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Nutrimetabolomic Strategies To Develop New Biomarkers of Intake and Health Effects

Rafael Llorach,[‖] Mar Garcia-Aloy,[‖] Sara Tulipani, Rosa Vazquez-Fresno, and Cristina Andres-Lacueva*

Nutrition and Food Science Department, XaRTA, INSA, Pharmacy Faculty, University of Barcelona, Avinguda Joan XXIII s/n, 08028 Barcelona, Spain; and INGENIO−CONSOLIDER Program, Fun-C-Food CSD2007-063, Ministry of Science and Innovation, Spain

ABSTRACT: Correctly assessing the metabolic status of subjects after consumption of specific diets is an important challenge for modern nutrition. Recently, metabolomics has been proposed as a powerful tool for exploring the complex relationship between nutrition and health. Nutritional metabolomics, through investigating the role that dietary components play in the maintenance of health and development of risk disease, aims to identify new biomarkers that allow the intake of these compounds to be monitored and related to their expected biological effects. This review offers an overview of the application of nutrimetabolomic strategies in the discovery of new biomarkers in human nutritional research, suggesting three main categories: (1) assessment of nutritional and dietary interventions; (2) diet exposure and food consumption monitoring; and (3) health phenotype and metabolic impact of diet. For this purpose, several examples of these applications will be used to provide evidence and to discuss the advantages and drawbacks of these nutrimetabolomic strategies.

KEYWORDS: *metabolomics, nutrimetabolomics, diet, biomarkers, nutrition, health phenotype*

INTRODUCTION

In the postgenomic era the integrated study of human physiology and pathology has emerged as a fundamental approach. In this regard, the Institute for Systems Biology has defined the term "systems biology" as "the study of an organism, viewed as an *integrated* and *interacting network* of genes, proteins and biochemical reactions which give rise to life", focusing the study on one organism and on all its components and the interactions among them, rather than studying them separately.[1] In the study of systems biology the "omics cascade" is a central concept.[2] It comprises the data obtained by genomics, transcriptomics, proteomics, and metabolomics, with the aim of integrating them to study the biochemical and biological mechanisms and elucidating the impact of diseases or environmental factors on the organism. Among ~omics levels, metabolomics is the one allocated at the end point of the omics cascade, because it is the nearest to the phenotype. Thus, it is an interesting tool in the study of phenotypic changes caused by the impact of environmental agents on several diseases.[2] The Metabolomics Society defines metabolomics as the "newly emerging field of omics research concerned with the comprehensive characterization of the small molecule metabolites in biological systems which can provide an overview of the metabolic status and global biochemical events associated with a cellular or biological system".[3] Two terms, close in meaning, have been used: metabonomics and metabolomics. Although both focus their research on the analysis of metabolites in the organism, metabonomics[4] focuses its efforts on studying the metabolic changes in an integrated biological system, whereas metabolomics[5] also considers the study of metabolites in cells, tissues, organs, and organisms. This discrepancy is also due to the origin of the word: the "metabolomics" concept is based on the metabolome, whereas the term "metabonomics" comes from the Greek roots "meta" and "nomos", which mean "change" and "rules" or "laws", respectively, in reference to the analysis of changes in metabolism.[6] In this regard, "metabolome" has been defined as the complete set of metabolites or chemicals that can be found in a cell, organ, or organism.[2,7] The term "metabolomics" is the one employed in this review. Two factors that influence the metabolome have been described: the endogenous metabolome, which involves all intrinsic metabolites related to the primary and intermediary metabolism, and the exogenous metabolome, which refers to all metabolites arising from extrinsic factors such as diet (i.e., food metabolome), microbiota, physical activity, stress, or drugs.[8,9] Specifically, "food metabolome" has been defined as all metabolites deriving from the ingestion of food.[10]

Metabolomic studies have shown a typical pipeline that has been broadly categorized into five main steps: sample collection, sample preparation, data acquisition, data analysis, and biological interpretation (Figure 1).[2,11] In addition, it is also necessary to highlight the importance of applying a quality control pipeline in metabolomic studies. A metabolomic approach has been applied in different kinds of samples, including single cells,[12] tissues, and biofluids. In this context, blood and urine are the most likely sources of biofluids for human metabolomics.[8] Other biofluids, such as saliva[13] and cerebrospinal fluid,[14] have also been used but to a lesser extent. A picture of the whole metabolome cannot be measured using a single analytical technology; however, bibliographic references about the use of multianalytical platforms are scarce. This means that various analytical techniques have emerged for

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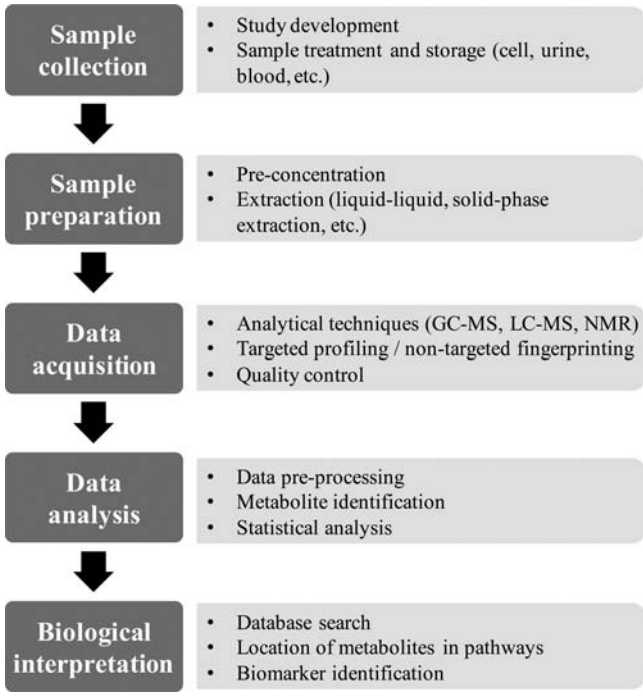

Figure 1. Metabolomics workflow scheme.

studying the metabolome. Among them, mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy are the most commonly applied in metabolomic analysis. An explanation and evaluation of their advantages and disadvantages have been reviewed previously.[2,7,9,15,16] With regard to data analysis and biological interpretation, comprehensive reviews and descriptions of bioinformatic tools have been published.[17−26]

