REVIEW

Metabolomics application for the design of an optimal diet

ZAKIRA NAUREEN[1], SIMONE CRISTONI[2], KEVIN DONATO[1,*], MARIA CHIARA MEDORI[3], MICHELE SAMAJA[4],
KAREN L. HERBST[5], BARBARA AQUILANTI[6], VALERIA VELLUTI[6], GIUSEPPINA MATERA[6], FRANCESCO FIORETTI[7],
AMERIGO IACONELLI[6], MARCO ALFONSO PERRONE[8], LORENZO DI GIULIO[9], EMANUELE GREGORACE[8],
PIETRO CHIURAZZI[10,11], SAVINA NODARI[7], STEPHEN THADDEUS CONNELLY[12], MATTEO BERTELLI[1,3,5,13]

[1] MAGI EUREGIO, Bolzano, Italy; [2] ISB Ion Source & Biotechnologies srl, Italy, Bresso, Milano, Italy; [3] MAGI'S LAB, Rovereto
(TN), Italy; [4] MAGI Group, San Felice del Benaco, Brescia, Italy; [5] Total Lipedema Care, Beverly Hills California and Tucson Arizona,
USA; [6] UOSD Medicina Bariatrica, Fondazione Policlinico Agostino Gemelli IRCCS, Rome, Italy; [7] Department of Cardiology,
University of Brescia and ASST "Spedali Civili" Hospital, Brescia, Italy; [8] Department of Cardiology and CardioLab,
University of Rome Tor Vergata, Rome, Italy; [9] Department of Vascular Surgery, University of Rome Tor Vergata, Rome Italy;
[10] Istituto di Medicina Genomica, Università Cattolica del Sacro Cuore, Rome, Italy; [11] UOC Genetica Medica, Fondazione Policlinico
Universitario "A. Gemelli" IRCCS, Rome, Italy; [12] San Francisco Veterans Affairs Health Care System, Department of Oral
& Maxillofacial Surgery, University of California, San Francisco, CA, USA; [13] MAGISNAT, Peachtree Corners (GA), USA

Summary

Precision nutrition is an emerging branch of nutrition science that aims to use modern omics technologies (genomics, proteomics, and metabolomics) to assess an individual's response to specific foods or dietary patterns and thereby determine the most effective diet or lifestyle interventions to prevent or treat specific diseases. Metabolomics is vital to nearly every aspect of precision nutrition. It can be targeted or untargeted, and it has many applications. Indeed, it can be used to comprehensively characterize the thousands of chemicals in foods, identify food by-products in human biofluids or tissues, characterize nutrient deficiencies or excesses, monitor biochemical responses to dietary interventions, track long- or short-term dietary habits, and guide the development of nutritional therapies. Indeed, metabolomics can be coupled with genomics and proteomics to study and advance the field of precision nutrition. Integrating omics with epidemiological and clinical data will begin to define the beneficial effects of human food metabolites. In this review, we present the metabolome and its relationship to precision nutrition. Moreover, we describe the different techniques used in metabolomics and present how metabolomics has been applied to advance the field of precision nutrition by providing notable examples and cases.

Introduction

Metabolomics is the study of small metabolites – such as amino acids, nucleic acids, lipids, or carbohydrates – and complex secondary metabolites present in biological systems inside the cells or extracellular fluids. Metabolomics uses analytical techniques, such as liquid or gas chromatography (LC or GC), mass spectrometry (MS), liquid chromatography-mass spectrometry (LC-MS), Fourier transformed infra-red spectroscopy (FTIR), and nuclear magnetic resonance (NMR) spectroscopy to create metabolomic profiles that enable identification and relative quantification of metabolites at a given time [1]. Metabolomic profiles are simply the secondary metabolites produced under extra- and intracellular environmental conditions. Qualitative and quantitative metabolomics compares and identifies specific metabolites produced under specific stimuli and generates metabolomic profiles based on various statistical methods. Metabolomics is becoming increasingly popular in nutritional research and provides a wealth of biological data, dependent upon intake of a specific diet, dietary pattern, age, gender, lifestyle, and health status [2]. Metabolomic approaches are deployed in nutritional research for the identification of metabolites in human bodies in response to certain dietary food regimens [3, 4], and the resulting metabolomic profiles are used to design personalized dietary and lifestyle interventions to improve overall health.

Precision nutrition

Precision nutrition is one of the most promising branches of nutritional sciences, combining genomics, proteomics, and metabolomics to identify an individual's metabotype and response to their dietary intake and lifestyle pattern [5]. Based on an individual's metabotype, tailor-made dietary regimes and physical activity plans are suggested to prevent or cure specific pathophysiological condition [6]. In addition, metabolomics is used to characterize thousands of chemical constituents of foods, to monitor biochemical response to specific food intake, to recognise food by-products in human tissues or biofluids, to identify nutrient deficiencies, to track dietary habits, and to devise nutritional regimes based on all obtained data [7, 8].

Metabolomics and Nutritional Science

Metabolomics is the branch of analytical chemistry that studies metabolites, which are small biological molecules (molecular weight under 1,500 Da) found in cells, tissues, and/or biological fluids. Metabolomics is different than other omics sciences because it uses a variety of sophisticated instrumentation to obtain detailed information about metabolites. In contrast, transcriptomics, proteomics, or genomics are single instrument-based techniques, and therefore metabolomics provides a better insight into biological data [9]. Over the past decade, three main spectroscopic techniques have emerged as well-known metabolomics pillars: nuclear magnetic resonance (NMR), gas chromatography-mass spectrometry (GC-MS), and liquid chromatography-mass spectrometry (LC-MS) [10]. NMR identifies and quantifies high-abundance molecules, whereas the other two techniques are good at quantifying and detecting low-abundance metabolite molecules [11]. Overall, these spectroscopic techniques are the backbone for the identification of organic compounds, such as amino acids, lipids, organic acids, and amines. The myriad of literature available on metabolomics studies using these sensitive analytical advanced techniques authenticates their sophistication and diversity [10-12].

