Chapter 7

Title: Metabolic phenotyping of diet and dietary intake

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Abstract

Nutrition provides the building blocks for growth, repair and maintenance of the body and is key to maintaining health. Exposure to fast foods, mass production of dietary components and wider importation of goods has challenged the balance between diet and health in recent decades and both scientists and clinicians struggle to characterise the relationship between this changing dietary landscape and human metabolism with its consequent impact on health. Metabolic phenotyping of foods, using high density data-generating technologies to profile the biochemical composition of foods, meals and human samples (pre and post food intake) can be used to map the complex interaction between the diet and human metabolism and also to assess food quality and safety. Here we outline some of the techniques currently used for metabolic phenotyping and describe key applications in the food sciences, ending with a broad outlook at some of the newer technologies in the field with a view to exploring their potential to address some of the critical challenges in nutritional science.

1. Introduction to metabolic profiling in nutritional research

Although nutrition studies in controlled clinical settings and epidemiological cohorts have unequivocally supported an underlying relationship between diet and health, the field is fraught

with limitations, particularly in free-living populations where dietary monitoring and misreporting are challenging. Whether we are evaluating the association of specific foods with a health claim, such as the anti-inflammatory activity of turmeric (largely due to the chemical curcumin) (1) or focus on the broader problems of over- or undernutrition, accurate reporting of dietary intake is key to making a true assessment of the impact of diet on health.

Nutritional recommendations from health policies, as well as Dietary Reference Intake values, are based on the nutritional needs of a population to keep that population healthy. Most Governments implement healthy eating policies based around increasing daily intake of high quality proteins and carbohydrates, low saturation fats, vitamins, minerals, fibre and water focussing on increasing portions of fruits and vegetables, non-animal proteins and whole grains whilst decreasing dietary salt, sugar, saturated fats and alcohol. Dietary advice, using food-based dietary guidelines, are the main cornerstone of the worldwide public health policies to reducing non-communicable diseases (NCDs) (2-4). The impact of dietary change in controlled environments such as metabolic wards, induces a 10% decrease in total cholesterol (5). But even in this controlled environment the response to standardised dietary change varies, suggesting there is individual variability in response to diet (6). In free-living people, total cholesterol is reduced to 5% following implementation of a dietary plan for weight-loss and/or metabolic improvement (7) and highlights the problem of compliance to one-size-fits-all dietary advice. This is compounded further by the fact that the use of self-reported food intake, wherein the prevalence of misreporting is estimated to be between 30-88% (8, 9), compromises understanding of the impact of dietary changes on preventing disease. For example, with present dietary tools it is difficult to assess if lack of effect at a population or individual level is due to there being no physiological effect, poor compliance to the recommended dietary change or high interpersonal variability in response to the same diet. Indeed, interpersonal variability has been observed in the amount of weight loss induced by caloric restriction (10)

and in the postprandial response to identical meals (11). However, it must also be borne in mind that foods often constitute a rich chemical composition such that different brands or types of food may represent a distinct panel of chemicals, for example apple juice is sometimes found to be adulterated with other fruits such as pears (12) while grape juice is commonly adulterated with apples (13). In addition to the complexity of food matrices the chemical composition changes during food processing (14) due to the large number of factors involved (temperature, pH, pressure, etc.) generating new chemicals, which in some cases has resulted in production of trace amounts of carcinogens, thereby posing a risk to human health (15).

Thus in assessing dietary intake amongst populations, new tools are urgently needed to promote progress in research, both in terms of being able to efficiently profile dietary components themselves, and in characterising the metabolic consequence of individual nutrients, foods and diets in humans. Moreover, based on evidence of inter-individual differences in metabolism of dietary components, a strategy for providing reliable individualised dietary advice is required.

There are multiple benefits of applying metabolic phenotyping to elucidate chemical profiles associated with particular diets or with specific metabolic responses to dietary intervention. The main applications of metabolic phenotyping in nutrition research are shown in Figure 1. These applications include i) quality control of food products, ensuring authenticity and provenance of material; ii) detection of toxicity of foods / food contaminants; iii) assessing metabolic response to diet at the individual and population levels; iv) stratification of individuals according to dietary response; v) identifying non-adherence to dietary interventions or plans.

By exploiting thousands of measured metabolites reflecting physiological status, food intake, metabolism and environmental exposure, the balance of traditional standardised nutritional advice can be shifted towards personalised nutritional management, accounting for an

individual's unique lifestyle, culture, environment and phenotype. Ultimately this may improve dietary compliance and effectiveness of the diet.