Two approaches have been described for the study of metabolomic data.[2,16] One of them is known as "targeted profiling", which is based on the analysis of a predefined metabolite group associated with a particular class of compounds or metabolic pathway. It provides quantitative results. Nevertheless, with this approach some information related to the whole metabolic network and its relationship with pathophysiological status might be lost. Instead, "non-targeted fingerprinting" attempts to obtain a broad picture of a metabolome with the aim of detecting the maximum number of metabolites in a sample, including those that are unknown or poorly characterized, giving a global approach of the whole metabolome of an organism. Its aim is to study metabolite patterns in response to a disease or an environmental or lifestyle factor, elucidating the new roles of some metabolites. This second metabolomic approach provides qualitative data and is considered to be a hypothesis-generating approach, whereas the first one is regarded as a hypothesis-driven method.[2]

Metabolomics, Health, and Nutrition. Metabolomics offers a wide range of applications in biomedical research. One of these is the capacity to predict or detect the stages of diseases and their progression or to monitor the compliance and response to therapy through the use of biomarkers.[2,3] Recent reviews have highlighted the relevance of metabolomics for obtaining biomarkers when this approach was applied in clinical conditions such as cardiovascular disease, cancer, or inborn errors of metabolism.[27] In this context, two phases in the discovery of biomarkers through metabolomics have been described.[27] The first one is the discovery phase, which provides potential biomarkers using nontargeted metabolomic approaches. Between 10 and 100 samples controlled by confounding factors are required in this stage. Fewer samples could be sufficient if samples were available before and after the perturbation of the same individual that was studied, allowing each subject to act as his own control, thereby reducing interindividual variability. Once the putative biomarker is identified, the process of validation in a general population is necessary. The aim of the second phase is to evaluate the specificity and selectivity of the biomarker using an absolute quantification by a targeted metabolic approach of a range of 100−1000 samples.

Currently, the main causes of morbidity and mortality worldwide are those pathologies known as noncommunicable diseases.[28] They include cardiovascular diseases, diabetes, cancers, and chronic respiratory diseases. All of them are often related to aging and are more influenced by lifestyle factors, such as tobacco use, insufficient physical activity, or unhealthy diet, than by genetic determinants. Due to the multifactorial etiology of these diseases, and because it has been established that a disease is characterized by exhibiting a particular pattern of metabolites, a new concept for biomarkers has emerged.[29,30] This focuses on the development of biomarker patterns, or metabolite signatures, instead of isolated biomarkers, as has been used traditionally in clinical practice.[30] This change means that more knowledge about perturbed pathways in pathological states is required. Furthermore, more complex technologies are needed to adequately characterize the novel biomarkers. Consequently, the combined use of both metabolomic approaches described previously offers an interesting scenario for the research of new biomarkers related to these pathologies.[2,27,29,31,32]

Focusing on the nutrition sciences, metabolomics is an interesting tool for assessing the nutritional status of an individual, the food consumption, the biological consequences of following a nutritional intervention, or the study of metabolic mechanisms in response to a diet depending on a particular metabolic phenotype.[7,10,16] With regard to the metabolome, which has been defined above, it should be noted that feeding induces alterations in the endogenous metabolome, and they can be evaluated by the detection of exogenous metabolites in biofluids.[9] They can be used as potential biomarkers of food consumption (i.e., food metabolome) and/or the effects of a dietetic intervention. The use of biomarkers in this field is interesting because they are objective and accurate tools. Recently, metabolomics has been proposed as a powerful tool for exploring the complex relationship between nutrition and health.[33] Several studies and reviews have pointed out the interest in applying metabolomics to the nutritional field of work.[7−9,16,31,33,34]

The aim of this review is to offer an overview of the state of the art of the application of nutrimetabolomic strategies in the discovery of new biomarkers in human health nutritional research.

REVIEW STRATEGY

To assess the current status of the application of nutrimetabolomic strategies in biomarker discovery, we have conducted an exhaustive search of the scientific bibliography. Our search was applied to the PubMed and Web of Science electronic databases. The search strategy used for the PubMed database was ("Metabolome"[Mesh] OR "Metabolomics"[-

Mesh]) AND ("Diet"[Mesh] OR "Food"[Mesh] OR "Nutritional Sciences"[Mesh]) AND ("Biological Markers"[Mesh]) AND ("Humans"[Mesh]). The search strategy used for the Web of Science database was (TS=(metabolom* OR metabonom*) AND TS=(diet* OR food* OR nutrition*) AND TS=(biomarker* OR biological marker*)) AND Document Types=(Article). The last search was run on January 25, 2012. Furthermore, to identify additional studies we also analyzed the reference lists of the publications selected. We included papers that used the metabolomics technology applied in nutritional studies. These had to be original investigations carried out in humans. No language or publication date restrictions were imposed. The search of the PubMed and Web of Science databases provided 24 and 146 publications, respectively. After adjusting for duplicates, 159 remained. Of these, we excluded 34 reviews and 7 other document types (congresses, comments, letters, etc.). The titles and abstracts of the remaining 118 articles were screened. Of these, 57 were excluded for the following reasons: 49 used animal models, 5 evaluated food matrices, 2 were conducted on bacteria, and 1 was an in vitro study. The 61 remaining studies were conducted in humans, but 25 were excluded because they were not related to the topic. Finally, we did not include 1 study because it used lipidomics technology, 7 studies because they used a targeted analysis, and 2 because they did not apply chemometric methods. Additionally, 14 publications in the field were detected among the references of the papers selected. Therefore, 40 studies were included in this review.