Targeted and untargeted metabolomics

Generally, metabolomics approaches are classified as targeted and untargeted. As stated by its name, targeted metabolomics deals with the identification of selected metabolites via cross comparison with their known standards, which in turn facilitates in developing biomarkers or hypotheses testing [13]. On the other hand, untargeted metabolomics mainly focuses on the discovery of novel, yet unknown compounds [14]. Given the high demand and rapidly growing interest in the identification and quantification of biologically active compounds, targeted metabolomics has a broad spectrum of applications, mainly in the diet and nutrition sector [13, 15], for example to identify nutritional disorders or deficiencies [16] and biomarkers of food intake (BFIs) [17], to analyze food composition, to estimate dietary intake [18] and to provide appropriate recommendations for chronic disease management [19].

Metabolomic tools and techniques

A large variety of spectroscopic techniques are employed as conventional characterization platforms in metabolomics studies, including Fourier transformed infrared (FT-IR) spectroscopy [20], high-performance liquid chromatography (HPLC) [15], mass spectrometry (MS) [21], and nuclear magnetic resonance (NMR) spectroscopy [22]. Ultra-performance LC (UPLC) is an advancement of conventional HPLC that operates at higher pressure, offering 2-3 times enhanced spectral sensitivity over conventional HPLC, alongside short measurement times and small analyte quantity requirement [23]. Mass spectrometry is sensitive in the detection of negligible analyte concentrations, but it requires laborious preliminary separation steps using GC/LC tools. On the other hand, NMR is preferred over the other spectroscopic techniques due to its non-destructive nature, high reproducibility, sample preparation feasibility, and both qualitative and quantitative modes of sample identification [24]. Nonetheless, weak NMR signal sensitivity in case of multicomponent analyte analysis is its main limitation [25], but it can be reduced by using cryogenically cooled probes, microprobes, and/or the dynamic nuclear polarization approach [26]. Overall, the huge diversity of metabolite structures – in terms of concentration, polarity, size, and stability – prevents the collective analysis of all metabolites using only one or two analytical techniques. Therefore, sequential coupling of different techniques has been proven to be beneficial to improve NMR signal. Currently, the main limitation of these coupled techniques is their cost-effectiveness, even though they will probably become the most prevalent metabolomics approach in future [27].

Metabolomics and comprehensive food characterization

The aim of precision nutrition revolves around the basic understanding of food composition and its correlated health benefits. Conventionally, food composition is analyzed in terms of macronutrient and essential nutrient content, but also by exploiting national food company databases (such as USDA or Health Canada) [28]. The few reported essential nutrients, however, do not cover the full spectrum of food composition, which refers to the micronutrient profile of a food product. Generally, the average fruit or vegetable consists of a cocktail of over 15,000 different components, belonging to over 100 chemical classes in variable concentration, ranging from 10^{-12}M (vitamins) to 10^{-3}M (sugars) [28]. These micronutrients impart basic properties to the food, including health benefits, food aroma, flavor, and color, which are due to polyphenols, terpenes, and pigments [29]. Metabolomics helps elucidate micronutrients present in food, thus enhancing our knowledge of various food constituents.

MS-NMR coupled spectroscopic metabolomics studies on a wide variety of foods (such as milk, banana, wine, beer, rice, tomato) have identified a vast majority of previously unknown nutrient species [10]. Moreover, these studies helped in developing food-nutrients/metabolome databases, including Phenol-Explorer, PhytoHub, and FooDB (Tab. I) [10, 30]. The statistics of these food metabolome databases is as follows:
- Phenol-Explorer: 501 polyphenols from 459 food varieties;
- PhytoHub: > 1,800 phytochemicals from 356 food varieties;
- FooDB, > 71,000 chemicals in nearly 800 food varieties [31].

All these databases act as a guide for nutrition scientists to develop precision nutrition and to understand the nutritional dynamics required to maximize expected health benefits.

Tab. I. Metabolite databases and repositories related to the food metabolome.

Database/ Repository	Website	Types of metabolites	Number of metabolites or foods	References
Food Metabolome Repository	http://metabolites.in/foods/	Food metabolites identified using LC-MS	222 food items analysed via LC-MS	[43]
FsDatabase	http://www.kazusa.or.jp/komics/en/tool-en/218-fstool.html	Flavonoids	6,867	[43]
HMDB	www.hmdb.ca	Microbial transformed Endogenous, and exogenous/ xenobiotic compounds identified in humans	Over 40,000	[42]
Exopome Explorer 2	www.ecmdb.ca	Dietary and pollution biomarkers	908	[40, 44]
FooDB	www.foodb.ca	Food constituents and additives	28,000	[28]
Phenol-Explorer	www.phenol-explorer.eu	Polyphenols in the diet	502	[45]
PhytoHub	www.phytohub.eu	Phytochemi-cals and their metabolites in the diet	1,500	[46]

FOOD METABOLOME

The term 'food metabolome' refers to the collection of all the metabolites of food that are derived by ingestion, digestion, and absorption. The term is broadly coined as 'human-food metabolome,' because humans consume a maximal amount of food metabolites [32]. Food consumed by humans contains approximately 25,000 compounds, which get further metabolized after ingestion, creating a complicated and extensive array of molecules [32, 33]. Nonetheless, the great diversity in human food metabolites is the biggest challenge in characterizing them completely: it can only be done by accurately monitoring dietary intake and any health effects defined in epidemiological and clinical investigations.

The food metabolome as part of the human metabolome

Human metabolomes are highly complex and vary depending on several factors, such as diet, health status, gender, age, genetic makeup, and physiology of an individual [34]. This is because humans, unlike laboratory animals, are free-living omnivores, and are exposed to multiple environments associated with a tremendous variety of ingested foods. Hence, the human metabolome comprises four different categories: endogenous metabolome (chemicals linked with cellular metabolism), food metabolome (derived from foodstuff), xenobiotics linked with drugs, and xenobiotics linked with environmental chemicals. The exact composition of the human metabolome is hard to ascertain; at least 50,000 detectable compounds have been identified in the human metabolome to date [35]. The composition of the human metabolome also varies depending on the type of biofluid and/or body part to which it is sampled from. For instance, the chemical composition of oral or gastric compounds is identical to the chemicals extracted from ingested food or drugs, whereas food constituents found in urine and blood are entirely different from the parent compounds because they get further metabolized

into secondary metabolites in the liver, kidneys, or intestines. Sometimes the parent compounds get extensively metabolized and thus turn into end products, which are similar to chemicals naturally produced by the body. In addition, the gut microbiota is a massive contributor to the composition of the human metabolome [36]. Typically, vitamins, certain amino acids, and fatty acids are specific microbial metabolites; however, there are other metabolites derived from biotransformation of both endogenous and food metabolomes by the gut microbiota. Gut microbiota-mediated metabolites include secondary bile acids, amino acid metabolites, short-chain fatty acids, and plant polyphenol metabolites [37].