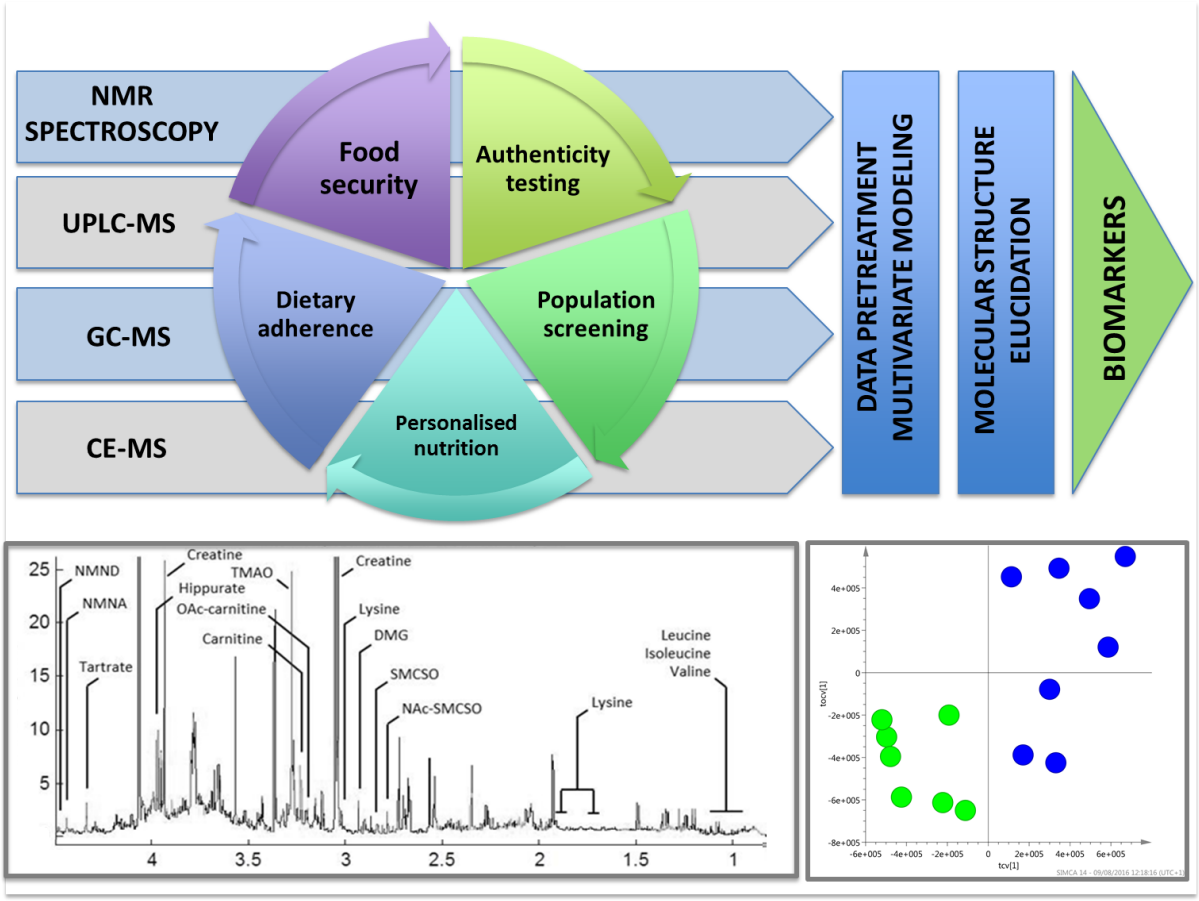

Figure 1 Applications of metabolic phenotyping in different food and nutritional settings

1.1 Metabolic phenotyping technology currently employed in nutrition research

Metabolic profiling strategies for analyzing biosamples, encompassing high-resolution spectroscopic methods in combination with multivariate statistical modelling tools, have been shown to be well-suited to generating metabolic signatures reflecting gene-environment interactions (16). Spectroscopic analysis has been applied across a wide range of studies with

the aim of characterizing classes of disease, different physiological states or response to particular therapies. In addition to endogenous metabolites reflecting the functionality of the human genome, spectroscopic profiles report on chemicals from food components and other xenobiotics and their metabolic transformation by the gut microbiota. The main players in metabolic phenotyping technologies are nuclear magnetic resonance spectroscopy (NMR) and mass spectrometry coupled to either gas-phase or liquid-phase chromatography (GC-MS and LC-MS respectively). These technology platforms are used to generate high fidelity profiles consisting of hundreds of molecules defining a biological sample which could be a homogenised diet, a blood or urine sample, or even a tissue biopsy (17-20). Other spectroscopic tools used less frequently include infrared spectroscopy and capillary electrophoresis mass spectrometry (CE-MS). These complex molecular profiles are subsequently analysed using computational modelling tools to accommodate the simultaneous analysis of multiple compounds.

No single analytical technology is capable of analysing the totality of compounds present in biological samples and in foods, nutrients, phytochemicals, dietary supplements, pharmaceutical derivatives, chemicals formed during storage, and food handling as well as microbiome-related chemicals. Each platform will deliver a different set of information and achieve only partial coverage of the metabolome. The combination of multiple analytical platforms for metabolic profiling analysis can provide a more holistic measure of food composition compounds and compounds derived from the ingestion of foods. However, the choice of analytical platforms is typically governed by practical and economic constraints.

NMR spectroscopy is rapid and non-destructive and has the advantage of high reproducibility and robustness producing metabolic profiles in a short period of time without the need for derivation and separation. It is the only spectroscopic tool that can deliver atom-centred

information giving it premium position in molecular structural elucidation. NMR spectroscopy is based on exploiting the molecular property of spin and the fact that small differences in local electronic environment around a molecule will result in differences in properties such as chemical shift that relate to specific molecules or chemical groups. Additionally, it is capable of providing wide selectivity with respect to analytes and definitive structural information for detecting them with no restrictions on the polarity, volatility, or chromophore content. However, NMR technology is not capable to detect inorganic ions or salts, and requires larger volumes of samples (typically 0.3–0.5 mL for high-throughput phenotyping studies) than the other analytical platforms. Mass spectrometry (MS), being inherently more sensitive than NMR, can reach very low LODs (sub nanomole level) and mass accuracy <1ppm and offers complementary molecular information.

Ultra performance liquid chromatography coupled to a mass spectrometer (UPLC-MS/LC-MS) used as a molecular separation phase prior to MS detection provides rapid analysis and delivers excellent chromatographic resolution (21). UPLC-MS/LC-MS has become the powerhouse of the pharmaceutical and biotechnology industries for the metabolic profiling of drugs. It is the most sensitive profiling technique of all, and requires minimal sample volume. However, it is not as robust as NMR, samples are not recoverable, analysis time is typically relatively slow and novel compound identification is more challenging since metabolite identification databases are highly dependent on the condition of the analytical method employed. In comparison to GC, it will yield a poor separation resolution and reproducibility. GC-MS has a good sensitivity, excellent separation reproducibility, detects most organic and some inorganic molecules, and requires low sample volumes. Like NMR it is robust and comprehensive databases are available for metabolite identification. Limitations of GC-MS include requirement for sample derivatization and separation, relatively slower analysis times, and non-recoverability of samples post analysis.

CE-MS is a fast, sensitive and inexpensive technique with high separation capabilities and rapid detection of ionic and highly polar metabolites that cannot be easily obtained by GC and LC/UPLC-MS. It requires low sample volume and less solvent. Poor reproducibility is inherently a limitation of CE-MS that makes it the least suitable of the platforms described for analysing large numbers of complex biological samples.

MS-profiling can be performed in screening mode to obtain broad coverage of the metabolome without the need for *a priori* hypotheses, or in targeted mode to give deep coverage for selected metabolite classes, for example amino acids, eicosanoids (inflammatory conditions), bile acids (liver disease) or short chain fatty acids.

Much effort has been directed towards the processing of spectral profiles and subsequent computational modelling in order to extract robust patterns related to biological endpoints. The main focus of these modelling technologies in nutrition and biomedical research in general is to i) identify trends, patterns and outliers in the data; ii) allow visualisation of chemically complex datasets by reducing the dimensionality of the data without losing biologically relevant variance in the data; iii) identify patterns of key metabolites (biomarker panels) related to a biological class or intervention; iv) define the dynamic behaviour of those profiles; v) derive predictive models for new samples introduced into the model e.g. prediction of response to a given dietary intervention; vi) assess authenticity or quality of biological materials based on multiple parameters. Key statistical techniques can be divided into those that are agnostic of class or biological information and operate solely on the inherent similarities / dissimilarities in biochemical composition of a group of samples (unsupervised techniques such as hierarchical clustering analysis (HCA), principal components analysis (PCA) or self-organizing maps (SOM)) and those that use information on sample class or biological response to maximize differences between classes and optimize recovery of biomarkers (supervised

techniques such as partial least squares discriminant analysis (PLS-DA), neural networks and machine learning techniques). For a comprehensive review of multivariate techniques refer to De Iorio et al. (22) and Smolinska et al (23).

