species – No differences in lipid profile stability between the treatment groups – 86 bacterial group-lipid pairs with notable correlations – 23 of the 131 genus-level taxa detectable by the HITChip
ITF (inulin/oligofructose 50/50 mix) prebiotics supplement *vs.* placebo (maltodextrin) *(Dewulf, et al. 2013)*	Impact of ITF prebiotics on the gut microbial ecosystem in obese women	RCT 3 m / Obese women (n=30)	Plasma Urine	^{1}H NMR	No significant clustering induced by the prebiotic – Subtle changes in the gut microbiota correlated with changes in fat mass, serum LPS and metabolism (hippurate, lactate and PC) – Patients with ↑Propionibacterium and Bacteroides vulgatus: ↑ lactate and PC
Chicory-derived inulin (Orafti inulin) 12 g of inulin (treatment) *vs.* maltodextrin (placebo control) *(Vandeputte, et al. 2017)*	Effect of chicory-derived inulin (Orafti inulin) on bowel function in healthy individuals with constipation	RCT crossover 4 wk / Healthy with constipation (n=44)	Faeces	GC-MS	↓ *Bilophila* abundances associated with softer stools and a favourable change in constipation-specific quality-of-life measures – Faecal metabolite profiles not altered by inulin consumption. ↑dodecanal – Changes in relative abundances of *anaerostipes*, *bilophila* and *bifidobacterium*
Oral probiotics mixture *vs.* a placebo *(Baldassarre, et al. 2018)*	Effectiveness and the safety of a probiotic-mixture for the treatment of infantile colic in breastfed infants	RCT 21 d / Infants between 30 and 90 days (n=66)	Faeces	^{1}H NMR	Probiotic modulated infantile colic symptoms by the end of treatment – Probiotic: ↑ acetate in subjects treated with the placebo and propylene glycol Placebo: ↑ 2-hydroxyisovalerate, alanine and 2-oxoisocaproate
DRINKS					
Beverages with carbohydrates and carbohydrates combined with proteins (low-carbohydrate, high carbohydrate, low-carbohydrate-protein and water) *(Chorell, et al. 2009)*	Effect of post- exercise ingestion of carbohydrates in combination with proteins on systemic metabolic response in the early recovery phase following exercise.	CT crossover 90 min of ergometer-cycling sessions / Males (n=24)	Serum	GC–TOF/MS	Impairments in insulin function or insulin resistance following ingestion of carbohydrates or carbohydrates + proteins – Pseudouridine suggested as a novel marker for pro-anabolic effect with LCHO-P ingestion (↑ insulin and availability of amino acids, and ↓ 3-methylhistidine) – LCHO-P improved metabolic status of less fit subjects in the recovery phase.↓ fatty acids and ↑ sugars, amino acids, insulin, and PSU
Green tea with carbohydrate-hydroelectrolyte drink or oligomineral water	Systemic effects of an isotonic sports drink on the metabolic status of athletes during recovery	CT crossover	Plasma Urine	^{1}H NMR	– Green tea–based sports drink had effect on glucose, citrate, and lactate levels in plasma and on acetone, 3-OH-butyrate, and lactate levels in urine – Absorption of green tea extract components: ↑ caffeine and hippuric acid levels in urine

(Miccheli, et al. 2009)		Strenuous cycling sessions / Male athletes (n=44)			