METABOLOMICS AND DIETARY BIOMARKERS

One of the important preludes of precision nutrition is to have a detailed understanding of an individual's diet and overall dietary status. Traditionally, assessing the nutritional status of an individual was done using several means, such as nutritional assessments, such as surveys, dietary diaries, 24 h dietary recalls, and food frequency questionnaires; however, these methods present several limitations. These include, but are not limited to, deliberate deception in reporting dietary intake, recall bias, memory lapses, and difficulties in estimating portion sizes. These limitations can lead to incorrect or inconsistent data collection, which leads to ambiguity in identifying dietary biomarkers, thus highlighting the necessity to deploy analytical tools that can correctly measure an individual's dietary intake and facilitate corresponding BFI detection. In this regard, a major initiative was launched in 2013: the so-called Food Biomarker Alliance, also known as FoodBAll [38].

Food Biomarker Alliance (FoodBAll)

The aim of FoodBAll was to use metabolomics in BFI identification and to create an inventory of metabolite biomarkers in biological fluids produced after intaking

a specific food [38]. This inventory helps to elucidate the metabolites produced in the human body as a response to dietary intake as well as overall metabolism. Understanding the metabolism of foods with respect to its type, quantity, and metabolic rate is a key to precision nutrition: such detailed information helps nutritional scientists and dieticians to tailor personalised dietary regimes both for healthy individuals and for patients to improve their overall health and wellbeing.

The members of the FoodBAll consortium identified several BFIs for various classes of foods and developed protocols and definitions for their identification and validation [39]. The BFIs have now been listed in dedicated databases, such as Exposome-Explorer [40], MarkerDB [41], and Human Metabolome Database (HMDB) [42].

Dietary biomarker discovery using dietary patterns

Dietary pattern analysis aids scientists and dieticians in gaining a broader insight into an individual's dietary intake, food preferences, and eating habits. Dietary pattern analysis encompasses the quantities, proportions, variety, and combinations of consumed foods/beverages as well as consumption frequency.

Identification of dietary biomarkers involves the combination of dietary and metabolomic patterns, which are analysed by applying chemometrics coupled with multivariate strategies to develop models for food intake behavior and metabolic patterns [47]. Both supervised and unsupervised methods can be used to identify similarities and differences in detected metabolites. The data are then subjected to various cluster analyses – such as hierarchical clustering – to identify similar groups [48]. Supervised learning methods, such as partial least-squares discriminant analysis (PLS-DA) and partial least squares regression (PLSR), are used to identify food metabolites that act as biomarkers to predict diet-related metabolic patterns [49, 50].

Food metabolites have an influential effect on human health. A person's metabolomic profile reflects the overall metabolic state under a particular environmental/pathophysiological condition and changes with respect to changes in said condition. In addition, an individual's metabolomic profile is dependent upon overall genetic makeup, phenotypic expression of genes, and dietary intake [51]. Here, dietary intake and choice of food play a dual role: not only do they affect the type of metabolites being produced, but they also influence the gut microbial community and the way this microbiota will metabolize the diet [51, 52]. It has been widely accepted now that gut microbiota plays a pivotal role in maintaining overall gut health, and changes in the gut microbiota may give rise to metabolic disorders and initiate or aggravate non-communicable diseases such as obesity, diabetes, or hypertension [53].

The gut microbiota is comprised of 1,000 different species of approximately 10^{14} individual microbes, with a total biomass of 2 kg [54]. These microbes not only help in metabolism and absorption of micronutrients, but they modulate the host's immunity against pathogens as well

[55]. Lactic acid bacteria and Bifidobacterial species residing in the human gut are essential for human health, as they synthesize vitamin K and several B vitamins, like thiamine, biotin, folates, cobalamin, pantothenic acid, nicotinic acid, pyridoxine, and riboflavin [56]. A cross talk exists between human gut microbiota, dietary intake, and the way it is metabolized: for instance, dietary intake influences the microbial community structure in the gut. In turn, the microbial community in the gut affects the way food components are metabolised and absorbed. The result of this cross talk determines the overall metabolomic state of an individual, which greatly influences their health and wellbeing [57]. Moreover, dietary changes affect the functionality of gut microbiota, thus increasing human dietary flexibility. In addition to dietary intake, the microbiota is affected by an individual's genetics, environment including psychology, bacteriophage action, and use of antibiotics or other treatments [58-61].

Untargeted metabolomics has been used to evaluate the physiology and metabolomic profile of gut microbiota in response to intake of dietary supplements (Table II). A recent study has reported the effect of seven dietary supplements on a consortium culture of bacteria containing *Blautia producta*, *Bifidobacterium longum*, *Anaerostipes caccae*, *Clostridium ramosum*, *Bacteroides thetaiotaomicron*, *Clostridium butyricum*, *Lactobacillus plantarum*, and *Escherichia coli*. GC-MS analysis of the dietary supplement's metabolism by the consortium (in comparison to placebo) detected 131 metabolites, which included organic acids, fatty acids, nucleic acids, amino acids (the predominant class of metabolites), phenolic compounds, steroids, sugars, alcohols, and inorganic nitrogenous compounds [54]. The study indicated a modulatory effect of dietary supplements on the microbial community and on gut metabolism, inhibiting or inducing specific metabolic pathways.