Once a systematic effect of a diet or food has been identified, the signals that define the response can be related to a series of chemicals that are identified either from databases relating spectral information to chemical structure or by performing specific analytical experiments on selected or pooled samples to recover further chemical information. For example, application of NMR pulse programs that allow derivation of information relating to neighbouring proton or carbon atoms (24), isolation of chemical components using solid, liquid or gas-phase chromatography followed by measurement using NMR and MS technologies (25), direct hyphenation of LC-NMR-MS (25), or by use of statistical spectroscopic correlation methods (see Robinette et al. for a summary of available methods (26)) based on identifying covariance of signals across an NMR or combined NMR and MS dataset.

The use of these technologies has provided an enormous step forward in nutritional sciences that allows a better understanding of the complex interactions between food, diet, microbiota, chronic diseases and metabolic phenotypes. Considering the nature and diversity of compounds which are metabolized, a single analytical technique is unlikely to yield a comprehensive and complete metabolic profile and frequently is used in a complementary manner.

2. Applications in food and nutritional research

Different approaches have been suggested for metabolic profiling applications in food and nutritional sciences. The following section addresses some scenarios where metabolic profiling has shown to be useful in specific food and nutritional contexts with promising results.

2.1 Food composition, organoleptic properties and food safety

Food composition has been traditionally evaluated by the standard methods suggested by the Food and Agriculture Organization of the United Nations (FAO) for the evaluation of carbohydrates, proteins, lipids, alcohol, polyols, organic acids and other food energy sources (27). These methodologies basically provide information about energy content of food, but the diversity of molecules and individual chemical forms are not detected. The use of metabolic phenotyping to analyse and identify food compounds enables identification of specific molecules and can provide the data required to validate the authenticity, quality and acceptability of some certain type of foods (28). This type of identification is fundamental for wines, vegetables, fruits and specific type of foods.

Protein content in food is determined by the Kjeldahl method, which evaluates the amount of nitrogen in food, but without the discrimination of the nitrogen source (27, 29). However, it is possible to artificially enhance the measured protein content of a food by adulteration of a product with melamine. Melamine contains 66.6% nitrogen and adding 1% to proteins leads to a false increase content by 4.16% in the results analysed with the Kjeldahl method (30, 31). Melamine has become one of the most effective adulterants used to increase the nitrogen content in food products and this type of adulteration has been a common occurrence in the last 10 years (32). In 2007 a massive pet food recall in United States was caused by the presence of melamine in pet food (33), which was present in wheat gluten, rice protein, and corn gluten imported from China and used as an ingredient (33, 34). Kidney stones and renal failure in cats and dogs were reported as some of the most common toxic effects of melamine-adulteration in pet foods (35). Toxicology evaluation of contaminated food and gluten protein from food recall using HILIC-(TOF) MS, identified the presence of melamine, cyanuric acid, and several other triazines as contaminants (34). Melamine also has been added to powdered infant formulas to increase falsely the content of proteins by increasing of non-protein nitrogen level (32). The

most famous case of melamine in food was in China in September of 2008, causing kidneys stones and urinary tract effects in more than 300.000 children and six infant deaths were reported as a consequence of melamine contamination (36, 37). After this event WHO/FAO recommended LC-MS and GC-MS, both targeted methodologies for screening, confirmation and quantification for the presence of melamine in food products (38). The use of non-targeted spectroscopic techniques has been employed for the detection of melamine in food and it is a suitable alternative to detect other types of adulterant components in food products (30). It has been suggested that renal toxicity of melamine is mediated by the gut microbiota in rats, where microbes transform melamine to cyanuric acid (39). A recent study from South Africa analysed the level of melamine in different sport products using LC-MS and found 46 % of them contained melamine in low levels which were within the Tolerable Daily intake (TDI) according to the WHO (38, 40). However, considering the low cost of melamine and its ability to increase the level of nitrogen, it is important to ensure food ingredients from well-known suppliers and especially if these food products are oriented for vulnerable groups as children so as to avoid future public health problems.

Wine is one the most widely consumed beverages of the world and several health benefits have been suggested to result from its moderate consumption (41). There are many varieties of wine according to their organoleptic characteristics such as aroma, flavour and colour to name a few and those characteristics are attributed to its geographical location, growing conditions and fermentation processes (42). The nature and structure of molecules found in wine are diverse and their concentration can vary depending on variety, making it an easy target for adulteration (43) The use of metabolic profiling techniques for wine analysis has been a useful tool to ensure the traceability and quality of wines (44). Metabolic profiling with NMR and HPLC–QTOFMS has permitted the identification of metabolite differences between grapes and wine varieties, for examples wines made with Campbell Early, Cabernet Sauvignon, and Shiraz grape and

Cabernet Sauvignon, Merlot and Pinot Noir wine varieties (45, 46). Additionally, the use of metabolic profiles has enabled detection of metabolite differences between the same wines varieties but produced in different stages of grape fermentation and different geographical locations (45, 47). In another study, samples of Riesling and Mueller-Thurgau wines from the Palatinate Region in Germany were analysed with NMR according to their quality classification assessed by a sensory panel. High and low quality wines showed a correlation of specific metabolites to its quality classification (48). The use of metabolic profiling in wine analysis provides a valuable tool to wine producers to ensure a good quality product and original denomination of origin.

Mozzarella cheese is a traditional dairy product from Italy, made with fresh buffalo milk and called Mozzarella di Bufala Campana (MBD) with Protected Designation of Origin (PDO): This characteristic means that the entire product must be traditionally and entirely manufactured (prepared, processed and produced) within the specific region and thus acquire unique properties attributed to this type of cheese (49). However, there are reports of this product being adulterated with the addition of another type of milk or claiming a false PDO status. The European Union (EU) has recommended the detection of bovine proteins in dairy products based on gel isoelectric focusing of γ-caseins after plasminolysis (EU Regulation Nº273/2008) to ensure the quality. The results of this method nevertheless are sometimes ambiguous with overlapped proteins and can result in false positive results (50). An alternative method for adulteration detection has been developed based on ultra-high performance LC-MS/MS for the detection of phosphorylated β-casein f33-48 tryptic peptide which is a specific and sensitive specie marker for MDB that can be detected in three magnitude orders lower than the methodology recommended by the EU (51) with a detection limit for bovine milk in buffalo cheese products of about 1%. Additionally, MDB has been profiled with HR-MAS-NMR (High Resolution Magic Angle Spinning Nuclear Magnetic Resonance) and this has been proven to

be a reliable methodology for detection of specific metabolic fingerprints that provides guarantee its PDO, thereby avoiding fraud (52).