After reviewing the selected articles, we propose a classification with three principal categories (Figure 2), namely,

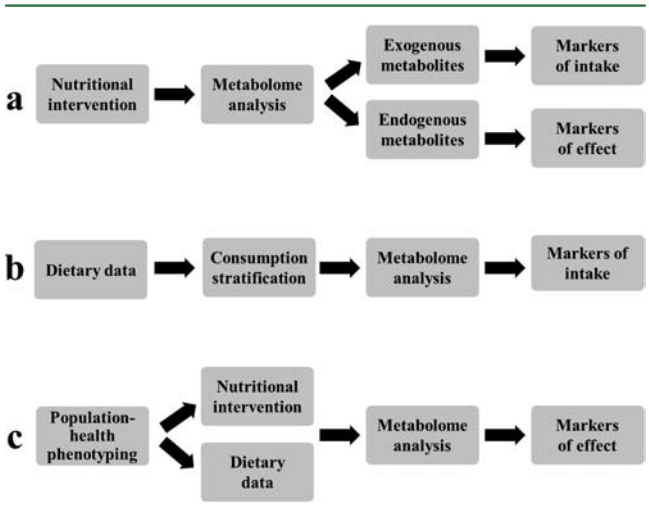

Figure 2. Main nutrimetabolomic approaches implemented: (a) assessment of nutritional and dietary interventions; (b) diet exposure and food consumption monitoring; (c) health phenotype and metabolic impact of diet.

"assessment of nutritional and dietary interventions", "diet exposure and food consumption monitoring", and "health phenotype and metabolic impact of diet". As well as these categories we have also considered a heterogeneous category termed "other studies".

NUTRIMETABOLOMIC STRATEGIES TO DEVELOP NEW BIOMARKER ASSESSMENT

An important challenge for modern nutrition is to correctly assess the metabolic status of subjects after consumption of specific nutrients, foods, or diets, with the aim of promoting health. Dietary components have been associated with an important diversity of mechanisms of action and biological effects in consumers. Furthermore, it is assumed that food consumption has an effect on the human metabolome. Consequently, the concept of nutritional metabolomics, or nutrimetabolomics, has emerged with the introduction of metabolomic technology into nutritional research.[35]

Assessment of Nutritional and Dietary Interventions. In the past few years a considerable number of studies using this approach have been published, most of them focused on evaluating the effect of an acute or chronic intake of a particular food (Figure 2a). Nevertheless, to our knowledge, there is only one human intervention study designed to evaluate the influence of dietary pattern interventions on the metabotype.[36]

A summary of selected studies is presented in Table 1. Almost all of the studies were performed on healthy adults,[36−46] whereas others were performed on healthy children,[47] pregnant women,[48] or mildly hypertensive,[49−51] metabolic syndrome,[52] or cancer[53] patients. The publications selected involved different types of design. Most of them were prospective studies,[36−39,44−54] but in five of them the volunteers consumed an acute dose of the food item evaluated.[40−43,55] The length of each intervention period in the prospective studies varied from 2 days[44,54] to 8 months.[48] Urine was the most widely used biological sample,[36,38−45,49,50,52,55] whereas three studies were performed on blood samples,[37,48,53] and two used both urine and blood samples.[47,54] Furthermore, two studies analyzed feces.[46,51] With regard to the analytical platform, ^{1}H NMR was the most common analytical technique used in these publications,[36−41,45−47,49−51,53,54] but MS has also been employed in five studies,[42,43,48,52,55] and in one article both platforms were applied.[44]

Effects on Metabolome and Biological Interpretation. The metabolic changes observed in these studies, together with their biological interpretation, are summarized in Table 2. This table shows several modifications either in food or in the endogenous metabolome. Broadly speaking, the effects on the food metabolome were more widely detected in urine samples, whereas blood samples showed the most changes in the endogenous metabolome. With regard to the modifications on the food metabolome, these can be classified into two classes: those directly derived from the digestion of food components by the human organism and those derived from the metabolism of human gut microbiota.

With regard to metabolites directly derived from the digestion of food components by the human organism, urinary levels of creatine, creatinine, and carnitine increased after the intake of meat.[36,47] High concentrations of creatine and carnitine are found in meats, and creatinine is formed from biodegradation of creatine and is carried to the kidneys by blood plasma to be eliminated by urine.[56] These metabolites have been proposed as potential biomarkers of dietary meat intake, although they have not yet been validated.[56] In support of this, carnitine was almost totally absent after 15 days of following a vegetarian diet,[36] and reduced levels of urinary creatinine were observed following the intake of chamomile tea[39] and fruit and vegetable juice.[44] However, Wang et al.[39] proposed that the lower levels of creatinine after chamomile consumption resulted from the antioxidative activity of chamomile. Among the reviewed studies, methylhistidine and anserine were associated with salmon consumption,[55] although

Table 1. Characteristics of Nutrimetabolomic Studies Assessing the Effect of Dietary Interventions in Humans

subjects: *n*	study design	intervention (dose per day)	duration	sample	analytical technique	ref
12 healthy	crossover randomized	(a) low-meat diet (60 g of red meat; 65 g of protein) (b) high-red-meat diet (420 g of red meat; 143−150 g of protein) (c) vegetarian diet (143−150 g of protein from nonmeat sources)	15 days each period	24 h urine	^{1}H NMR	36
5 healthy	single group	60 g of soy protein (45 mg of isoflavones)	1 complete menstrual cycle	plasma	^{1}H NMR	37
9 healthy	parallel	(a) 60 g tof extured vegetable protein (45 mg of conjugated isoflavone glucosides) (b) 50 g of miso (25 mg of unconjugated isoflavones)	1 complete menstrual cycle	24 h urine	^{1}H NMR	38
14 healthy	single group	200 mL of chamomile tea (5 g of powder in 200 mL of water)	2 weeks	urine	^{1}H NMR	39
3 healthy	single group	3 g of decaffeinated black tea extract	acute	urine	^{1}H NMR[a]	40
17	crossover randomized	(a) 6 g of black tea in water (b) 6 g of green tea in water (c) 360 mg of caffeine in gelatin capsules (control)	2 days each period	24 h urine plasma	^{1}H NMR	54
20 healthy	crossover randomized double-blind	>200 mL of water with a capsule containing 2500 mg of (a) dried black tea extract powder (800 mg of polyphenols) (b) sucrose (placebo)	acute	48 h urine	^{1}H NMR	41
10 healthy	crossover randomized	(a) 40 g of cocoa powder + 250 mL of water (b) 40 g of cocoa powder + 250 mL of milk (c) 250 mL of milk (control)	acute	24 h urine	LC-q-ToF-MS	42
24 healthy	parallel randomized blind	10 capsules containing (a) almond skin extract (3.5 g of almond extract + 0.5 g of microcrystalline cellulose) (b) 4 g of microcrystalline cellulose (placebo)	acute	24 h urine	LC-q-ToF-MS	43
42 metabolic syndrome	parallel randomized	(a) low-fat diet with mixed nuts (30 g: 15 g of walnuts + 7.5 g of almonds + 7.5 g of hazelnuts) (b) low-fat diet (control)	3 months	24 h urine	LC-q-ToF-MS	52
21 healthy	single group	4 × 100 mL of apple, carrot, and strawberry drinks	2 days	urine	^{1}H NMR LC-q-ToF-MS	44
20 healthy	single group	500 g of broccoli and Brussels sprouts	2 weeks	48 h urine	^{1}H NMR	45
29 mildly HTA	crossover double-blind	(a) mix of wine and grape juice extract (800 mg of polyphenols) (b) microcrystalline cellulose (placebo)	1 month each period	24 h urine	^{1}H NMR	49