Beverages containing glucose (maltodextrin (MD) + glucose (2:1 ratio)), galactose (MD + galactose (2:1)), or fructose (MD) + fructose (2:1)) *(Bruce, et al. 2010)*	Effects of three different carbohydrate based recovery beverages after sessions of ergometer cycling in a human exercise study	CT crossover cycling sessions / Trained male cyclists (n=10)	Plasma	GC–TOF/MS	– Galactose beverage: ↑ galactonic acid throughout the recovery period – Fructose beverage: ↑ fructose
Red wine (272mL/day) *vs* dealcoholized red wine (272mL/day) or gin (100mL/day) *(Vazquez-Fresno, et al. 2012)*	Effect of moderate wine intake on the metabolome of subjects with CDV risk, identifying both markers of consumption and endogenous changes	RCT crossover 4 wk / High-risk subjects ≥55 y without documented CHD (n=61)	Urine	^{1}H NMR	– Metabolites from wine metabolism: mannitol in RWA and tartrate in RWA and RWD – Endogenous modifications after wine consumption: BCAA metabolites – Ethanol robust biomarker of alcohol consumption in GIN and RWA diets – 4-hydroxyphenylacetate and hippurate different effect in combination with alcohol
Functional beverage containing grape skin extract *vs.* a control beverage as a placebo *(Khymenets, et al. 2015)*	Impact of acute and sustained consumption of a functional beverage based on grape skin extracts on the urinary metabolome by applying an untargeted metabolomic approach	RCT crossover 15 d / Healthy (n=31)	Urine	HPLC–qTOF/MS	– Prolonged FB consumption: microbial metabolites of flavanols, hydroxyvaleric acid and hydroxyvalerolactone derivatives – Acute FB consumption: ↑ tyrosine – Representative markers of FB consumption: epicatechin and phenolic acid metabolites (tissular and microbiota origin)
MISCELANEOUS					
Dried black tea extract powder (capsule containing 2500 mg of dried black tea extract poweder, red grape extract or sucrose-placebo) *(van Velzen, et al. 2009)*	Integration of metabolomics and pharmacokinetics (or nutrikinetics) data to describe a human study population with different metabolic phenotypes	RCT crossover 2 d / Healthy non-smoking males (n=20)	Urine	^{1}H NMR	– ↑ urinary excretion of gut mediated metabolites of tea flavonoids – The nutrikinetic properties of phenolic biomarkers describe metabolic phenotypes – Hippuric acid and 4-hydroxy derivate of hippuric acid (4-hydroxyhippuric acid) important contributors to RP
Vitamin E supplementation (capsule containing 400 mg of α-tocopheryl acetate) *(Wong and Lodge 2012)*	Changes induced by vitamin E supplementation on plasma metabolome	Single arm trial 4 wk / Males (n=10)	Plasma	LC–qTOF/MS	– Supplementation ↑ plasma vitamin E, ↑ Lysophosphatidylcholine species (16:0, 18:0, 18:1, 18:2, 20:3 and 22:6) – Vitamin E influences phospholipid metabolism and induces lysoPC generation
n-3 PUFA supplement (capsule with 3 g/day) *(Rudkowska, et al. 2013)*	Molecular and metabolic changes following *n*-3 PUFA supplementation (traditional biomarkers, transcriptome and metabolome analyses)	Run in period followed by single arm trial 6-wk / Healthy (n=30)	Plasma	LC–MS	n-3 PUFA supplementation suggest cardioprotective effects – ↓ Triglycerides and ↑ erythrocyte *n*-3 PUFA, ↓ plasma glycerophosphatidylcholine and lysophosphatidylcholine in both genders – ↑ Plasma HDL-cholesterol and fasting glucose levels in women after *n*-3 PUFA – *n*-3 PUFA changed expression of 610 genes in men and 250 genes in women – *n*-3 PUFA in men ↑ acylcarnitines, hexose and leucine. In women ↓ SM C20:2 and ↑ SM C22:3