Early detection of pathogens in crops is fundamental for the agricultural sector, the use of metabolic profiles may prove to be helpful to this purpose (53). The non-cultivable bacteria *Candidatus Liberibacter spp* is the responsible of the disease known as Huanglongbing (HLB) in citrus trees (54). There is no known cure for this disease and infected trees die after a few years. Furthermore this disease produces a change in the flavour of oranges, which then cannot be consumed because of the extremely bitter- acid flavour they acquire (54). Recent studies have described the use of metabolic profiling by NMR in orange juice, leaves and roots from asymptomatic and symptomatic trees detecting differences in their metabolites (55, 56). The implementation of new strategies to tackle and detect timely the presence of HLB are important for citrus industry, where metabolic profiling techniques could be useful in developing new protocols to reduce HLB infected plants.

Metabolic profiling techniques have been studied to detect food pathogens in various types of food with promising results (28). Foodborne pathogens are a constant threat for the food industry and public health, consequently rapid techniques are required to obtain reliable detection of spoiled or dangerous foods. A proof concept of a rapid method detection for *Listeria monocytogenes* has been developed using gas chromatography coupled to orthogonal acceleration time-of-flight mass spectrometry (GC-oaToFMS) capable of detecting metabolic fingerprints in laboratory media and milk (57). Additionally, an alternative method for pathogens detection has been developed to detect *Escherichia coli O157:H7*, *Salmonella Typhimurium, Salmonella Muenchen,* and *Salmonella Hartford* in laboratory samples with GC-MS (58). The unmet need for rapid techniques for accurate and sensitive detection of food pathogens in food presents an analytical challenge, particularly when metabolite patterns are required to be specific down to the level of bacterial strain and food matrix. However, new

technologies capable of real-time metabolite profiling linked to extensive databases of bacterial profiles are on the horizon and are described in Section 3.

2.2 The use of dietary biomarkers to improve the assessment of dietary intake

Collection of dietary data is generally carried out by different methods like weighed food records, food frequency questionnaires, food diaries and 24 hour recall methods, to name but a few (59). These techniques provide information about eating habits, portion size, food and nutrient consumption, but these data tend to lack accuracy considering the different sources of food composition tables estimating energy, nutrients and other food elements (60, 61). Additionally, dietary assessment usually requires highly trained personnel, careful validation, time and cognitive ability from the respondent and skill of the researcher to obtain reliable results (62).

A major limitation of nutritional science is the objective assessment of dietary intake at both individual and population levels. The prevalence of misreporting is estimated at 30-88% (63, 64) based on underreporting biased towards unhealthy foods and over-reporting towards fruits and vegetables (65). Moreover, underreporting dietary energy intake is exacerbated in obese individuals which is a major concern considering the increasing prevalence of obesity globally (66-68).

With the present dietary tools it is difficult to assess if lack of effect at a population or individual level is due to there being no physiological effect, poor compliance to the recommended dietary change, differences in chemical composition of foods sourced from different origins or high inter-individual variability in response to the same diet.

The urgent need to improve methods for the assessment of dietary intake for nutrition studies has been widely discussed, where the use of dietary biomarkers (DBs) and metabolic profiling techniques have been proposed as a promising approach for this purpose (69).

DBs are defined as measurable metabolite or metabolites excreted in biofluids that derive directly or indirectly from a given nutrient, food or diet. This concept is based on the principle that excretion levels of metabolites are highly correlated to the dietary intake of a food or nutrient over a fixed period of time (70). The assessment and validation of DBs consist in identifying a candidate metabolite or metabolites and subsequently evaluating them in a nutritional trial under controlled conditions (71). The application of high- throughput analytical techniques has permitted the identification and discovery of several DBs in urine and blood (72, 73). Dietary biomarkers from different food groups identified from 2006 to 2016 using multiplatform metabolic profiling strategies are summarised in **Table 1**.

The approaches that have been used to DBs discovery can be either hypothesis led or data driven (72, 74). In the hypothesis driven case, a prior knowledge of different DB is selected according to their food composition data and information about *in vivo* metabolism. If a dietary study is hypothesis driven, for example the assertion that conferred health benefits are due to a specific component or class of components e.g. polyphenols or citrus fruits, then targeted detection methods are chosen according to the type of metabolites to be evaluated allowing easy identification and potentially quantification (75, 76). Alternatively where the food or diet is associated with improved health but the chemical components responsible are not known, then a data driven approach is adopted and multivariate analysis techniques are used to identify potential DBs. The samples to study DBs are mainly obtained from two types of studies: 1) the study of associations of dietary intake and metabolites analysis from biofluids in cross sectional studies or 2) controlled nutritional interventions where participants consume known amounts

of certain foods (72). Dietary biomarker validation must be done in a cross-sectional study after its detection in a controlled nutrition intervention study if the results from the controlled trial are to be useful in the general population (76). Conversely, the identification of DBs from cross-sectional or epidemiological studies generally only consider associations between DBs levels, amount of food consumed and presence or absence of disease, in some cases. However, in most cases, direct relationships between foods and disease are not demonstrated and neither are the biochemical mechanisms regarding metabolism of nutrients and their relationship with DBs explained. Thus the DBs identified in population studies often require interrogation *via in vitro* or *in vivo* models to ascertain mechanisms of action.

Nevertheless, the information provided by DBs is a useful adjunct to complement the dietary data obtained from traditional methods of dietary assessment and provides an important support in epidemiological studies of association between diet and diseases (53, 77).

2.3 Dietary effects on metabolic profile in health and disease

Different life style factors have a deep impact on health and diseases status, especially the impact of dietary habits and food consumption on the development of NCDs (78). NCDs are one the main cause of mortality and morbidity and their prevalence is projected to continue to rise during following years (79). The main NCDs include obesity, diabetes, hypertension, cardiovascular diseases, cancer and other metabolic disorders (78). Diet and nutrition play a key role in the aetiology and progression of NCDs: different studies have found epidemiological associations between specific dietary patterns and disease prevalence (80-83).

For example, adherence to Mediterranean diet has been associated with cardiovascular disease (CVD) prevention and with lower mortality among patients with a history of CVD. Likewise, sodium reduction in combination with a DASH-type diet has been associated with optimal BP reduction. However, there is a need to understand the complexity of interactions among metabolites, dietary pattern, specific food consumption, onset and progression of healthy and diseases states that cannot be detected by traditional medical screening programmes.

a. The Role of Diet in Influencing Metabolic Syndrome and Obesity

Metabolic syndrome (MS) is defined according to ATP III criteria (based on meeting at least three of the next five parameters: waist circumference >102 cm (men) and >88 cm (women), blood pressure ≥130/≥85, fasting triglycerides >1.7 mmol/L, fasting HDL cholesterol <1.04 (men) and <1.03 mmol/L (women)and fasting glucose ≥6.1 mmol/L) and its diagnosis can be made on the basis of presence of three altered symptoms for fasting glucose, blood pressure, HDL cholesterol, waist circumference and triglycerides (84). Obesity is defined as an excessive fat accumulation that may impair health. Obesity is frequently diagnosed by body mass index (BMI). A BMI > 30 is classified as clinically obese, and can be further categorized into grade I (BMI 30 to 34.9), grade II (BMI 35 to 39.9) and grade III (BMI >40) (85). There is evidence that suggests that obesity and MS are precursors of other metabolic diseases as 2 diabetes mellitus (T2DM), hypertension and cardiovascular disease (CVD) associated with high rates of mortality (68, 86). Understanding of the significance of metabolic phenotypes in NCDs could be a helpful tool to improve clinical treatment of patients and subsequent approaches to therapeutic management.