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Table 1. continued

subjects: *n*	study design	intervention (dose per day)	duration	sample	analytical technique	ref
58 mildly HTA	crossover randomized double-blind	(a) mix of wine and grape juice extract or red grape juice extract (800 mg of polyphenols) (b) microcrystalline cellulose (placebo)	1 month each period	24 h urine	^{1}H NMR[b]	50
39[c] mildly HTA	crossover randomized double-blind	(a) mix of wine and grape juice extract or grape juice extract (800 mg of polyphenols) (b) microcrystalline cellulose (placebo)	1 month each period	feces	^{1}H NMR	51
96 healthy	parallel	(a) fortified wheat flour (300 g: 20 mg of folic acid + 3.5 mg of vitamin B_1 + 3.5 mg of vitamin B_2 + 30 mg of ferric sodium edetate +25 mg of zinc oxide/kg) (b) placebo	8 months	serum	UPLC-ToF-MS	48
17 prostate cancer	crossover randomized	(a) diet rich in whole-grain rye and rye bran products (485 g) (b) diet of refined whole-grain products with added cellulose (485 g) (control)	1.5 months each period	plasma	^{1}H NMR	53
16 healthy	single group	synbiotic (0.5 g of fructooligosaccharides + 10^9 CFU *B. longum* + 10^9 CFU *L. acidophilus* per serving, twice a day)	1 month	feces	^{1}H NMR	46
24 healthy	parallel	(a) 1.5 L of skimmed milk (53 g of protein) (b) 250 g of low-fat meat (53 g of protein)	1 week	24 h urine serum	^{1}H NMR	47
36	crossover randomized	orange juice, tea with skimmed milk and sugar, butter croissant, and (a) cornflakes with milk (control) (b) smoked salmon trimmings (60 g) (c) steamed broccoli florets (200 g) (d) raspberries (200 g) (e) 2 biscuits (37.5 g) with 125 mL of semiskimmed milk	acute	4.5 h urine	FIE-MS[d]	55

[a]HPLC NMR-MS was used to identify an unknown metabolite. [b]GC-MS was used for targeted profiling of urinary phenolic acids. [c]Although there were 53 volunteers who participated in the study, researchers analyzed fecal samples from 39 subjects. [d]GC-ToF-MS was used for targeted analysis.

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Table 2. Effects Observed on Metabolome following a Nutritional Intervention and Their Biological Interpretation