Moreover, metabolomics can help identifying food-induced shifts in metabolites, thus providing useful information about an individual's diet. For instance, an intervention study classified two dietary patterns, the New Nordic Diet (NND) and the Average Danish Diet (ADD), using untargeted metabolomics coupled with multivariate analysis, with a low misclassification error rate (19%) [62]. This reveals that untargeted metabolomics can be used as a powerful screening tool to estimate compliance to a certain dietary pattern [62]. A similar study was conducted to explore the effects of an isocaloric Mediterranean diet (MD) intervention on overall metabolic health, systemic metabolome, and gut microbiome in individuals having lifestyle risk factors for metabolic diseases. The results revealed that switching to the Mediterranean diet whilst retaining the overall caloric intake resulted in changes in the gut microbiome as well as the metabolites in urine, with a marked reduction in blood cholesterol levels and an improvement in overall health [63].

In another study, 1H NMR was used to analyse the urinary metabolome of 1,848 Americans, which revealed 46 metabolites that can help differentiate healthy and unhealthy individuals. These metabolites indicated the correlation of vitamin C, glucose, and fructose with

Tab. II. Targeted and untargeted metabolomic approaches for food metabolites biomarker identification.

Techniques	Purposes of the study	Study groups	Findings	References
Targeted Metabolomics				
Fluorescence spectroscopy	Comparative analysis of the effects of dietary levels of proline betaine on glycine betaine excretion, homocysteine, and betaine concentrations in plasma	8 healthy males Age: 18-50	Proline and betaine had little effect on plasma total homocysteine concentrations in healthy humans	[65]
Mass spectrometry	Blood metabolites that correlate red meat consumption to the onset of type 2 diabetes	790 males and 1,257 females, including 801 with type 2 diabetes Age: 35-64	Six biomarkers were linked to elevated red meat consumption and diabetes risk	[66]
Mass spectrometry	Analysis of demographics, dietary habits, and metabotypes	740 males and 760 females Age: 18-90	Two subgroups identified for postprandial insulin levels and fasting metabolic profile	[67]
Targeted mass spectrometry	Effect of Western dietary patterns on metabotypes	16 females and 21 males Age: 18-50	Western dietary pattern with high saturated fat intakes resulted in higher levels of short chain acylcarnitine and amino acids as dietary biomarkers	[68]
Untargeted metabolomics				
NMR	Identification of coffee consumption biomarkers	7 females and 1 male Age: 28-45	Identification of putative biomarker 2-furoylglycine	[69]
Mass spectrometry	Characterization of dietary walnut fingerprinting	275 subjects, both male and female Age: 55-80 (males) and 60-80 (females)	Identification of 18 markers of fatty acid metabolism and intermediate metabolites of the tryptophan/serotonin pathway	[70]
Mass spectrometry	Compliance tool development based on metabotyping strategies to compare Average Danish Diet vs New Nordic Diet for 6 months	79 females and 28 males Age: 18-65	Identification of 22 unique food markers for 7 food groups (chocolate, cabbage, beetroot, citrus, green beans, strawberry, and walnut)	[71]

biomarkers of citrus fruit consumption, such as 2-hydroxy-2-(4-methylcyclohex-3-en-1-yl) propoxy glucuronide, 4-hydroxyprolinebetaine, and proline betaine. In addition, these metabolites highlighted the association of calcium and sodium with citrate, and formate with hypertension, renal function, and adiposity [64].

These studies indicate the potentials of metabolomic approaches in revealing the metabolomic status of an individual in response to dietary intake and pathophysiological conditions, in detecting gut microbial changes, and in applying precision nutrition.

METABOLOMICS AND CUSTOMISED DIET DESIGN

The most impressive use of metabolomics data in nutrition sciences is perhaps the design of tailor-made diets, based on an individual's metabolomic profile, dietary preferences, gut microbiome, lifestyle, and pathophysiological status. In addition to that, the integration of the other omics technologies provides an excellent platform for prevention and management of metabolic disorders like diabetes, obesity, hyperlipidemia, and hypercholesterolemia [72]. For instance, one study reported the integrated use of metagenomics, metabolomics, and precision nutrition coupled with machine learning to devise a dietary regime to manage postprandial blood glucose levels. The algorithm was based on anthropometric data, physical activity, metabolomic-based blood parameters, self-reported dietary intake, and gut microbiome composition of 800 participants considered healthy or prediabetic [73]. A high interpersonal variability was observed in postprandial glycemic responses of participants, irrespective of the fact that they were given the same food. Based on these findings, DayTwo Inc., the first precision nutrition company, was established, with the aim to design custom diets for prevention and control of prediabetes [74].

Similarly, another study used LC-MS based metabolomic profiling on blood samples from 40 healthy adults with normal blood sugar levels to predict their risk of acquiring type 2 diabetes, resistance to insulin, and associated comorbidities. Based on their metabolomic profiling, subjects were given customised diets, nutritional supplements, physical activity, and lifestyle recommendations for 100 days. The follow-up metabolomic analysis after 100 days, showed a significant decline in the risk of developing type 2 diabetes and associated comorbidities [75].

These and similar studies indicate that precision nutrition guided by metabolomic profiling is a promising arena for further research. Thus far, most of the metabolomic studies had focused on identifying metabotypes and metabolomic markers associated with obesity, diabetes, and metabolic disorders. Further studies in this regard will enable scientists and dietitians to design customised diets, based on an individual's metabolomic status for achieving better health and controlling lifestyle-mediated diseases.

Conclusions

Metabolomic studies coupled with genomics, proteomics, and multivariate analysis, provide an excellent platform for new advancements in the field of precision nutrition. Not only does this approach generate a repertoire of novel human food metabolome biomarkers, but it will also greatly enhance the development of molecular nutritional epidemiology, thus contributing to a better prescription of dietary regimes and physical activity for managing a healthy lifestyle and preventing and curing lifestyle-mediated diseases. In addition, precision nutrition not only brings hope for patients suffering from various metabolic disorders, but also provides nutritionists with a tool for designing diets that match the nutritional requirements, metabolic function, and gut microbiota to achieve maximum benefits.

Acknowledgements

Conflicts of interest statement

Authors declare no conflict of interest.

Author's contributions

MB: study conception, editing and critical revision of the manuscript; SC, KD, MCM, MS, KLH, BA, VV, GM, FF, AI, MAP, LDG, EG, PC, SN, STC: literature search, editing and critical revision of the manuscript. All authors have read and approved the final manuscript.