Four isoenergetic diets differing in *n*-3 FA and polyphenols content *(Bondia-Pons, et al. 2014)*	Effects of *n*-3 fatty acid and polyphenol rich diets on plasma and HDL fraction lipidomic profiles in subjects at high cardiovascular risk	RCT parallel 8 wk / Individuals at high cardiovascular risk (n=78)	Plasma	UPLC–qTOF/MS	– Inverse correlation between long-chain TG with high number of double bonds (≥6), PCs and PEs with low number of double bonds (≤4) and with lipids containing arachidonic acid in plasma – Observation of two patterns – PCs and PEs major lipids altered in the HDL fraction
High–palmitic acid (HPA) *vs* a low–palmitic acid and high–oleic acid (HOA) diet *(Kien, et al. 2014)*	Effect of dietary fatty acids and their metabolism on CVD risk; Identification of a metabolomic signature in blood lipid concentrations and whole-body fat oxidation	CT crossover 3 wk / Healthy (n=18)	Muscle Serum	GC–MS DIMS	– PA with OA ↓ blood LDL concentration and whole-body fat oxidation – ↑ production and accumulation of acylcarnitines in women – HOA ↓ PA:OA ratio in serum and muscle phosphatidylcholine – Inhibitory effects of the HOA diet on mRNA expression of INSIG-1
Gelatin and whey protein supplements (20-g/d) *(Piccolo, et al. 2015)*	Differences in plasma metabolites from obese women consuming gelatin *vs.* whey protein supplements (weight-loss trial)	RCT parallel 8 wk / Obese women with metabolic with MetSyn (n=29)	Plasma	GC–qTOF/MS	– Supplemental protein source rich in BCAAs modifies innate BCAA metabolism – Whey-based vs gelatin-based protein supplement: ↓ fasting plasma abundance of Pro- and Cys-related metabolites
Commercial meal with blueberry and chocolate flavour (—*Nutrilett Intensive*—, Axellus A/S, Denmark) *(Schmedes, et al. 2015)*	Potential of NMR-based metabolomics and impact of a 6-week very low-calorie diet and weight reduction on the serum and faecal metabolome in overweight healthy subjects	CT 6 wk / Healthy females (n=70)	Serum Faeces	^{1}H NMR	– Highest weight loss: ↑ serum ketone bodies (3-HBA, acetoacetate) and lactate – Lowest weight loss: ↑ serum lipids – Pre- and post-weight loss faecal samples ↓ acetate, butyrate and propionate and ↑ lactate and lipids after weight reduction
Two breakfast meals: Cereal breakfast and egg *vs* ham breakfast, both with coffee or tea	Comparison of the acute metabolic response to two equicaloric breakfasts using ^{1}H NMR metabolomics	CT crossover 4 d / Healthy s (n=24)	Urine	^{1}H NMR	– EHB *vs* CB ↑ phosphocreatine/creatine, citrate and lysine. CB:↑ erythrose – Coffee drinkers: ↑ 2- furoylglycine and Sumiki's acid in postprandial – Tea drinkers: ↑ 3-hydroxyisovalerate in postprandial – Coffee and tea drinkers:↑ trigonelline and hippuric acid postprandial

(Radjursoga, et al. 2017)					
Daily intake of dark chocolate during (25 g for breakfast and lunch) *(Martin, et al. 2012)*	Identify metabolic phenotypes indicative of specific responses to dark chocolate consumption	Single arm trial 1 wk / Healthy (n=73) (n=20 final study)	Plasma Urine	^{1}H NMR FIA–QTrap LC–qTOF	– Urinary excretion of cocoa-derived metabolites: ↑ 7-methylxanthine, theobromine, and their products by endogenous and microbial metabolism *e.g.,* hippurate, 3-(3-hydroxyphenyl)- propionate – NMR signals; aromatic compounds associated to metabolism of cocoa polyphenols – CD subjects ↑ urinary content of butyrate, 3-hydroxybutyrate, 3-hydroxyisovalerate, p-cresol sulfate, phenyacetylglutamine and phenylacetate, and ↓ creatinine – CD *vs* CI subjects: consistent pattern ↓ 3-hydroxyisovalerate, p-cresol sulfate, creatinine, phenylacetylglutamine, and phenylacetate,