effects on metabolome[a]	biological interpretation[b]	ref
↑ creatine (high-meat), ↑ creatinine (high-meat)	markers of meat intake	36
↑ carnitine (high-meat), ↓ carnitine (vegetarian)	markers of red-meat intake/↑ β-oxidation	
↑ trimethylamine-*N*-oxide (high-meat)	marker of meat intake/metabolite from microbiota	
↑ taurine (high-meat), ↑ *N*-acetyl-5-hydroxytryptamine (high-meat)	markers of protein intake	
↑ *p*-hydroxyphenylacetic acid (vegetarian)	metabolite from microbiota	
↓ N^6,N^6,3N^6-trimethyllysine (vegetarian)	↑ synthesis of carnitine from N^6,N^6,N^6-trimethyllysine	
↑ acetylcarnitine (high-meat), ↑ glutamine (high-meat)		
↑ lactate	↑ anaerobic metabolism/↓ gluconeogenesis	37
↓ carbohydrate concentrations	↑ anaerobic metabolism	
subject-specific changes in isoleucine, valine, triglycerols, choline, acetate, acetoacetate, and alanine	changes in carbohydrate and energy metabolism	
↑ *N*-acetyl glycoproteins	changes in protein turnover	
↑ 3-hydroxybutyrate, changes in lipoproteins		
↑ trimethylamine-*N*-oxide	improved glomerular function or general kidney function/ metabolite from microbiota	38
changes in choline and betaine	improved glomerular function or general kidney function/ metabolite from microbiota/changes in lipid and cholesterol metabolism and transport	
changes in creatinine and creatine	improved glomerular function or general kidney function	
↑ glutamate, ↑ glutamine	changes in tricarboxylic acid cycle/↑ protein breakdown	
↓ citrate, changes in sugar region	↓ glycolysis	
changes in methylamines, glycine, and acetate	changes in lipid and cholesterol metabolism and transport	
↑ hippuric acid	metabolite from microbiota	39
↑ unknown metabolite	metabolite from chamomile	
↓ creatinine	↑ antioxidative activity	
↑ glycine		
↑ hippuric acid, ↑ 1,3-dihydroxyphenyl-2-*O*-sulfate	metabolites from microbiota	40
↑ gallic acid (tentative identification), ↑ para-substituted aromatic compound		
urine: ↓ tyrosine (black tea), ↓ alanine (black tea), ↓ glutamine/glutamate, ↑ taurine (black tea), ↑ glycine, ↑ valine, ↑ methionine (black tea), ↑ pyruvate, ↑ α-ketoglutarate, ↑ glucose (black tea), ↑ betaine (green tea), ↑ dimethylamine, ↑ *N*-acetyl glycoproteins; changes in unknown metabolites		54
↑ hippuric acid, ↑ 1,3-dihydroxyphenyl-2-*O*-sulfate, ↑ unidentified aromatic compounds (green tea)	metabolites from microbiota	
↑ citrate (green tea), ↑ succinate, ↑ oxaloacetate (green tea), ↑ 2-oxoglutarate	↑ oxidative energy metabolism/changes in fatty acid biosynthesis, gluconeogenesis, and glyceroneogenesis/↑ insulin secretion	
↑ β-hydroxybutyrate (black tea)	changes in ketogenesis and fatty acid oxidation	
plasma: ↓ acetate (black tea), ↑ acetate (green tea), ↑ β-hydroxybutyrate (green tea); changes in lipoprotein distribution		
↓ glucose	↑ insulin activity	
↓ lactate (green tea), ↓ alanine (green tea)	↓ anaerobic glycolysis	
↑ hippuric acid, ↑ 4-hydroxyhippuric acid, ↑ 1,3-dihydroxyphenyl-2-*O*-sulfate	metabolites from microbiota	41
↑ 6-amino-5-[*N*-methylformylamino]-1-methyluracil, ↑ methyluric acids, ↑ 3,7-dimethyluric acid, ↑ methylxanthines, ↑ theobromine, ↑ caffeine	purine alkaloid derivatives	42
↑ vanillic acid, ↑ vanilloylglicine, ↑ epicatechin-*O*-sulfate, ↑ *O*-methylepicatechin	markers of cocoa or chocolate intake	
↑ 4-hydroxy-5-(3,4-dihydroxyphenyl)valeric acid, ↑ phenylvalerolactone derivatives	metabolites from microbiota	
↑ 3,5-diethyl-2-methylpyrazine, ↑ hydroxyacetophenone, ↑ cocoa diketopiperazines	cocoa flavor and taste compounds	
↑ hydroxynicotinic acid, ↑ trigonelline	nicotinic acid derivatives	
↑ tyrosine	amino acid from cocoa	
↑ flavonoid conjugates, ↑ phenylvalerolactones, ↑ 4-hydroxy-5-(phenyl)valeric acid conjugates, ↑ hydroxyphenylacetic acid conjugates, ↑ hydroxyphenylpropionic acid conjugates, ↑ vallinic acid glucuronide, ↑ hydroxyhippuric acid, ↑ ferulic acid glucuronide	metabolites from microbiota and almond skin extract	43
↑ 10-hydroxydecene-4,6-diynoic acid sulfate, ↑ tridecadienoic/tridecynoic acid glucuronide, ↑ dodecanedioic acid	↑ β-oxidative metabolism and turnover	52
↑ pyrogallol sulfate, ↑ *p*-coumaryl alcohol glucuronide, ↑ *p*-coumaryl alcohol sulfate, ↑ conjugates of urolithin A	metabolites from microbiota	
↑ *N*-acetylserotonin sulfate, ↑ hydroxyindolacetic acid	tryptophan/serotonin metabolites	
↑ hippuric acid	marker of benzoic acid intake/metabolite from microbiota	44
↓ methylhistidine	marker of myofibrillar protein turnover	
↓ creatinine	changes in muscle metabolism	
↑ unknown metabolite		
↑ SMCSO, ↑ 3 metabolites structurally related to SMCSO	markers of cruciferous vegetable intake	45
↑ hippuric acid, ↑ 4-hydroxyhippuric acid, ↑ 4-hydroxyphenylacetic acid, ↑ unknown aromatic metabolite	metabolites from microbiota	49
↑ formic acid, ↓ alanine, ↓ unknown metabolite		
↑ 3- and 4-hydroxyhippuric acid, ↑ 3-hydroxyphenylpropionic acid, ↑ 3- and 4-hydroxyphenylacetic acid, ↑ hippuric acid (mix)	metabolites from microbiota	50
↑ citrate (mix)	changes in mitochondrial tricarboxylic acid cycle turnover	
↑ betaine (mix)	changes in homocysteine homeostasis	

Table 2. continued

effects on metabolome[a]	biological interpretation[b]	ref
↓ isobutyrate; changes in unknown metabolites	↓ protein fermentation/modulation of the gut microbial ecology	51
↑ fructose 6-phosphate, ↑ lactic acid	↑ glycolysis/↑ antioxidative capacity	48
↑ sphingosine 1-phosphate	↑ pentose phosphate pathway → ↑ normal development of the nervous system	
↑ docosahexaenoic acid	↑ neuroprotection	
↓ hexadecanoic acid	↑ biosynthesis pathway of unsaturated fatty acids	
↑ sebacic acid, ↓ indoleacrylic acid, ↑ L-aspartyl-L-phenylalanine, ↓ harderoporphyrin, ↑ glycolithocholate, ↑ tryptophan, ↓ C18:2, ↑ C18:3, ↑ C20:1, ↓ C20:3, ↓ C20:4, ↑ C20:5, ↓ C22:4, ↓ C22:5, ↑ C24:1		
↑ 3-hydroxybutyric acid, ↑ acetone	↑ energy metabolism	53
↑ betaine	marker whole grain and bran intake/changes in homocysteine metabolism	
↑ *N*,*N*-dimethylglycine	changes in homocysteine metabolism	
↑ dimethyl sulfone	metabolite from microbiota	
↓ tyrosine, ↓ phenylalanine, ↓ alanine, ↓ glutamate, ↓ lysine, ↓ glycine, ↓ valine, ↓ isoleucine	↑ amino acid assimilation	46
↑ propionate, ↑ butyrate	metabolite from microbiota	
↓ succinate, ↑ acetate, ↑ lactate, changes in unknown metabolites		
urine: ↑ urea (meat), ↑ unknown metabolite (meat)		47
↓ hippuric acid (milk)	metabolites from microbiota	
↑ creatine (meat), ↑ histidine (meat)	markers of meat intake	
serum: minor changes in lipid profile (milk)		
↑ 1-methylhistidine (salmon), ↑ anserine (salmon)	metabolites of histidine and histidine-derived peptides	55
↑ trimethylamine-*N*-oxide (salmon)	degradation product of carnitine/marker of seafood consumption	
↑ ascorbate (broccoli and raspberry)	marker of fruit and vegetable intake	
↑ tetronic acid and derivatives (broccoli)	putative ascorbate catabolism products	
caffeoyl sulfate (raspberry), methylepicatechin sulfate (raspberry)	phase II metabolism products	