References

[1] Radhakrishnan EK, Ajay Kumar, Aswani R. Biocontrol mechanisms of endophytic microorganisms. Academic Press 2021.

[2] Tang Y, Zhu Y, Sang S. A novel lc-ms based targeted metabolomic approach to study the biomarkers of food intake. Mol Nutr Food Res 2020;64:2000615. https://doi.org/10.1002/mnfr.202000615

[3] Beckmann M, Lloyd AJ, Haldar S, Favé G, Seal CJ, Brandt K, Mathers JC, Draper J. Dietary exposure biomarker-lead discovery based on metabolomics analysis of urine samples. Proc Nutr Soc 2013;72:352-61. https://doi.org/10.1017/S0029665113001237

[4] Zhu Y, Shurlknight KL, Chen X, Sang S. Identification and pharmacokinetics of novel alkylresorcinol metabolites in human urine, new candidate biomarkers for whole-grain wheat and rye intake. J Nutr 2014;144:114-22. https://doi.org/10.3945/jn.113.184663

[5] Pigsborg K, Magkos F. Metabotyping for Precision Nutrition and Weight Management: Hype or Hope? Curr Nutr Rep 2022;11:117-23. https://doi.org/10.1007/s13668-021-00392-y

[6] Hillesheim E, Brennan L. Metabotyping and its role in nutrition research. Nutrition research reviews 2020;33:33-42. https://doi.org/10.1017/S0954422419000179

[7] Palmnäs M, Brunius C, Shi L, Rostgaard-Hansen A, Torres NE, González-Domínguez R, Zamora-Ros R, Ye YL, Halkjær J, Tjønneland A, Riccardi G, Giacco R, Costabile G, Vetrani C, Nielsen J, Andres-Lacueva C, Landberg R. Perspective: Metabotyping-A Potential Personalized Nutrition Strategy for Precision Prevention of Cardiometabolic Disease. Adv Nutr 2020;11:524-32. https://doi.org/10.1093/advances/nmz121

[8] Bush CL, Blumberg JB, El-Sohemy A, Minich DM, Ordovás JM, Reed DG, Behm VAY. Toward the Definition of Personalized Nutrition: A Proposal by The American Nutrition Association. J Am Coll Nutr 2020;39:5-15. https://doi.org/10.1080/07315724.2019.1685332

[9] Dettmer K, Hammock BD. Metabolomics--a new exciting field within the "omics" sciences. Environ Health Perspect 2004;112:A396-7. https://doi.org/10.1289/ehp.112-1241997

[10] Emwas AH, Roy R, McKay RT, Tenori L, Saccenti E, Gowda GAN, Raftery D, Alahmari F, Jaremko L, Jaremko M, Wishart DS. NMR Spectroscopy for Metabolomics Research. Metabolites 2019;9:123. https://doi.org/10.3390/metabo9070123

[11] Diémé B, Lefèvre A, Nadal-Desbarats L, Galineau L, Madji Hounoum B, Montigny F, Blasco H, Andres CR, Emond P, Mavel S. Workflow methodology for rat brain metabolome exploration using NMR, LC-MS and GC-MS analytical platforms. J Pharm Biomed Anal 2017;142:270-8. https://doi.org/10.1016/j.jpba.2017.03.068

[12] Amberg A, Riefke B, Schlotterbeck G, Ross A, Senn H, Dieterle F, Keck M. NMR and MS Methods for Metabolomics. Methods Mol Biol 2017;1641:229-58. https://doi.org/10.1007/978-1-4939-7172-5_13

[13] Cao G, Song Z, Hong Y, Yang Z, Song Y, Chen Z, Chen Z, Cai Z. Large-scale targeted metabolomics method for metabolite profiling of human samples. Anal Chim Acta 2020;1125:144-51. https://doi.org/10.1016/j.aca.2020.05.053

[14] Schrimpe-Rutledge AC, Codreanu SG, Sherrod SD, McLean JA. Untargeted Metabolomics Strategies-Challenges and Emerging Directions. J Am Soc Mass Spectrom 2016;27:1897-905. https://doi.org/10.1007/s13361-016-1469-y

[15] González-Domínguez R, Jáuregui O, Mena P, Hanhineva K, Tinahones FJ, Angelino D, Andrés-Lacueva C. Quantifying the human diet in the crosstalk between nutrition and health by multi-targeted metabolomics of food and microbiota-derived metabolites. Int J Obes (Lond) 2020;44:2372-81. https://doi.org/10.1038/s41366-020-0628-1

[16] Yoon HR. Screening newborns for metabolic disorders based on targeted metabolomics using tandem mass spectrometry. Ann Pediatr Endocrinol Metab 2015;20:119-24. https://doi.org/10.6065/apem.2015.20.3.119

[17] Ulaszewska M, Garcia-Aloy M, Vázquez-Manjarrez N, Soria-Florido MT, Llorach R, Mattivi F, Manach C. Food intake biomarkers for berries and grapes. Genes Nutr 2020;15:17. https://doi.org/10.1186/s12263-020-00675-z

[18] Collins C, McNamara AE, Brennan L. Role of metabolomics in identification of biomarkers related to food intake. Proc Nutr Soc 2019;78:189-96. https://doi.org/10.1017/S002966511900048X

[19] Milošević M, Arsić A, Cvetković Z, Vučić V. Memorable food: fighting age-related neurodegeneration by precision nutrition. Front Nutr 2021;8:688086. https://doi.org/10.3389/fnut.2021.688086

[20] Yusof NA, Isha A, Ismail IS, Khatib A, Shaari K, Abas F, Rukayadi Y. Infrared-metabolomics approach in detecting changes in Andrographis paniculata metabolites due to different harvesting ages and times. J Sci Food Agric 2015;95:2533-43. https://doi.org/10.1002/jsfa.6987

[21] Clarke NJ. Mass Spectrometry in Precision Medicine: Phenotypic Measurements Alongside Pharmacogenomics. Clin Chem. 2016;62:70-6. https://doi.org/10.1373/clinchem.2015.239475