Soluble cocoa powder (40 g of cocoa with 250 mL of water and 40 g of cocoa with 250 mL of milk *vs* 250 mL of milk as a control) *(Llorach, et al. 2009)*	Metabolomic strategy to analyse the influence of a single cocoa intake on the 24 h kinetic trajectory	CT crossover Single dose / Healthy (n=10)	Urine	HPLC–qTOF	– 27 metabolites related to cocoa-phytochemicals – Main changes after cocoa powder intake: alkaloid derivatives, polyphenol metabolites (both host and microbial metabolites) and processing-derived products such as diketopiperazines
Cocoa powder (40 g with 250 ml of milk) *(Llorach-Asuncion, et al. 2010)*	Changes in urinary metabolome after cocoa powder consumption. Capacity to improve metabolome visualization and interpretation after a meal consumption study	Post-prandial study 24 h / Healthy (n=10)	Urine	HPLC–qTOF	– Metabolites characterized by several mass features – Two-way clustering tool for discovering the possible source of metabolites
Ready-to-eat meals supplemented with 1.4 g of cocoa extract (645 mg polyphenols) *vs* control meal *(Ibero-Baraibar, et al. 2016)*	Effect of consuming ready-to-eat meals containing a cocoa extract	RCT parallel 4 wk / Middle-aged volunteers (n=50)	Urine	HPLC–TOF/MS	– Metabolites in cocoa group related to theobromine metabolism (3-methylxanthine and 3-methyluric acid), food processing (L-beta-aspartyl-L-phenylalanine), flavonoids (2,5,7,3',4'-pentahydroxyflavanone-5-*O*-glucoside and 7,4'-dimethoxy-6-*C*-methylflavanone), catecholamine (3-methoxy-4-hydroxyphenylglycol-sulphate) and endogenous metabolism (uridine monophosphate)

Abbreviations: CE-UV, capillary electrophoresis-ultraviolet; RCT, randomized controlled crossover trial; n, number of people initially enrolled in the study; HPLC, high pressure liquid chromatography; qTOF, quadropole time of flight; FIA-MS, flow injection-mass spectrometry analysis; LTQ-FT, linear ion trap mass spectrometer coupled with Fourier Transform

Table 2. Dietary biomarkers proposed in humans through the use of high throughput metabolomics based approaches (human dietary intervention studies + cross sectional studies)

FOOD	METABOLIC APPROACH	SAMPLE	CANDIDATE BIOMARKERS	REF
VEGETABLES				
Cruciferous	^{1}H NMR	Urine	S-methyl-L-cysteine sulphoxide	(Edmands, et al. 2011)
Cruciferous	LC-MS/MS	Urine	Sulforaphane	(May, et al. 2013)
Onion	^{1}H NMR	Urine	N-acetyl-S-(1Z)-propenyl-cysteine-sulfoxide	(Posma, et al. 2017)
Beetroot	UPLC-qTOF-MS	Urine	4-Ethyl-5-amino-pyrocatechol sulphate; 4-Methylpyridine-2-carboxylic acid glycine conjugate	(Andersen, et al. 2014)
Radish sprouts	UHPLC-QqQ-MS/MS	Urine	Sulforaphene; Sulforaphane-N-acetyl-l-cysteine; 3,3'-Diindolylmethane	(Baenas, et al. 2017)
Broccoli	FIE-MS	Urine	Ascorbate; Tetronic acids; L-Xylonate/L-lyxonate; Naringenin glucuronide	(Lloyd, et al. 2011)