In one metabolic phenotyping study applied to obesity, plasma metabolites were analysed in a cohort of adults classified as normal weight and overweight/obese (OW) individuals with or without metabolic syndrome from the INFOGENE study. MS was used to detect differences

between metabolic profiles of normal vs OW participants. Metabolite differences in OW individuals with MS were especially enriched for metabolites such as PCaa (Phosphatidylcholines diacyl), PCae (Phosphatidylcholines acyl-alkyl) and medium chain sphingomyelins that were associated with a deteriorated metabolic state, which was more related to an obese metabolic profile rather than a healthy metabolic profile(87).

Other studies have explored the association between the urinary metabolome and BMI. In a unique cohort of samples from the INTERMAP epidemiologic study (88), 24 h urine collection samples were analysed by NMR to derive an untargeted metabolic profile and a targeted ion exchange chromatography (IEC) method was used to measure amino acid metabolites and related compounds. Urinary metabolites directly associated with BMI included trimethylamine, dimethylamine, 4-cresyl sulfate, phenylacetylglutamine and 2-hydroxyisobutyrate (gut microbial co-metabolites), succinate and citrate (Tricarboxylic acid c cycle intermediates), ketoleucine and the ketoleucine/leucine ratio (linked to skeletal muscle mitochondria and branched-chain amino acid metabolism), 3-methylhistidine (skeletal muscle turnover and meat intake) and ethanolamine (skeletal muscle turnover) (89). These metabolites could be useful in clinical settings where obese urinary phenotypess detected in normal weight individuals may indicate a heightened risk of developing obesity or related metabolic diseases.

Most obese individuals present with at least one altered characteristic for metabolic parameters and this condition is termed metabolic unhealthy obesity (MUO). Interestingly, 10 to 30 % of obese individuals present healthy metabolic parameters for insulin sensivity, blood pressure and lipid profiles (90), referred to as metabolic healthy obesity (MHO) (85, 91, 92). Currently, there is little knowledge about the role of abnormal metabolic phenotypes and factors that switch from MHO to MUO. Serum metabolic profiling of a paired group of 34 individuals with MUO and MHO was carried out using LC-MS for targeted analysis and GC-MS for both target and untargeted analysis. Metabolites that systematically differentiated between MUO and

MHO individuals included L-kynurenine, glycerophosphocholine (GPC), glycerol 1-phosphate, glycolic acid, tagatose, methyl palmitate and uric acid. In addition, these metabolites were related to several metabolic pathways, including fatty acid biosynthesis, phenylalanine metabolism, propanoate metabolism, and valine, leucine and isoleucine degradation (93).

Another similar study described distinct metabolic phenotypes for MUO, MHO and lean healthy (LH) subjects after the consumption of a high calorie meal challenge at breakfast. Plasma amino acid and fatty acid profiles were analysed by CE-MS and GC, respectively. MHO subjects were capable to easily adapt to the caloric challenge compared to MUO, showing a preserved insulin sensitivity. The metabolic profile presented some significant differences in amino acid levels between fasting and postprandial states for asparagine (LH v/s MUO), cystine (LH v/s MUO and LH v/s MHO), glutamine (LH v/s MUO and MUO v/s MHO) and serine (LH v/s MUO and LH v/s MHO). Lipid metabolic profiles were significantly different between fasting and postprandial for palmitoleic acid (LH v/s MUO; LH v/s MHO), Linoleic acid (MUO v/s MHO), γ-linolenic acid (LH v/s MUO and MUO v/s MHO) and arachidonic acid levels (MUO v/s MHO). Additionally, positive correlations were found between fasting levels of isoleucine, fasting insulin and insulin area under curve (IAUC). Also, a positive association of leucine with both HOMA- IR and fasting insulin was described confirming the role of branched amino acids (BCAA) to identify possible obese people at cardiometabolic risk (94). These fasting metabolites and associations should be considered as potential predictive indicators of postprandial response, independently of the BMI or metabolic diseases diagnosis in patients.

A recent study proposed a regression model of prediction for successful weight loss for patients with overweight, obesity and morbid obesity based on metabolic signatures at baseline before the consumption of caloric restriction diet for 8 weeks. Participants received a liquid diet of

approximately 800 kcal during 8 weeks. Metabolic profiles changes in plasma were measured before and after weight loss period using NMR for low weight molecular metabolites and LC-MS for lipidomics. By looking at baseline parameters, a maximum of 57% of participants weight loss could be predicted. However, the best predictions were obtained with the morbid obesity participants in comparison with obese participants (10). Weight loss prediction was based on metabolites related to energy metabolism such as acetoacetate, triacylglycerols, phosphatidylcholines, amino acids, creatine and creatinine. Hence, it is suggested that a successful weight loss in morbid obesity is modulated by a high energy metabolism status previous to a calorie restriction diet period allowing a personalised advice in this type of patients in near future.

2.4 Feeding the gut microbiome

No nutrition-associated metabolic phenotyping chapter would be complete without mentioning the gut microbiome and its role in metabolism of nutrients and foods, with consequent impact on metabolic signalling between host and bacterial community. The microbiome is key to the status of the immune system with capacity to affect a diverse range of tissues and organs including the liver and intestinal tract and is implicated in the aetiology or development of many diseases including inflammatory bowel disease, fatty liver diseases and several cancers. Given the close relationship between the gut and liver (the "gut-liver axis"), the intestinal microbiome has been widely recognized for playing a key role in the maintenance of gut-liver health. Ingested foods and nutrients processed by the gut bacteria are transformed into metabolically active chemicals, some of which act directly as signalling molecules in gut-brain axis communication.