[22] Pandey R, Caflisch L, Lodi A, Brenner AJ, Tiziani S. Metabolomic signature of brain cancer. Mol Carcinog 2017;56:2355-71. https://doi.org/10.1002/mc.22694

[23] Gray N, Lawler NG, Yang R, Morillon AC, Gay MCL, Bong SH, Holmes E, Nicholson JK, Whiley L. A simultaneous exploratory and quantitative amino acid and biogenic amine metabolic profiling platform for rapid disease phenotyping via UPLC-QToF-MS. Talanta 2021;223:121872. https://doi.org/10.1016/j.talanta.2020.121872

[24] Cao R, Liu X, Liu Y, Zhai X, Cao T, Wang A, Qiu J. Applications of nuclear magnetic resonance spectroscopy to the evaluation of complex food constituents. Food Chem 2021;342:128258. https://doi.org/10.1016/j.foodchem.2020.128258

[25] Huang B, Xu L, Wang N, Ying J, Zhao Y, Huang S. trans-4-Fluoro-l-proline: A Sensitive 19F NMR Probe for the rapid simultaneous enantiomeric analysis of multicomponent amines. Anal Chem 2022;94:1867-13. https://doi.org/10.1021/acs.analchem.1c04823

[26] Zhao L, Pinon AC, Emsley L, Rossini AJ. DNP-enhanced solid-state NMR spectroscopy of active pharmaceutical ingredients. Magn Reson Chem 2018;56:583-609. https://doi.org/10.1002/mrc.4688

[27] Soininen P, Kangas AJ, Würtz P, Tukiainen T, Tynkkynen T, Laatikainen R, Järvelin MR, Kähönen M, Lehtimäki T, Viikari J, Raitakari OT, Savolainen MJ, Ala-Korpela M. High-throughput serum NMR metabonomics for cost-effective holistic studies on systemic metabolism. Analyst 2009;134:1781-5. https://doi.org/10.1039/b910205a

[28] Wishart DS. Metabolomics: applications to food science and nutrition research. Trends Food Sci Technol 2008;19:482-93. https://doi.org/10.1016/j.tifs.2008.03.003

[29] Cory H, Passarelli S, Szeto J, Tamez M, Mattei J. The role of polyphenols in human health and food systems: a mini-review. Front Nutr 2018;5:87. https://doi.org/10.3389/fnut.2018.00087

[30] Rothwell JA, Perez-Jimenez J, Neveu V, Medina-Remón A, M'hiri N, García-Lobato P, Manach C, Knox C, Eisner R, Wishart DS, Scalbert A. Phenol-Explorer 3.0: a major update of the Phenol-Explorer database to incorporate data on the effects of food processing on polyphenol content. Database (Oxford) 2013;2013:bat070. https://doi.org/10.1093/database/bat070

[31] Forte G, Bocca B, Senofonte O, Petrucci F, Brusa L, Stanzione P, Zannino S, Violante N, Alimonti A, Sancesario G. Trace and major elements in whole blood, serum, cerebrospinal fluid and urine of patients with Parkinson's disease. J Neural Transm (Vienna) 2004;111:1031-40. https://doi.org/10.1007/s00702-004-0124-0

[32] Scalbert A, Brennan L, Manach C, Andres-Lacueva C, Dragsted LO, Draper J, Rappaport SM, van der Hooft JJ, Wishart DS. The food metabolome: a window over dietary exposure. Am J Clin Nutr 2014;99:1286-308. https://doi.org/10.3945/ajcn.113.076133

[33] Khakimov B, Engelsen SB. Resveratrol in the foodomics era: 1:25,000. Ann N Y Acad Sci 2017;1403:48-58. https://doi.org/10.1111/nyas.13425

[34] Holmes E, Loo RL, Stamler J, Bictash M, Yap IK, Chan Q, Ebbels T, De Iorio M, Brown IJ, Veselkov KA, Daviglus ML, Kesteloot H, Ueshima H, Zhao L, Nicholson JK, Elliott P. Human metabolic phenotype diversity and its association with diet and blood pressure. Nature 2008;453:396-400. https://doi.org/10.1038/nature06882

[35] Smith CA, O'Maille G, Want EJ, Qin C, Trauger SA, Brandon TR, Custodio DE, Abagyan R, Siuzdak G. METLIN: a metabolite mass spectral database. Ther Drug Monit 2005;27:747-51. https://doi.org/10.1097/01.ftd.0000179845.53213.39

[36] van der Werf MJ, Overkamp KM, Muilwijk B, Coulier L, Hankemeier T. Microbial metabolomics: toward a platform with full metabolome coverage. Anal Biochem 2007;370:17-25. https://doi.org/10.1016/j.ab.2007.07.022

[37] Nicholson JK, Holmes E, Kinross J, Burcelin R, Gibson G, Jia W, Pettersson S. Host-gut microbiota metabolic interactions. Science 2012;336:1262-7. https://doi.org/10.1126/science.1223813

[38] Brouwer-Brolsma EM, Brennan L, Drevon CA, van Kranen H, Manach C, Dragsted LO, Roche HM, Andres-Lacueva C, Bakker SJL, Bouwman J, Capozzi F, De Saeger S, Gundersen TE, Kolehmainen M, Kulling SE, Landberg R, Linseisen J, Mattivi F, Mensink RP, Scaccini C, Skurk T, Tetens I, Vergeres G, Wishart DS, Scalbert A, Feskens EJM. Combining traditional dietary assessment methods with novel metabolomics techniques: present efforts by the Food Biomarker Alliance. Proc Nutr Soc 2017;76:619-27. https://doi.org/10.1017/S0029665117003949

[39] Gao Q, Praticò G, Scalbert A, Vergères G, Kolehmainen M, Manach C, Brennan L, Afman LA, Wishart DS, Andres-Lacueva C, Garcia-Aloy M, Verhagen H, Feskens EJM, Dragsted LO. A scheme for a flexible classification of dietary and health biomarkers. Genes Nutr 2017;12:34. https://doi.org/10.1186/s12263-017-0587-x

[40] Neveu V, Moussy A, Rouaix H, Wedekind R, Pon A, Knox C, Wishart DS, Scalbert A. Exposome-Explorer: a manually-curated database on biomarkers of exposure to dietary and environmental factors. Nucleic Acids Res 2017;45:D979-84. https://doi.org/10.1093/nar/gkw980