White cabbage/Brussels sprout	UPLC-qTOF-MS	Urine	N-acetyl-S-(N-3- methylthiopropyl)cysteine; N-acetyl-S-(Nallylthiocarbamoyl)cysteine; Iberin N-acetyl-cysteine; Erucin N-acetyl-cysteine; N-Acetyl-(N'-benzylthiocarbamoyl)-cysteine; Sulforaphane N-acetyl-cysteine; Sulforaphane N-cysteine	(Andersen, et al. 2013)
Red cabbage *(brussels sprouts, pointed cabbage)*	UPLC-qTOF-MS	Urine	3-Hydroxy-hippuric acid sulphate; 3-Hydroxy-hippuric acid; Iberin N-acetyl-cysteine; N-acetyl-S-(N-3-methylthiopropyl)cysteine; N-acetyl-S-(N-allylthiocarbamoyl)cysteine; Sulphoraphane N-acetyl-cysteine	(Andersen, et al. 2014)
Greens: lettuce, spinach, green peppers	UPLC-MS/MS	Serum	3-carboxy-4-methyl-5-propyl-2- furanpropanoic	(Guertin, et al. 2014)
Vegetable	HPLC-ESI-MS/MS	Urine	Enterolactone + kaempferol	(Mennen, et al. 2006)
Vegetarian diet	^{1}H NMR	Urine	p-hydroxyphenylacetate	(Stella, et al. 2006)
Lactovegetarian diet	^{1}H NMR	Urine	Hippurate; N-acetyl glycoprotein; Succinate	(Xu, et al. 2010)
Vegetarian diet	^{1}H NMR	Urine	Phenylacetylglutamine; Glycine	(O'Sullivan, et al. 2011)
FRUITS				
Citrus	HPLC-ESI-MS/MS	Urine	Hesperetin; Naringenin	(Mennen, et al. 2006)
Citrus	^{1}H NMR	Urine	Proline betaine	(Heinzmann, et al. 2010)
Citrus	FIE-MS	Urine	Proline betaine and conjugates	(Lloyd, et al. 2011)
Citrus	UPLC-QTOF-Micro UPLC-LTQ-Orbitrap	Urine	Proline betaine; Limonene 8,9-diol glucuronide; Nootkatone 13,14- diol glucuronide; Hesperetin 3'-Oglucuronide; Hydroxyproline betaine; N-Methyltyramine sulfate; Naringenin 7- Oglucuronide	(Pujos-Guillot, et al. 2013)
Citrus	LC-MS/MS	Urine	Proline betaine	(May, et al. 2013)
Citrus	UPLC-MS/MS	Serum	Stachydrine; Scyllo- and chiro-inositol	(Guertin, et al. 2014)
Orange/Citrus	UPLC-qTOF-MS	Urine	Proline betaine; Hesperetin glucuronide	(Andersen, et al. 2014)
Citrus	UPLC-qTOF	Urine	Naringenin glucuronide	(Edmands, et al. 2015)
Orange juice	UPLC-MS/MS GC/MS	Urine	N-methyl proline; Methyl glucopyranoside (alpha+beta); Stachydrine; Betonicine; N-Acetyl putrescine; Dihydroferulic acid	(Rangel-Huerta, et al. 2017)

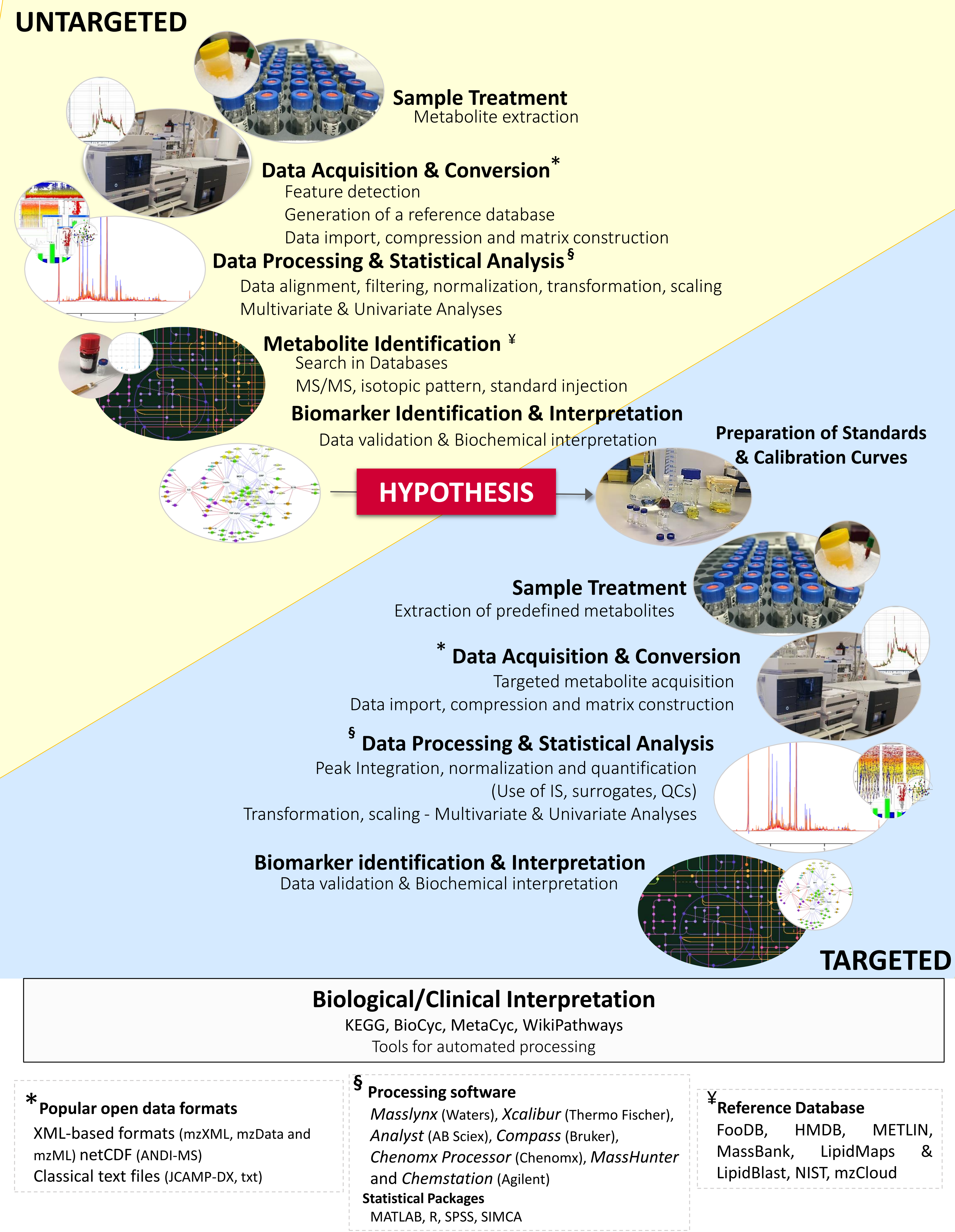
Aronia-citrus juice	HPLC-qTOF-MS	Urine	Proline betaine; Ferulic acid; Mercapturate derivatives	(Llorach, et al. 2014)
Apple	HPLC-ESI-MS/MS	Urine	Phloretin	(Mennen, et al. 2006)
Apples / pears	UPLC-qTOF	Urine	Phloretin glucuronide	(Edmands, et al. 2015)
Apple	^{1}H NMR	Urine	Rhamnitol	(Posma, et al. 2017)
Raspberries	FIE-MS	Urine	Sulphonated caffeic acid; Methyl-epicatechin sulfate ; 3-Hydroxyhippuric acid; Naringenin glucuronide; Ascorbate	(Lloyd, et al. 2011)
Bilberries	UHPLC-qTOF-MS	Plasma	Hippuric acid	(Hanhineva, et al. 2015)
Strawberry	LC-MS	Urine	4-Hydroxyhippuric acid; 4-Hydroxy-2,5-dimethyl-3(2H)-furanone (furaneol) glucuronide; Pelargonin glucuronide; p-coumaric acid sulphate; Dihydrokaempferol glucuronide; Furaneol sulphate; 2,5-dimethyl-4-methoxy-2,3-dihydro-3-furanone (mesifurane); Mesifurane sulphate; Leucopelargonidin; Catechin sulphate	(Cuparencu, et al. 2016)
Grapefruit	HPLC-ESI-MS/MS	Urine	Naringenin	(Mennen, et al. 2006)
Combination of fruits and/or fruit juices	HPLC-ESI-MS-MS	Urine	Gallic acid, 4-O-methylgallic acid; Isorhamnetin; Kaempferol; Hesperetin; Naringenin; Phloretin	(Mennen, et al. 2006)