At the broadest level, high calorie diets and obesity have been shown to be associated with distinct microbiomes with an increased ratio of *Firmicutes* to *Bacteroidetes* identified as being modulated by a weight reduction diet (95) However, other research groups have failed to

reproduce consistent differences in the *Firmicutes to Bacteroidetes* in the microbiota of obese and non-obese individuals (96) suggesting a more complex relationship between nutrition and the microbiota with regard to its role in metabolic syndrome and obesity. Nevertheless, the weight of evidence heavily favours a strong relationship between obesity and the microbiome based on both metagenomic data (97) and on metabolic phenotyping data showing clear differences in gut microbial metabolites of dietary aromatic amino acids, phenolics and short chain fatty acids (98) . One of the strongest modulators of the intestinal microbes and their activity is caloric restriction. Low fat diets and other diets aiming to achieve weight loss result in a robust panel of metabolic changes, mirrored by an alteration in the gut microbiome. Higher urinary concentrations of hippurate, phenylacetylglutamine and 4-cresyl sulphate, all gut microbial metabolites or bacterial-host co-metabolites have been associated with lean body mass and with weight loss (88, 99). Microbial modulation of dietary choline, specifically phosphatidylcholine to trimethylamine-*N*-oxide (TMAO) has been implicated in cardiovascular disease (100) and yet high levels of TMAO are found in urine samples obtained from Japanese populations originating from a diet rich in fish containing high levels of TMAO (88). Clearly heart disease is not overtly high in the Japanese, in fact quite the contrary, again pointing to the extreme complexity surrounding the microbe-host relationship in processing of foods and nutrients and pointing to conditional relationships of gene-environmental interactions in disease aetiology.

One of the most credible pieces of evidence highlighting the interaction of diet and the microbiome in disease is that there are three distinct metabolic phenotypes of individuals found with respect to gut bacterial metabolism of the dietary soy component daidzein: individuals who metabolize daidzein to O-desmethylangolensin (ODMA), equol or both (101). Equol production is associated with beneficial effects of soy in cardiovascular disease. Individuals who produced ODMA and equol have been reported to have lower levels of the gut microbial

metabolite trimethylamine yet were associated with increased pro-inflammatory cytokines further underscoring the complexity of the metabolic interaction between man and his microbiome.

Dietary impact on cancer risk also demands interrogation of the tripartite relationship between diet, inflammation and the microbiome. Unequivocal evidence has shown that migrant populations assume the colon cancer incidence of the host population after only one generation. A cross-over study in African Americans and rural Africans showing a switch of diet induces a rapid change in metrics of colon cancer risk (102). Other research programmes have shown clear links between bacterial and host modulation of dietary choline and cancer both in terms of risk and also prognosis (103, 104).

It is widely accepted that short chain fatty acids (SCFA) play an important role in maintaining the epithelial integrity of the mucosa in inflammatory bowel disease. Acute studies in both animals and humans demonstrate that SCFA can have a favourable effect on inflammatory bowel disease activity markers. The challenge of using SCFAs as a mainstream therapy has been in developing a methodology that will increase colonic SCFAs over a prolonged period of time to assess disease remission. Recent research has produced a method of delivering short chain fatty acid to the colon over a prolonged (>6month) period of time based on SCFA inulin esters (105). There are many potential metabolic intersections between the human host and their microbiome and this arena provides a broad landscape in which to develop nutriceuticals and health-promoting foods.

3. Future of nutrimetabonomics

3.1 -REIMS and DESI imaging technologies

Several promising new MS-based methods for profiling and imaging of foods, and for profiling bacterial communities in foods are on the horizon. Mass spectrometric methods have been used

to differentiate and to phenotype bacteria for several decades based on the composition of their lipid coat and fatty acid profiling, beginning with pyrolysis MS (106, 107) and GC-MS (108) methods. Matrix Assisted Laser Desorption/Ionization (MALDI) mass spectrometry has also been applied to taxonomic characterization of bacteria (109), but is limited by the requirement for extensive sample preparation including embedding within a chemical matrix. Rapid Evaporative Ionisation Mass Spectrometry (REIMS) is a recently developed matrix independent method that can accurately speciate microorganisms based upon their species-specific phospholipid fingerprint (110). The technique operates by applying a radiofrequency derived electrical current directly to the bacterial colony or biological sample and generating a vapour from the bacterial biomass, which contains ions that can be directly imported into a mass spectrometer. The technique is rapid and could be applied directly to foods to identify harmful microorganisms present in foodstuffs.

In a similar manner the REIMS technology can be applied directly to foods to monitor composition directly. This has obvious application in food adulteration, and its major strength lies in the rapidity of the method allowing high throughput screening of food products. The best known exemplar for this technique is the differentiation of meat burgers according to the species the meat originated from, detecting horse, beef and venison and establishing the relative proportion of each (111) .

The complexity of gene-environment interactions that drives the association between nutrition, metabolic phenotypes and disease is underpinned by vast interactive networks of signaling molecules that bridge the various layers of biomolecular organization in humans. Improved methods and systems biology frameworks for conducting analysis at the interface between epigenetics and molecular phenotypes (112).

3.2- Nutrition in the intensive care setting

Metabolic phenotyping studies in the critical care setting are relatively sparse but have been used to characterise a wide range of patients from pneumonia and acute lung injury to trauma patients (113). There is growing recognition of the role of nutritional management in the intensive care setting and recent studies have shown the value of applying profiling studies to evaluate various nutritional management strategies. For example, recovery of mitochondrial function has been found to be predictive of recovery form multiple organ dysfunction. Metabolic phenotyping has been highlighted as a vehicle for patient stratification with respect to therapeutic management with respect to administration of potentially beneficial agents such as thiamine, ascorbic acid, tocopherol, selenium, zinc and potential metabolic resuscitators (coenzyme Q10 (CoQ10), cytochrome oxidase (CytOx), L-carnitine, melatonin) (114). Other metabolic profiling studies have identified altered plasma levels of sucrose, mannose, 3D-hyroxybutyrate, lactate, methionine, arginine, and various acylcarnitines to be associated with sepsis (115). These molecules may provide a useful biomarker panel against which to measure nutritional and other therapeutic management strategies in intensive care patients. Further profiling studies have shown the association between nutritional status and prognosis in the clinical care setting. Mogensen *et al* reported a correlation between metabolic profiles in critical care patients with malnutrition and 28 day survival, with dysregulation of glutathione and purine metabolism being particularly important (116). The term metabolic resuscitation has become popular and is indicative of the growing recognition of the need for specific nutritional care in the Critical care patient. A recent study reported that amino acids fluctuate and that their levels correlated with prognosis as assessed by the APACHE and SOFA scores, with a particularly strong correlation between worsening prognosis and a decline in sulfur-containing amino acids, such as taurine (117) .Thus, metabolic profiling technology has clear potential as a tool for nutritional support in sepsis patients.

3.3- Neonatal nutrition

Pregnancy offers a window of opportunity to shape the health of both the mother and foetus, and good nutrition is considered a vital part of managing the pregnancy journey. Metabolic phenotyping applied to pregnant women has shown differential profiles between individuals undergoing a healthy pregnancy and those with intrauterine growth restriction (IUGR) which was characterized by lower levels of urinary acetate, trimethylamine, tyrosine and formate and higher levels of N-acetyl glycoproteins (118). These biomarkers of growth restriction also provide a panel of markers to target via nutritional management. Breast milk is generally considered to be healthier than formula milk and breast-feeding has been associated with lower risk of infants and children developing asthma, eczema and downstream obesity (119, 120). Several studies have examined early feeding regimens using metabolic phenotyping, some of these studies in preterm or intrauterine growth restriction infants. After just three days of formula nutrition urinary excretion of glucose, galactose, glycine and myo-inositol increased whilst breast bed babies had higher urinary levels of adipic acid, citric acid, homoserine and aminomalonic acid (121).