[41] Wishart DS, Bartok B, Oler E, Liang KYH, Budinski Z, Berjanskii M, Guo A, Cao X, Wilson M. MarkerDB: an online database of molecular biomarkers. Nucleic Acids Res 2021;49:D1259-67. https://doi.org/10.1093/nar/gkaa1067

[42] Wishart DS, Feunang YD, Marcu A, Guo AC, Liang K, Vázquez-Fresno R, Sajed T, Johnson D, Li C, Karu N, Sayeeda Z, Lo E, Assempour N, Berjanskii M, Singhal S, Arndt D, Liang Y, Badran H, Grant J, Serra-Cayuela A, Liu Y, Mandal R, Neveu V, Pon A, Knox C, Wilson M, Manach C, Scalbert A. HMDB 4.0: the human metabolome database for 2018. Nucleic Acids Res 2018;46:D608-17. https://doi.org/10.1093/nar/gkx1089

[43] Akimoto N, Ara T, Nakajima D, Suda K, Ikeda C, Takahashi S, Muneto R, Yamada M, Suzuki H, Shibata D, Sakurai N. FlavonoidSearch: A system for comprehensive flavonoid annotation by mass spectrometry. Sci Rep 2017;7:1243. https://doi.org/10.1038/s41598-017-01390-3

[44] Neveu V, Nicolas G, Salek RM, Wishart DS, Scalbert A. Exposome-Explorer 2.0: an update incorporating candidate dietary biomarkers and dietary associations with cancer risk. Nucleic Acids Res 2020;48:D908-12. https://doi.org/10.1093/nar/gkz1009

[45] Rothwell JA, Urpi-Sarda M, Boto-Ordoñez M, Knox C, Llorach R, Eisner R, Cruz J, Neveu V, Wishart D, Manach C, Andres-Lacueva C, Scalbert A. Phenol-Explorer 2.0: a major update of the Phenol-Explorer database integrating data on polyphenol metabolism and pharmacokinetics in humans and experimental animals. Database (Oxford) 2012;2012:bas031. https://doi.org/10.1093/database/bas031

[46] Giacomoni F, Da Silva AL, Bronze M, Gladine C, Hollman P, Kopec R, Yanwen DL, Micheau P, Dos Santos MC, Pavot B, Schmidt G. PhytoHub, an online platform to gather expert knowledge on polyphenols and other dietary phytochemicals. 8. International Conference on Polyphenols and Health (ICPH 2017) 2017. Available at https://hal.archives-ouvertes.fr/hal-01697081. Accessed on: 01/07/2022.

[47] An Y, Feng L, Zhang X, Wang Y, Wang Y, Tao L, Qin Z, Xiao R. Dietary intakes and biomarker patterns of folate, vitamin B6, and vitamin B12 can be associated with cognitive impairment by hypermethylation of redox-related genes NUDT15 and TXNRD1. Clin Epigenetics 2019;11:139. https://doi.org/10.1186/s13148-019-0741-y

[48] Hanhineva K, Barri T, Kolehmainen M, Pekkinen J, Pihlajamäki J, Vesterbacka A, Solano-Aguilar G, Mykkänen H, Dragsted LO, Urban JF Jr, Poutanen K. Comparative nontargeted profiling of metabolic changes in tissues and biofluids in high-fat diet-fed Ossabaw pig. J Proteome Res 2013;12:3980-92. https://doi.org/10.1021/pr400257d

[49] Kjeldahl K Bro R. Some common misunderstandings in chemometrics. J Chemometr 2010;24:558-64. https://doi.org/10.1002/cem.1346

[50] Wold S, Martens H, Wold H, Ruhe A, Kågstrøm B, eds. The multivariate calibration problem in chemistry solved by the PLS methods. In: Ruhe A Kågstrøm B, eds. Proceedings of the Conference on Matrix Pencils, Lecture Notes in Mathematics. Heidelberg, Germany: Springer Verlag 1983:286-93.

[51] Guo Y, Bian X, Liu J, Zhu M, Li L, Yao T, Tang C, Ravichandran V, Liao P, Papadimitriou K, Yin J. Dietary Components, Microbial

Metabolites and Human Health: Reading between the Lines. Foods 2020;9:1045. https://doi.org/10.3390/foods9081045

[52] Roager HM, Dragsted LO. Diet-derived microbial metabolites in health and disease. Nutrition Bull 2019;44:216-27.. https://doi.org/10.1111/nbu.12396

[53] Danneskiold-Samsøe NB, Dias de Freitas Queiroz Barros H, Santos R, Bicas JL, Cazarin CBB, Madsen L, Kristiansen K, Pastore GM, Brix S, Maróstica Júnior MR. Interplay between food and gut microbiota in health and disease. Food Res Int 2019;115:23-31. https://doi.org/10.1016/j.foodres.2018.07.043

[54] Farag MA, Abdelwareth A, Sallam IE, El Shorbagi M, Jehmlich N, Fritz-Wallace K, Serena Schäpe S, Rolle-Kampczyk U, Ehrlich A, Wessjohann LA, von Bergen M. Metabolomics reveals impact of seven functional foods on metabolic pathways in a gut microbiota model. J Adv Res 2020;23:47-59. https://doi.org/10.1016/j.jare.2020.01.001

[55] Yoo JY, Groer M, Dutra SVO, Sarkar A, McSkimming DI. Gut microbiota and immune system interactions. Microorganisms 2020;8:1587. https://doi.org/10.3390/microorganisms8101587

[56] LeBlanc JG, Milani C, de Giori GS, Sesma F, van Sinderen D, Ventura M. Bacteria as vitamin suppliers to their host: a gut microbiota perspective. Curr Opin Biotechnol. 2013;24:160-8. https://doi.org/10.1016/j.copbio.2012.08.005

[57] Shen J, Obin MS, Zhao L. The gut microbiota, obesity and insulin resistance. Mol Aspects Med 2013;34:39-58. https://doi.org/10.1016/j.mam.2012.11.001