Sea buckthorn	LC-MS	Urine	Catechin Sulphate; xi-2,3-dihydro-2-oxo-1H-indole-3-acetic acid; Hippuric acid; 5-Hydroxyindole-3-acetic acid; Cyclohexane carboxylic acid glycine; 1-Cyclohexene carboxylic acid glycine; Cyclohexadiene carboxylic acid glycine; *N*-methyl hippuric acid; Isorhamnetin glucuronide; Pyrocatechol sulphate; Dihydroxycyclohexane carboxylic acid; Protocatechuic acid glucoside	(Cuparencu, et al. 2016)
LEGUMES				
Chickpeas, lentils, beans	^{1}H NMR	Urine	Glutamine; Dimethylamine; 3-methylhistidine	(Madrid-Gambin, et al. 2017)
Peas	^{1}H NMR	Urine	N-methylnicotinic acid (NMNA, trigonelline)	(Posma, et al. 2017)
SOY PRODUCTS				
Soy	LC-MS/MS	Urine	Isoflavones	(May, et al. 2013)
Soy Drink	GC-MS ^{1}H NMR	Urine	D-Pinitol; Maltol; Trigonelline; Pyridoxine; Trans-aconitate	(Munger, et al. 2017)
GRAINS				
Whole grain sourdough rye bread	FIE-MS	Urine	Benzoxazinoid derivatives; Hydroxylated phenyl acetamide derivatives	(Beckmann, et al. 2013)
Whole-grain sourdough rye bread/ white wheat bread with rye bran	LC-qTOF-MS	Plasma	Sulfonated hydroxyl-*N*-(2-hydroxyphenyl) acetamide; *N*-(2-hydroxyphenyl)acetamide; 2,4-dihydroxy-1,4-benzoxazin-3-one; 1,3-benzoxaxazol-2-one	(Hanhineva, et al. 2014)
Whole-grain rye	LC-qTOF-MS	Urine	Alkylresorcinol metabolites; Caffeic acid sulfate; Hydroxyhydroxyphenyl acetamide sulfate; 3,5-dihydroxyphenylpropionic acid sulfate; Hydroxyphenyl acetamide sulfate	(Hanhineva, et al. 2015)
Whole-grain bread	UHPLC-qTOF-MS	Plasma	Glucuronidated alk(en)ylresorcinols	(Hanhineva, et al. 2015)
Whole-grain bread	HPLC-qTOF-MS	Urine	2-hydroxy-N-(2-hydroxyphenyl) acetamide; 2-hydroxy-1,4-benzoxazin-3-one glycoside;	(Garcia-Aloy, et al. 2015)

			3-(3,5-dihydroxyphenyl) propanoic acid glucuronide; 5-(3,5-dihydroxyphenyl) pentanoic acid sulphate; Dihydroferulic acid sulphate; Enterolactone glucuronide; Pyrraline; 3-Indolecarboxylic acid glucuronide; 2,8-Dihydroxyquinoline glucuronide	
MEAT				
Meat (omnivore diet)	IEC	Urine	1-methylhistidine; 3-methylhistidine	(Myint, et al. 2000)
Atkins diet	^{1}H NMR	Urine	Taurine	(Lenz, et al. 2004)
Ground Beef (raw/broiling)	HPLC	Plasma	Carnosine	(Park, et al. 2005)
Meat	^{1}H NMR	Urine	Acetyl-carnitine; Creatinine; Taurine; Carnitine; Trimethylamine-N-oxide; 1-methylhistidine; 3-methylhistidine	(Stella, et al. 2006)
Low fat meat	^{1}H NMR	Urine	Creatine; Histidine; Urea	(Bertram, et al. 2007)
Red meat	IEC	Urine	1-methylhistidine; 3-methylhistidin	(Cross, et al. 2011)
Red meat	^{1}H NMR	Urine	O-acetylcarnitine	(O'Sullivan, et al. 2011)
Beef	GC-MS	Plasma	β-alanine; 4-hydroxyproline; 2-aminoadipic acid; Leucine	(Ross, et al. 2015)
Chicken	HPLC-QTRAP ^{1}H NMR	Plasma Urine	3-methylhistidine; Guanidoacetate	(Yin, et al. 2017)
Chicken/ Red meat	UHPLC-MS/MS	Urine Plasma	3-methylhistidin; Anserine; Carnosine	(Cheung, et al. 2017)
COOKED MEATS				
Fried meat (lean beef)	GC–MS	Urine	2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP); 4′-OH-PhIP	(Reistad, et al. 1997)
Char-broiled beef	GC–MS	Urine	PhIP metabolites	(Strickland, et al. 2002)
Fried chicken breasts	LC-MS/MS	Urine	PhIP metabolites; N2-OH-PhIP-N2-glucuronide; N2-PhIP-glucuronide	(Kulp, et al. 2004)
Grilled/stir-fried meat	LC–MS	Hair	PhIP	(Kobayashi, et al. 2005)
FISH				
Fish	^{1}H NMR	Urine	Trimethylamine-*N*-oxide	(Lenz, et al. 2004)
Salmon	FIE-MS	Urine	Anserine; Trimethylamine-N-oxide; 1-methylhistidine	(Lloyd, et al. 2011)