[a]Biofluids not specified in studies of this table are in Table 1. [b]Biological interpretation corresponds to author's discussions about the effects observed on metabolome including endogenous and exogenous metabolites.

both metabolites could be markers of meat, chicken, or fish intake.[56] Several markers related to cocoa flavor and taste have also been identified.[42] Higher levels of *S*-methyl-L-cysteine sulfoxide (SMCSO) and three other metabolites structurally related to SMCSO were observed after 14 days of a daily consumption of broccoli and Brussels sprouts, and the authors proposed the use of SMCSO as a biomarker of cruciferous vegetable consumption.[45] In another study, consumption of broccoli and raspberries was associated with higher urinary excretion of ascorbate and derivatives of this metabolite, which could be used as markers of fruit and vegetable intake.[55] However, tetronic acids were specific to broccoli intake, whereas caffeoyl sulfate and methylepicatechin sulfate were characteristics of raspberry consumption. In a recent publication, Tulipani et al.[52] identified phase II metabolites of medium-chain polyunsaturated fatty acids (C10−C13), which were the most significant urinary markers following a 12 week nut consumption. Moreover, urinary markers of serotonin metabolism were also related, for the first time, to nut intake, although their origin (dietary or endogenous) remains to be elucidated.[52]

Changes in microbiota-derived metabolites were observed in urine samples after the consumption of vegetables or plant-derived products. In most NMR analyses of urinary metabolome, modifications in hippuric acid excretion have been detected. The excretion pattern of this metabolite showed an increase after the consumption of chamomile,[39] black and green tea,[40,41,54] fruit and vegetable juice,[44] and a mix of wine and grape juice extract.[49,50] Hydroxyhippuric, hydroxyphenylacetic, and hydroxyphenylpropionic acids are other metabolites from dietary polyphenols produced by gut microbiota[57] the urinary concentrations of which have also been raised following the consumption of a vegetarian diet,[36] black tea,[41] almond skin extract,[43] and wine and grape juice extract or grape juice extract.[49,50] Additionally, phenylvaleric acids and phenylvalerolactone have been identified after consumption of food sources of flavan-3-ols.[42,43] Some of these compounds were still being excreted in urine 24 h after the intake of a single dose.[42,43] Other microbial-derived metabolites, such as conjugates of urolithin A, were detected in the urinary metabolome after the intake of nuts.[52] In other studies, which are not included in this review, these metabolites have also been detected after the intake of cocoa, almonds, or nuts.[58,59]

With regard to endogenous metabolite alterations, Solanky et al.[37,38] suggested that soy isoflavones had an inhibitory effect on glycolysis associated with a general alteration in energy metabolism. More changes in carbohydrate and lipid metabolism have been detected with the intake of green and black tea[54] and fortified wheat flour.[48] Alterations in amino acid metabolism were determined following the consumption of nuts,[52] a fruit and vegetable drink,[44] and a synbiotic product.[46] It is also important to bear in mind that similar food items with different chemical compositions could have different effects on the modifications of metabolome. In this field, Solanky et al.[38] observed a difference in the magnitude of the biological effects of soy isoflavones depending on their conjugation. Furthermore, in the study of van Dorsten et al.,[50] the effects observed on the urinary metabolome following consumption of grape juice extract or a mix of wine and grape juice extract differed slightly, suggesting that a small change in the phytochemical composition of a food item could have a different impact on the metabolome.

Diet Exposure and Food Consumption Monitoring. As depicted in Figure 2b, this strategy focuses on applying the

metabolomic approach to explore the relationship between dietary information and metabolic profiles in producing new insights (biomarkers) that allow the intake of a particular dietary pattern or component to be assessed. It is important to note that this approach could be applied in interventional studies and in observational studies in which participants are in free-living conditions with ad libitum diets.

In large epidemiological studies in which it is possible to obtain a large amount of information about dietary habits (e.g., either food frequency questionnaires (FFQs) or 24 h recall), metabolomics may be used to evaluate this information.[33] Pere-Trepat et al.[60] described a strategy to discriminate between different dietary patterns and ^{1}H NMR plasma metabolic profiles in a cohort of Danish twins. After a principal component analysis (PCA) of FFQs, five dietary patterns emerged: energy intake, plant versus animal-based diet, "traditional diet" versus sugar-rich diet, "traditional" versus "modern" diets, and consumption of skimmed versus whole dairy products. Moreover, metabolic phenotypes related to the dietary patterns obtained by partial least squares discriminant analysis (PLS-DA) were essentially based on differences in lipids and amino acid profiles in plasma.[60] Another recent study also focused on the relationship between dietary patterns and metabolomic profiles.[61] Recorded 3 day food diaries and biofluids (plasma and urine) from 160 subjects were collected in a double-blind, randomized, placebo-controlled dietary intervention study. The authors analyzed the data by *K*-means clustering for dietary information and ^{1}H NMR for biofluids. Among the results, ^{1}H NMR spectra of urine allowed the identification of metabolites associated with different dietary patterns. In addition, the authors identified several biomarkers of particular food groups, for instance, phenylacetylglutamine for vegetable intake.[61]

With regard to those studies related to the application of metabolomics to identify a particular biomarker or biomarker pattern that could help us to check the adherence to the intervention in large epidemiological studies or the free-living population intake of a particular dietary component, Heinzmann et al.[62] have developed a strategy for food biomarker discovery that combines nutritional intervention with metabolic phenotyping and biomarker validation in a large-scale epidemiological study. The authors identified the urinary excretion of proline betaine (a metabolite from citrus) as a specific and sensitive biomarker of citrus fruit intake. First, they identified proline betaine as a urinary biomarker of citrus consumption in a study with eight participants. Then the authors quantified the relative concentrations of proline betaine in citrus products and evaluated the urinary excretion profile after orange juice consumption. Finally, the validation was carried out, generating a receiver operating characteristic (ROC) curve in U.K. participants in the INTERMAP study in which citrus intake was established from four 24 h dietary recalls per person. The area under the curve (AUC) was 92.3% with a specificity and sensitivity of 90.6% and 86.3%, respectively. The platform selected to produce the untargeted urinary metabolic profiles was ^{1}H NMR.[62] A strong relationship between citrus intake and excretion of proline betaine has also been observed by Lloyd et al.[63] In this work, the authors used nontargeted metabolite urinary fingerprinting (FIE-MS and FT-ICR-MS) to study the relationships between citrus exposure in free-living human subjects, estimated by a FFQ, and the chemical content of urine. The results showed that an acute intake of orange juice resulted in the appearance of proline betaine and several biotransformed metabolites in postprandial urine. In addition, a process of biomarker validation showed sensitivities of 80.8−92.2% and specificities of 74.2−94.1% (false discovery rate-adjusted P values < 0.05) for elevated proline betaine in those volunteers who reported a high consumption of citrus.[63]