The gut bacterial community, introduced at, or even before, birth undergoes dynamic colonization of the intestinal tract resulting in microbial communities that support our immune function, our ability to harvest calories from our food and perform chemical signaling functions between the gut and other organs and tissues. The gut microbiome is inextricably linked with dietary processing and is largely symbiotic with its human host. Dysfunction of the microbiome has been associated with multiple diseases ranging from inflammatory bowel disease to obesity-related conditions and even neurodevelopmental disorders such as autism (122-124). The transmissibility of microbiomes, be they beneficial or adverse, is a controversial subject, particularly in the case of their association with obesity and insulin resistance (125). Collado *et al* reported higher levels of fecal *Bacteroides* spp., *Staphylococcus* spp. and *Clostridium*

difficile counts in 6 month old babies from overweight mothers and also showed that weight gain during pregnancy impacted on the microbial composition (126). Preterm babies have been shown to have increased risk of metabolic syndrome and cardiovascular disease later on in life and are also at higher risk of neurodevelopmental complications (127, 128). One school of thought proposes that it is the nutritional management of preterm or low birth weight infants that is responsible for the downstream health implications rather than the phenomenon of being born with immature organs and systems. In a mouse model of feeding to achieve catch up growth in neonatal undernourished mice, the gut microbial metabolites phenyllactate (plasma) and 4-cresyl sulphate (urine) remained different from wither undernourished counterparts fed normally or from control mice (129) implicating a nutritionally modified microbiome.

Human milk oligosaccharides, present in breast milk have been found to promote growth in models of infant undernutrition (130) and protect against pathodenic infections such as Campylobacter and Group B Streptococcus. Metabolic profiling methods for determining the glycomic profile have been developed for both nano-liquid chromatography chip time-of-flight mass spectrometry (131) and NMR platforms (132). The association between nutrition and cognitive development is intriguing and under researched. A few metabolic profiling studies have begun to probe the relationship between the diet and the gut-brain axis. One such study in pigs has shown that early life supplementation with phospholipids and gangliosides alters the brain biochemistry and improves spatial learning in piglets (133). Several methodologies and strategies for profiling human breast milk have been developed and should prove useful in investigating the tripartite relationship between the early colonising microbiome, infant nutrition and the metabolic profiles (134, 135).

3.4- Sports nutrition

Appropriate nutrition enhances physical performance and recovery (136-138). Athlete's dietary choices, amounts and timing of food intake and supplements can help reduce the risk of injury whilst providing a more effective training (139). To date, the majority of exercise-diet-metabolism studies rely on single targeted markers. On the other hand, metabolic phenotyping captures information of the athlete's biochemistry that reflects, physiological status, genetic, microbiome and other environmental interactions such as dietary exposure and lifestyle (17, 140). This has the potential to provide personalized recommendations that will enhance the sportsman's performance.

Metabolic profiling studies have been applied to identify exercise-related metabolites (141), inter-individual variation in response to exercise(142) and to compare the effect of different exercise sessions and different levels of training exercises (143). Likewise, the use of metabolic profiling is essential to objectively monitor and assess athletes' food intake at an individual level that allows accurate understanding of the impact of diet on performance and recovery. This will enable the development of optimised personalised food and training plans.

Currently, there is growing interest in increasing our understanding of the impact of functional foods and supplements on athletes' metabolic behaviour, before, during and after physical activity. Several metabolic profiling studies have evaluated the impact of formulated sports drinks, enriched with macronutrients and/or micronutrients or plant based extract to improve sportsman's performance and recovery. Serum metabolic profiles revealed different systemic metabolic response in the early recovery phase post exercise when comparing the ingestion of water, low-carbohydrate beverages, high-carbohydrate beverages, and low-carbohydrate-protein beverages, immediately after 90 min of ergometer-cycling (144). This suggests that the post exercise intake of low-carbohydrate-protein beverages improved the metabolic status of less fit subjects by increasing the serum levels of pseudouridine and decreasing levels of 3-methyl histidine, a marker of muscle turnover. A similar study, evaluated the metabolic effect

of a green tea based sports drink, rich in polyphenols, in comparison to oligomineral water, which resulted in improving energy metabolism and glucose homeostasis (145). Post exercise serum and urinary metabolic profiles of rowing athletes revealed lower lactate and higher glucose and citrate plasma levels and an increment of urinary acetone and 3-hydroxybutyrate during rehydration.

The benefit of phenolic ingestion during and post exercise on athletes' performance and recovery was investigated by MS metabolic profiling strategies. The acute effect of banana intake compared to 6% carbohydrate drink during and after 75-km cycling performance and post exercise resulted in no detectable differences in performance, blood glucose levels, oxidative stress, inflammation and innate immune levels (146). However, further studies compared the effect of the ingestion of banana, water and pear before and during 75-km cycling indicated that banana and pear intake was associated with a meaningful performance enhancement, diminished inflammation, decreased fatty acid mobilization and oxidation and contributes unique phenolics that elevate antioxidant capacity (147).

On the other hand, metabolic profiling strategies are well suited to investigate changes in gut microbial metabolites after supplements and/or food intake. Long distance runners were supplemented with blueberry and a green tea polyphenol rich soy protein-based product after 3 days of intensified training which increased ketogenesis during recovery and a distinct gut-derived phenolic signature (hippurate, 4-hydroxyhippurate, 4-methylcathecol sulphate) which the authors propose is mediated through increased gastrointestinal permeability (148).

Nieman *et al* also investigated the influence of 2 weeks intake of pistachio nuts on cyclists' performance and post exercise recovery. Although, traditional biomarkers for exercise-induced inflammation and oxidative stress did not showed differences between pistachio and non-pistachio consumption, the metabolic profiling analysis revealed differences in 19 blood metabolites related to leukotoxic effects and oxidative stress. In addition, gut derived raffinose,

sucrose and myoinositol were present in the circulation of endurance athletes as a result of the prolonged and intense exertion and the 2-weeks pistachio intake (149).

To summarise, the application of metabolic profiling strategies have the potential to revolutionise sports nutrition since they provide athletes with a personalised toolset to enhance their performance, reduce likelihood of muscle injury and potentially extend the working life of elite sportsmen.

Concluding Remarks

As the applications for metabolic phenotyping in nutrition expand, the analytical technologies for metabonomics and lipidomics are becoming more robust and compound libraries are growing, together with innovative methods of modelling and mining spectroscopic data. We are beginning to see the routine use of these technologies in food screening and sales of specialised products such as the FoodScreener, an automated NMR-based system for authenticity testing of fruit juices and wines are increasing. Newer technologies such as the rapid evaporative ionization mass spectrometry (REIMS) for direct MS-based testing of food provenance hold great promise for identifying food fraud in a matter of minutes, for example detecting adulteration of beef burgers with horse meat and have potential to revolutionise the food screening industry. MS libraries of bacteria present in foods also opens the door to interesting opportunities in food security.