	UPLC-qTOF-MS		Trimethylamine N-oxide	(Andersen, et al. 2013)
Fish	UPLC-qTOF-MS	Urine	Trimethylamine N-oxide	(Andersen, et al. 2014)
Fish	UPLC-MS/MS	Serum	3-carboxy-4-methyl-5-propyl-2- furanpropanoic acid; DHA; EPA; 1-Docosahexaenoylglycero-phosphocholine	(Guertin, et al. 2014)
Fatty Fish	UHPLC-qTOF-MS	Plasma	3-carboxy-4-methyl-5-propyl-2-furanpropionic acid; EPA; DHA	(Hanhineva, et al. 2015)
Herring	GC-MS	Plasma	DHA; Cetoleic acid	(Ross, et al. 2015)
Fish	UHPLC-MS/MS	Urine	Trimethylamine-*N*-oxide	(Cheung, et al. 2017)
Meat / fish	UHPLC-MS/MS	Plasma	Acetylcarnitine; Propionylcarnitine; 2-methylbutyrylcarnitine	(Cheung, et al. 2017)
DAIRY PRODUCTS				

Cheese	UPLC-qTOF/MS	Urine	Indoxyl sulfate; Xanthurenic acid; Tyramine sulfate; 4-hydroxyphenylacetic acid; Isovalerylglutamic acid; Acylglycines	(Hjerpsted, et al. 2014)
Butter	UPLC-qTOF/MS	Urine	3-phenyllactic; alanine, proline; pyroglutamic acid	(Hjerpsted, et al. 2014)
Butter	UPLC-MS/MS	Serum	Methyl palmitate (15 or 2); Pentadecanoate (15:0); 10-Undecenoate (11:1n–1)	(Guertin, et al. 2014)
Milk	GC-MS ^{1}H NMR	Urine	Lactose; Galactose; Galactonate; Allantoin; Hippurate; Galactitol; galactono-1,5-lactone	(Munger, et al. 2017)
Milk	LC-MS GC-MS FIA-MS/MS	Serum/ Plasma Urine	Trimethyl-N-aminovalerate; Uridine; Hydroxysphingomyelin C14:1; Diacylphosphatidylcholine C28:1	(Pallister, et al. 2017)
NON-ALCOHOLIC BEVERAGES				
Sugar-sweetened beverage	^{1}H NMR	Urine	Formate; Citrulline; Taurine; Isocitrate	(Gibbons, et al. 2015)
Coffee	HPLC-ESI-MS/MS	Urine	Caffeic; Chlorogenic acid	(Mennen, et al. 2006)
Coffee	HPLC-PDA-MS	Urine	Dihydrocaffeic acid-3-*O*-sulfate; Feruloylglycine	(Stalmach, et al. 2009)
Coffee	LC-MS/MS	Plasma	Dimethoxycinnamic acids	(Nagy, et al. 2011)
Coffee	UPLC-qTOF-MS	Urine	Atractyligenin glucuronide; Diketopiperazine cyclo(isoleucyl-prolyl); Trigonelline; Paraxanthine; 1-methylxanthine, 1-methyluric acid, 1,7-dimethyluric acid, 1,3 or 3,7 dimethyluric acid; 1,3,7-trimethyluric acid; 5-acetylamino-6-formylamino-3-methyluracil	(Rothwell, et al. 2014)
Coffee	UPLC-MS/MS	Serum	Trigonelline (N'-methylnicotinate); Quinate; 1-Methylxanthine; Paraxanthine; N-2-furoyl-glycine; Catechol sulfate	(Guertin, et al. 2014)
Coffee	UPLC-qTOF	Urine	Dihydroferulic acid sulfate	(Edmands, et al. 2015)
	^{1}H NMR	Urine	2-furoylglycine	(Heinzmann, et al. 2015)
Black tea	^{1}H NMR	Urine	Hippuric acid; 1,3-dihydroxyphenyl-2-*O*-sulfate	(Daykin, et al. 2005)
Black tea	HPLC-ESI-MS-MS	Urine	Gallic; 4-O-methylgallic acids	(Mennen, et al. 2006)
Black tea/green tea	HPLC-MS/MS	Urine	Hippuric acid	(Mulder, et al. 2005)
Black tea/green tea	^{1}H NMR	Urine	Hippuric acid; 1,3-dihydroxyphenyl-2-*O*-sulfate	(Van Dorsten, et al. 2006)
Black tea/green tea	HPLC-FTMS(n) HPLC-TOFMS-SPE-NMR	Urine	Hippuric acid; Hydroxybenzoic glycine conjugate; Vanilloylglycine; Pyrogallol-2-*O*-sulfate	(van der Hooft, et al. 2012)
Tea	UPLC-qTOF	Urine	4-*O*-methylgallic acid	(Edmands, et al. 2015)
Wine	^{1}H NMR	Urine	Tartrate; Ethyl glucuronide; 2,3-butanedio; Mannitol; Ethanol; 3-Methyl-2-oxovalerate	(Vazquez-Fresno, et al. 2015)
Wine	HPLC-ESI-MS/MS	Urine	m-coumaric acid; Gallic acid; 4-*O*-methylgallic acid	(Mennen, et al. 2006)