[58] Hasan N, Yang H. Factors affecting the composition of the gut microbiota, and its modulation. PeerJ 2019;7:e7502. https://doi.org/10.7717/peerj.7502

[59] Naureen Z, Dautaj A, Anpilogov K, Camilleri G, Dhuli K, Tanzi B, Maltese PE, Cristofoli F, De Antoni L, Beccari T, Dundar M, Bertelli M. Bacteriophages presence in nature and their role in the natural selection of bacterial populations.Acta Biomed 2020;91:e2020024. https://doi.org/10.23750/abm.v91i13-S.10819

[60] Kiani AK, Anpilogov K, Dhuli K, Paolacci S, Benedetti S, Manara E, Guerri G, Dautaj A, Beccari T, Dundar M, Bertelli M. Naturally-occurring and cultured bacteriophages in human therapy. Eur Rev Med Pharmacol Sci 2021;25:101-7. https://doi.org/10.26355/eurrev_202112_27339

[61] Ingrosso G, Saldi S, Marani S, Wong A, Bertelli M, Aristei C, Zelante T. Breakdown of symbiosis in radiation-induced oral mucositis. J Fungi (Basel) 2021;7:290. https://doi.org/10.3390/jof7040290

[62] Andersen MB, Rinnan Å, Manach C, Poulsen SK, Pujos-Guillot E, Larsen TM, Astrup A, Dragsted LO. Untargeted metabolomics as a screening tool for estimating compliance to a dietary pattern. J Proteome Res 2014;13:1405-18. https://doi.org/10.1021/pr400964s

[63] Meslier V, Laiola M, Roager HM, De Filippis F, Roume H, Quinquis B, Giacco R, Mennella I, Ferracane R, Pons N, Pasolli E, Rivellese A, Dragsted LO, Vitaglione P, Ehrlich SD, Ercolini D. Mediterranean diet intervention in overweight and obese subjects lowers plasma cholesterol and causes changes in the gut microbiome and metabolome independently of energy intake. Gut 2020;69:1258-68. https://doi.org/10.1136/gutjnl-2019-320438

[64] Posma JM, Garcia-Perez I, Frost G, Aljuraiban GS, Chan Q, Van

Horn L, Daviglus M, Stamler J, Holmes E, Elliott P, Nicholson JK. Nutriome-metabolome relationships provide insights into dietary intake and metabolism. Nat Food 2020;1:426-36. https://doi.org/10.1038/s43016-020-0093-y

[65] Atkinson W, Downer P, Lever M, Chambers ST, George PM. Effects of orange juice and proline betaine on glycine betaine and homocysteine in healthy male subjects. Eur J Nutr 2007;46:446-52. https://doi.org/10.1007/s00394-007-0684-5

[66] Wittenbecher C, Mühlenbruch K, Kröger J, Jacobs S, Kuxhaus O, Floegel A, Fritsche A, Pischon T, Prehn C, Adamski J, Joost HG, Boeing H, Schulze MB. Amino acids, lipid metabolites, and ferritin as potential mediators linking red meat consumption to type 2 diabetes. Am J Clin Nutr 2015;101:1241-50. https://doi.org/10.3945/ajcn.114.099150

[67] Li K, Brennan L, McNulty BA, Bloomfield JF, Duff DJ, Devlin NF, Gibney MJ, Flynn A, Walton J, Nugent AP. Plasma fatty acid patterns reflect dietary habits and metabolic health: A cross-sectional study. Mol Nutr Food Res 2016;60:2043-52. https://doi.org/10.1002/mnfr.201500711

[68] Bouchard-Mercier A, Rudkowska I, Lemieux S, Couture P, Vohl MC. The metabolic signature associated with the Western dietary pattern: a cross-sectional study. Nutr J 2013;12:158. https://doi.org/10.1186/1475-2891-12-158

[69] Heinzmann SS, Holmes E, Kochhar S, Nicholson JK, Schmitt-Kopplin P. 2-Furoylglycine as a candidate biomarker of coffee consumption. J Agric Food Chem 2015;63:8615-21. https://doi.org/10.1021/acs.jafc.5b03040

[70] Garcia-Aloy M, Llorach R, Urpi-Sarda M, Tulipani S, Estruch R, Martínez-González MA, Corella D, Fitó M, Ros E, Salas-Salvadó J, Andres-Lacueva C. Novel multimetabolite prediction of walnut consumption by a urinary biomarker model in a free-living population: the PREDIMED study. J Proteome Res 2014;13:3476-83. https://doi.org/10.1021/pr500425r

[71] Andersen MB, Kristensen M, Manach C, Pujos-Guillot E, Poulsen SK, Larsen TM, Astrup A, Dragsted L. Discovery and validation of urinary exposure markers for different plant foods by untargeted metabolomics. Anal Bioanal Chem 2014;406:1829-44. https://doi.org/10.1007/s00216-013-7498-5

[72] de Toro-Martín J, Arsenault BJ, Després JP, Vohl MC. Precision Nutrition: A Review of Personalized Nutritional Approaches for the Prevention and Management of Metabolic Syndrome. Nutrients 2017;9:913. https://doi.org/10.3390/nu9080913

[73] Zeevi D, Korem T, Zmora N, Israeli D, Rothschild D, Weinberger A, Ben-Yacov O, Lador D, Avnit-Sagi T, Lotan-Pompan M, Suez J, Mahdi JA, Matot E, Malka G, Kosower N, Rein M, Zilberman-Schapira G, Dohnalová L, Pevsner-Fischer M, Bikovsky R, Halpern Z, Elinav E, Segal E. Personalized nutrition by prediction of glycemic responses. Cell 2015;163:1079-94. https://doi.org/10.1016/j.cell.2015.11.001

[74] LeVatte M, Keshteli AH, Zarei P, Wishart DS. Applications of Metabolomics to Precision Nutrition. Lifestyle Genom 2022;15:1-9. https://doi.org/10.1159/000518489

[75] Anwar MA, Barrera-Machuca AA, Calderon N, Wang W, Tausan D, Vayali T, Wishart D, Cullis P, Fraser R. Value-based healthcare delivery through metabolomics-based personalized health platform. Healthc Manage Forum 2020;33:126-34. https://doi.org/10.1177/0840470420904733