Scale up of metabolic profiling technology for profiling of biofluids opens the door to MWAS and MWAS-GWAS studies facilitating disease-diet correlations to be extracted from large cohorts with the ability to simultaneously address dietary reporting accuracy, adherence to diet and inter-individual differences in metabolism of foods and nutrients. For the first time personalised nutrition, informed by accurate phenotyping technology, is a tangible prospect

both in terms of achieving required sample throughput and economic viability. Realisation of this goal has the potential to significantly impact disease risk and drive improved health initiatives, particularly if the nutrition community joins forces to drive method standardisation and creation of shared databases.

Table 1 Dietary Biomarkers

Dietary Interventions studies for detection of biomarkers using metabolic profiling approach

Food Group	Specific Group	Biomarkers	Biofluid	Techniques	N of participants	References
Dairy	Casein and whey protein	Blood:Methionine sulfoxide, *N*-phenylacetyl-methionine, Urine: N-phenylacetyl-Methionine sulfoxide, *N*-phenylacetyl-methionine, β-asp-Leu	Blood	LC-QTOF-MS.	11	Stanstrup et al, 2014.
	Cheese	Tyramine sulfate, Isobutyrylglycine, Acylglycine, Xanthurenic acid, Isovalerylglycine, 4-hydroxyphenylacetic acid,	Urine	UPLC-MS/QTOF	33	Bousgaard et al. 2014
	Cheese	↑ Prolinebetaíne, urea	Urine	NMR	15	Zheng et al. 2015
	Semi Skimmed Milk	↑ Citrate, creatinine, creatine, urea) in urine	Urine	UPLC-MS/QTOF	33	Zheng et al. 2015
Meat Products	Meat Products	Creatine, carnitine, acetyl-carnitine, and trimethylamine-*N*-oxide (TMAO)	Urine	NMR	12	Stella et al. 2006.
	Salmon	Anserine, 1-3-Methylhistidine, TMAO	Urine	FIE-MS and GC-tof-MS	24	Lloyd et al. 2011.
	Red meat	1-methylhistidine and 3-methylhistidine	Urine	Ion exchange chromatography	17	Cross et al. 2011
	Fish	TMAO	Urine	UPLC-TOF-MS	17	Andersen et al. 2013
	Meat protein (Beef, pork, chicken)	Meat protein (Beef, pork, chicken)	Urine	LC – MS/MS	52	van der Kuil et al.2013.
	Oily fish	Methylhistidine	Urine	FIE- FTICR-MS	68	Lloyd at al. 2013
	Cod	Blood: TMAO, creatine, Proline Arsenobetaine 1-Methyl-Histidine and 3-Methyl- Histidine mixture 1,2,3,4-Tetrahydro-β-carboline-3- carboxylic acid Phenylalanine Taurine Docosahexaenoi acid Urine: TMAO, N6,N6,N6-trimethyl-lysine, 1,2,3,4-Tetrahydro-β-carboline-3-carboxylic acid, Arsenobetaine, 1-Methyl-Histidine and 3-Methyl-Histidine mixture	Blood and urine	LC-QTOF-MS.	11	Stanstrup et al, 2014.
	Beef	B-alanine, 4-hydroxyproline	Blood	GC- MS	17	Ross at al. 2015
	Herring	DHA and cetoleic acid	Blood	GC- MS	17	Ross at al. 2015
Vegetables	Broccoli and Brussel sprouts	SMCSO (S-methyl-L-cysteine sulfoxide)	Urine	NMR	20	Edmands et al. 2011
	Isothiocyanate hydrolysis product from cruciferous vegetables	Sulforaphane	Urine	LC – MS/MS	10	May et al. 2013
Fruits	Orange and apple juice	Proline betaine	Blood	HPLC	8	Atkinson et al. 2007
	Citrus fruits	Proline betaine	Urine	FIE- ICR- MS	23	Lloyd et al. 2011.
	Raspberries	Sulphonated caffeic acid and sulphonated methyl-epicatechin	Urine	FIE-MS and GC-TOF-MS	24	Lloyd et al. 2011.
	Citrus fruits	Proline betaine, hydroxyproline betaine, Hesperetin 3′-O-glucuronide , naringenin 7-O-glucuronide	Urine	MS	24	Pujos-Guillot et al. 2013.

	Aronia-citrus juice	Proline betaine, ferulic acid, and two unknown mercapturate derivatives	Urine	HPLC-QTOF	51	Llorach et al. 2014
	Grapes	Tartaric acid	Urine	MNR	25	García- Pérez et al. 2016.
Grains and cereals	Grain protein (Wheat protein, bran, rice and maize)	Lysine, valine, threonine, a-aminobutyric acid, proline, ornithine, arginine	Blood	LC – MS/MS	52	van der Kuil et al.2013
	Whole grain bread (rye)	Alkylresorcinol metabolites derivatives . .Hydroxyhydroxyphenyl acetamide sulfate, 3,5-dihydroxyphenylpropionic acid sulfate, caffeic acid sulfate, hydroxyphenyl acetamide sulfate .	Urine	LC-MS	72	Hanhineva et al. 2015.
Coffee and Cocoa	Cocoa beverage with and without milk addition	(Epi)catechin-O-sulfate (urine), (–)-Epicatechin-O-glucuronide (urine), (Epi)catechin-O-sulfate (urine and plasma), O-Methyl-(epi)catechin-O-sulfate (urine and plasma)	Urine and Blood	HPLC- MS	9	Mullen et al. 2009
	Coffee	dihydrocaffeic acid-3-O-sulfate and feruloylglycine	Urine and Blood	HPLC-MS	11	Stalmach et al. 2009
	Coffee	2-furoylglycine (2-FG)	Urine and Blood	H NMR	8	Heinzmann et al. 2015
Others	Isoflavone in soya	Genistein, Daidzein, Glycitein	Urine	LC – MS/MS	10	May et al. 2013
	Wine	Tartaric Acid	Urine	LC–ESI-MS/MS	21	Regueiro et al.2014
	Sucrose	Fructose, Sucrose, Erythronic acid	Urine and Blood	MS (FIE-MS) and GC-TOF-MS	90	Beckman et al., 2015

LC: Liquid Chromatografy, QTOF: Quadrupole time of flight, MS: Mass spectrometry, UPLC: Ultra high performance liquid chromatography, NMR: Nuclear magnetic resonance, FIE: Flow infusion electrospray ionisation, FTICR: Fourier transform ion cyclotron resonance, HPLC: High performance liquid chromatography, ESI: Electrospray ionisation

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