Wine	UPLC-MS/MS	Plasma Urine	Gallic acid and ethylgallate metabolites; Resveratrol and resveratrol microbial metabolites; 2,4-Dihydroxybenzoic acid; (epi)catechin; Valerolactone metabolites	(Urpi-Sarda, et al. 2015)
Red wine	UPLC-qTOF	Urine	Gallic acid ethyl ester	(Edmands, et al. 2015)
Beer	UPLC-MS/MS	Serum	16-Hydroxypalmitate	(Guertin, et al. 2014)

MISCELLANEOUS				
Cocoa	LC-qTOF	Urine	Vanilloylglycine; Dihydroxyphenyl valerolactone glucuronide; Furoylglycine; 7-methylxanthine; 3-methylxanthine; Theobromine; Xanthurenic acid	(Llorach-Asuncion, et al. 2010)
Cocoa	HPLC-qTOF-MS	Urine	Theobromine metabolism (AMMU; 3-methyluric acid; 7-methylxanthine; 3-methylxanthine; 3,7-dimethyluric acid; Theobromine) Polyphenol microbial metabolites (Methoxyhydroxyphenylvalerolactone; Glucuronide and sulphate conjugates of 5-(3' ,4' -dihydroxyphenyl)-valerolactone)	(Garcia-Aloy, et al. 2015)
Chocolate	UPLC-qTOF-MS	Urine	6-Amino-5-[N-methylformylamino]- 1-methyluracil; Theobromine; 7-Methyluric acid	(Andersen, et al. 2014)
Chocolate products	UPLC-qTOF	Urine	Methyl(epi)catechin sulfate	(Edmands, et al. 2015)
NUTS				
Almond Skin	LC-qTOF	Urine	Flavonoids; Valerolactone conjugates	(Llorach, et al. 2010)
Walnut	HPLC-QTOF-MS	Urine	10-hydroxy-decene-4,6-diynoic acid sulfate; Tridecadienoic/tridecynoic acid glucuronide; Sulfate conjugates of urolithin A; 3-indolecarboxylic acid glucuronide	(Garcia-Aloy, et al. 2014)
Peanut	UPLC-MS/MS	Serum	4-vinylphenol sulfate; Tryptophan betaine	(Guertin, et al. 2014)
Walnut	UPLC-qTOF-MS	Urine	5-Hydroxyindole-3-acetic acid	(Andersen, et al. 2014)

Abbreviations: FIA-MS, flow injection-mass spectrometry analysis; HPLC, high pressure liquid chromatography; qTOF, quadrupole time of flight; QqQ, triple quadrupole; UHPLC, ultrahigh performance liquid chromatography



UNTARGETED
Sample Treatment
Metabolite extraction
Data Acquisition & Conversion*
Feature detection
Generation of a reference database
Data import, compression and matrix construction
Data Processing & Statistical Analysis§
Data alignment, filtering, normalization, transformation, scaling
Multivariate & Univariate Analyses
Metabolite Identification ¥
Search in Databases
MS/MS, isotopic pattern, standard injection
Biomarker Identification & Interpretation
Data validation & Biochemical interpretation
HYPOTHESIS
Preparation of Standards & Calibration Curves
Sample Treatment
Extraction of predefined metabolites
* Data Acquisition & Conversion
Targeted metabolite acquisition
Data import, compression and matrix construction
§ Data Processing & Statistical Analysis
Peak Integration, normalization and quantification
(Use of IS, surrogates, QCs)
Transformation, scaling - Multivariate & Univariate Analyses
Biomarker identification & Interpretation
Data validation & Biochemical interpretation
TARGETED
Biological/Clinical Interpretation
KEGG, BioCyc, MetaCyc, WikiPathways
Tools for automated processing
* Popular open data formats
XML-based formats (mzXML, mzData and mzML) netCDF (ANDI-MS)
Classical text files (JCAMP-DX, txt)
§ Processing software
Masslynx (Waters), Xcalibur (Thermo Fischer), Analyst (AB Sciex), Compass (Bruker), Chenomx Processor (Chenomx), MassHunter and Chemstation (Agilent)
Statistical Packages
MATLAB, R, SPSS, SIMCA
¥ Reference Database
FooDB, HMDB, METLIN, MassBank, LipidMaps & LipidBlast, NIST, mzCloud