Health Phenotype and Metabolic Impact of Diet. An overall representation of this strategy is illustrated in Figure 2c. Usually, nutritional studies collect anthropometrical, biochemical, and medical data (i.e., health data) that could be used to split the population into phenotypes, allowing a deeper study of either the dietary intervention or dietary pattern effect. Selection of phenotypes could be carried out intuitively, for instance, by selecting only those who were diabetic and obese from the obese population and then studying the effect of a particular food consumption, or it could be achieved by using unsupervised multiparametric statistical algorithms such as PCA or *K*-means clustering.[64]

In this context, Martin et al.[65] studied the metabolic responses of free-living subjects (n = 30), classified into low- and high-anxiety trait phenotypes, to a daily consumption of 40 g of dark chocolate for up to 14 days. Plasma and urine samples were collected at the beginning, in the middle, and at the end of a 2 week study and were analyzed by NMR and MS. Results indicated that a daily intake of chocolate was strongly dependent on the dispositional stress state of the individuals, as noted with statistically significant metabolic effects only in subjects with a high-anxiety trait. In fact, intake of dark chocolate resulted in the diminution of the levels of catecholamines and cortisol in the urine from subjects with high dispositional stress.[65] In another study, O'Sullivan et al.[64] applied this kind of strategy in a double-blind, randomized, placebo-controlled dietary intervention to study whether metabolic phenotyping could identify responders to vitamin D supplementation in terms of the metabolic syndrome. After 4 weeks of intervention with 15 g of vitamin D or placebo, a *K*-means clustering of basal biochemical parameters of metabolic syndrome was carried out. With this analysis, authors defined five metabolic phenotypes with different biochemical profiles. In addition, a ^{1}H NMR analysis of urine and plasma was performed. First results did not show any significant effects of vitamin D supplementation on biomarkers of metabolic syndrome. However, fasting insulin concentrations, homeostatic model assessment (HOMA) score, and C-reactive protein (CRP) concentrations were significantly reduced following supplementation with vitamin D in the metabotype characterized by low concentrations of vitamin D and altered adipokine profile.[64]

Other Studies. As described above, this category groups several studies showing particular approaches to the application of metabolomics in nutritional studies. Population-based studies in the field of nutrimetabolomics showed differences in the urinary metabolome among subjects from different geographic zones,[66−69] leading to the hypothesis that diet greatly influences the endogenous urinary metabolome.[66] After analysis of the urinary metabolome of four populations (Japan, China, United States, and United Kingdom) from the INTERMAP study, two metabolic phenotypes (i.e., metabotypes) were differentiated.[67] Each corresponded to East Asian and Western populations, showing contrasting dietary patterns between them. Furthermore, two differentiated metabotypes among Chinese populations were observed.[67,68] In line with these results, previous analyses of these populations showed

differences in metabolite profiles among them.[69] Thus, consumption information should be available to allow successful interpretation of metabolomic data.[66]

The major subgroup of this heterogeneous category involves a wide range of study designs. They include novel approaches ranging from the integration of high-dimensional biological data[70] to the exploration of a new biomarker concept around the connection between exposition and disease,[71] through the evaluation of associations between metabotypes and dietary preferences,[72] the metabolic responses induced by a nutritional intervention following physical exercise,[73,74] or the comparison of the extent of dietary and physiological effects on the urinary metabolome.[75] Bakker et al.[70] proposed the simultaneous study of biochemical parameters, gene expression, proteins, and metabolites in a nutritional intervention to study the effects of specific dietary components in overweight men. This global approach allows an integrated biological interpretation of the data obtained to be carried out, with the aim of studying the molecular processes involved in a particular health or disease status. Chadeau-Hyam et al.[71] applied a "meet-in-the-middle" approach in a subcohort of the EPIC study. This approach was focused on the identification of putative intermediate metabolic biomarkers related to both exposure and disease outcomes, with the aim of reinforcing the knowledge of dietary factors that influence disease development. In the analyzed EPIC cohort, authors identified plasma metabolomic signatures linking colon cancer with dietary fiber. The strategy of Rezzi et al.[72] showed different metabotypes according to chocolate preferences independent of the ingested food. With these observations, the authors suggested that specific dietary preferences may influence metabolic status and gut microbiota, which may have long-term consequences on health status. Finally, Xu et al.[75] explored the differences in the magnitude of the effect of diet and gender on the metabolic urinary profiles of men and women who followed a lactovegetarian or omnivorous diet. Researchers concluded that their results indicated that food patterns played a larger role in the metabolome than gender.

As well as these examples of nutrimetabolomic studies, we have included in this category a subgroup of recent works focused on the development and validation of methods and protocols in nutrimetabolomic studies. In this context, a three-step validation approach was suggested for identifying dietary biomarkers in complex metabolomic data sets influenced by inter- and intraindividual variation.[76] Pereira et al.[77] developed and validated a simple and robust UPLC-MS method for the nutrimetabolomic analysis of human plasma samples. They tested and compared five methods including three protein precipitation procedures and two anticoagulants. Their results showed that the method utilizing heparin as an anticoagulant and protein precipitation with methanol provided the most reproducible results. Fave et al.[78] proposed a standardized protocol designed to unify experimental strategies in the study of intake biomarkers with the aim of minimizing the potential confounding effects of pathophysiological and environmental factors. They described a robust and reproducible protocol for analyzing urinary metabolite profiles from individuals in fasting and different feeding statuses (during the period between the evening meal and breakfast and after food ingestion), suggesting that this could be a tool for obtaining data from habitual diet or specific food consumption. The novelty of this study is that this protocol would help to obtain homogeneous data in nutrimetabolomic studies and would facilitate the comparison of results obtained in different studies.[78]

Summary of Nutrimetabolomics Studies Findings. Modifications on food metabolome included variations in the concentrations of metabolites directly derived from the ingestion of food components and from human gut microbiota. These metabolites could be used as isolated biomarkers, or biomarker patterns, of the exposure to specific dietary components, foods, or dietary patterns to discover the actual ingestion of a subject or population. Most published studies refer to the analysis of changes or differences in metabolome according to nutritional data. To obtain more specific details of potential biomarkers of intake, a second approach for nutrimetabolomic studies has been described. Along with this metabolomic approach, some intake biomarkers of specific foods have been proposed, for instance, proline betaine (marker of citrus consumption). The strong relationship between citrus intake and this metabolite has been confirmed in different population samples by different research groups, validating its biological robustness. However, in most cases the corresponding validation required to evaluate its specificity and sensitivity is missing. Therefore, it might be concluded that the research of food metabolome biomarkers in the nutrimetabolomic field is currently in the previously described first stage, that is, the discovery phase. To improve both phases, the extensive bibliography derived from published studies about metabolism and bioavailability should be taken into account. Additionally, because the nontargeted fingerprinting approach is first used to discover novel biomarker candidates of intake and health effects, it is also necessary to implement a targeted approach postdiscovery phase with the aim of obtaining more detailed data of these potential biomarkers. Furthermore, in the scientific bibliography we can also find studies demonstrating that the metabotype could have an impact on nutritional responses.

Alterations in carbohydrate, lipid, amino acid, and/or energy metabolism have been detected through the increase or reduction of endogenous metabolites. These metabolites could be potential biomarkers of the effects on human health that exert a nutritional intervention, showing more specific data about the relationship between diet and pathophysiological states. The generated biomarkers could be used globally in all subjects or specifically for a target population group according to phenotyping criteria.

CURRENT LIMITATIONS

The application of nutrimetabolomics in large epidemiological studies opens new perspectives in the field of food metabolome biomarker discovery and validation. An important point in these studies is that very often it is necessary to realize that it is not possible to obtain a homogeneous metabolome sample collection, mainly because the metabolome will be different as a result of different factors, for example, the elapsed time since the last meal. This point is especially significant in observational studies in which there are no restrictions or parameters such as diet or food intake.

Most of the analytical methods used in nutrimetabolomic studies have been NMR spectroscopy, which is characterized by a high reproducibility but with a limited sensitivity, followed by MS techniques, which are more sensitive. Therefore, there is no analytical platform that is capable of identifying all of the metabolites present in a metabolome. Furthermore, there are still unknown signals, and currently it is still difficult to unify data from different nutrimetabolomic studies.

PERSPECTIVES

Differences in metabolome have been observed among different populations, leading to the hypothesis that food patterns could have a key role. Although the application of metabolomics in the nutritional field is quite recent, numerous studies suggesting different approaches have been published. The most used approach until now is that which assesses the changes observed in the endogenous and food metabolome after a nutritional intervention.

The bibliographic data about the excretion behavior of microbiota metabolites (present in urine even after 24 h of a single-dose intake) could be a new source of food intake biomarkers. Therefore, increasing the ability to identify these metabolites, for example, by using highly specialized bioinformatic tools, could open new windows in the field of monitoring the consumption of certain foods as a prerequisite for its association with health. An important advantage of this hypothesis is that some gut microbial metabolites are quite specific for particular compounds (i.e., urolithin for ellagitannins or hydroxyphenylvalerolactones for flavan-3-ols). Even if we consider that several dietary sources provide flavan-3-ols (i.e., tea or cocoa), the phenylvalerolactone and phenylvaleric acid profiles could discriminate between tea and cocoa consumers.

It is also important to extend the understanding of the role and weight exerted by endogenous factors such as gender, age, circadian rhythm, lifestyle, and microbiota. This knowledge will enable researchers to take these factors into account and thus reduce the intra- and interindividual variations of metabolic profiles. In this way, with the characterization of the physiological endogenous metabolome (i.e., metabotype), researchers will be able to classify individuals in accordance with their metabolite concentrations. More specifically in nutritional sciences, it is interesting to have a complete description of the metabolites present in humans depending on their nutritional status or food pattern. Therefore, future nutrimetabolomic studies should use standardized diets and well-defined populations in randomized, placebo-controlled, and double-blind crossover trials. This fact will also help in the evaluation of the prognostic and development of new therapeutic nutritional strategies. At the same time, it is also necessary to improve the technology around metabolomics.

This evolution will allow metabolomics to be introduced in the personalized nutritional field, with the aim of either recommending the best dietary choices for maintaining health or implementing a particular nutritional risk-lowering or therapeutic treatment, as well as to measure the compliance and response of dietary treatments. Furthermore, with technology advances their efficiency and precision will be enhanced, and therefore they will become more economical. These facts will facilitate the use of metabolomics in personalized nutrition. Personalized dietary recommendations are usually associated with the employment of functional foods, and the application of metabolomics in the functional food sector is also interesting. It can be used in the improvement of traditional products or in the development of new ones, with the aim of adapting their nutritional value to the requirements of specific consumer groups. The nutritional properties and health benefits of these products could be investigated, and the derived information would enable the reporting of nutritional and health claims.

AUTHOR INFORMATION

Corresponding Author

*Phone: +34 93 403 4840. Fax: +34 93 403 5931. E-mail: candres@ub.edu.

Author Contributions

‖Equal contribution.

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ABBREVIATIONS USED

CFU, colony-forming unit; ESI, electrospray ionization; FFQ, food frequency questionnaire; FIE, flow infusion electrospray ionization; FT-ICR, Fourier-transform ion cyclotron resonance; GC, gas chromatography; ^{1}H NMR, hydrogen-1 nuclear magnetic resonance; HPLC, high-performance liquid chromatography; HTA, hypertensive; LC, liquid chromatography; MS, mass spectrometry; PCA, principal component analysis; q, quadrupole; SMCSO, *S*-methyl-L-cysteine sulfoxide; ToF, time-of-flight; UPLC, ultraperformance liquid chromatography.

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