ANNUAL REVIEWS

Annual Review of Food Science and Technology

Polyphenol Exposure, Metabolism, and Analysis: A Global Exposomics Perspective

Ian Oesterle,[1,2] Dominik Braun,[1] David Berry,[3,4] Lukas Wisgrill,[5] Annette Rompel,[2] and Benedikt Warth[1]

[1]Department of Food Chemistry and Toxicology, Faculty of Chemistry, University of Vienna, 1090 Vienna, Austria; email: ian.oesterle@univie.ac.at, dominik.braun@univie.ac.at, benedikt.warth@univie.ac.at

[2]Department of Biophysical Chemistry, Faculty of Chemistry, University of Vienna, 1090 Vienna, Austria; email: annette.rompel@univie.ac.at

[3]Centre for Microbiology and Environmental Systems Science, Department of Microbiology and Ecosystem Science, Division of Microbial Ecology, University of Vienna, 1090 Vienna, Austria; email: david.berry@univie.ac.at

[4]The Joint Microbiome Facility of the Medical University of Vienna and the University of Vienna, 1090 Vienna, Austria

[5]Division of Neonatology, Pediatric Intensive Care and Neuropediatrics, Department of Pediatrics and Adolescent Medicine, Medical University of Vienna, 1090 Vienna, Austria; email: lukas.wisgrill@meduniwien.ac.at

Annu. Rev. Food Sci. Technol. 2021. 12:461–84

First published as a Review in Advance on December 22, 2020

The *Annual Review of Food Science and Technology* is online at food.annualreviews.org

https://doi.org/10.1146/annurev-food-062220-090807

Keywords

polyphenols, human biomonitoring, food metabolomics, microbiome, food bioactives, biotransformation, exposome

Abstract

Polyphenols are generally known for their health benefits and estimating actual exposure levels in health-related studies can be improved by human biomonitoring. Here, the application of newly available exposomic and metabolomic technology, notably high-resolution mass spectrometry, in the context of polyphenols and their biotransformation products, is reviewed. Comprehensive workflows for investigating these important bioactives in biological fluids or microbiome-related experiments are scarce. Consequently, this new era of nontargeted analysis and omic-scale exposure assessment offers a unique chance for better assessing exposure to, as well as metabolism of, polyphenols. In clinical and nutritional trials, polyphenols can be investigated simultaneously with the plethora of other chemicals to which we are exposed, i.e., the exposome, which may interact abundantly and modulate bioactivity. This research direction aims at ultimately eluting into a

true systems biology/toxicology evaluation of health effects associated with polyphenol exposure, especially during early life, to unravel their potential for preventing chronic diseases.

INTRODUCTION

Polyphenols are a large class of bioactive compounds present in plant-based foods that each contains at least one phenyl ring and one hydroxyl group. These molecules can be divided into two main classes, flavonoids and nonflavonoids, with either of these two classes containing a variety of subclasses, as shown in **Figure 1**, such as flavonols, anthocyanins, and isoflavones (Crozier et al. 2009, del Rio et al. 2012).

The presence of dietary polyphenols has been described in numerous plants, vegetables, and other food sources, such as coffee or chocolate, each having a different polyphenolic profile. Notably, 452 food items containing a total of 502 different polyphenols can be found in the Phenol-Explorer database, an important resource for polyphenol content in foods, metabolism, and fate during food processing (Neveu et al. 2010). For instance, in leaves of *Moringa oleifera* up to 291 different polyphenols have been annotated (Rocchetti et al. 2020). The phenolic profile may vary slightly for the same food item depending on factors such as region, climate, and soil. This was, for example, demonstrated in the *M. oleifera* leaves from trees that were grown in different regions of China (Zhu et al. 2020).

Polyphenols have been described as protective agents for neurodegenerative and other chronic diseases, mainly via their antibacterial, anticancer, and anti-inflammatory properties (Shahidi & Yeo 2018). The health and protective effects of polyphenols are related to not only their antioxidant activity but also a variety of other modes of action, such as modulatory interaction with enzymes or receptors (Figueira et al. 2017). For instance, ferulic acid may induce anticancer activity by affecting the behavior of cells in a human pancreatic cancer cell line by changing the expression of certain apoptosis and cell cycle genes (Fahrioğlu et al. 2016). Another example is kaempferol, which displays antioxidant activity by reducing reactive oxygen species and anti-inflammatory activity by inhibiting proinflammatory enzymes such as cyclooxygenase-1 and -2 enzymes (Devi et al. 2015).

However, polyphenols can also have prooxidant activity, especially when high amounts of certain polyphenols are present, e.g., when complementing a diet with concentrated polyphenols (Granato et al. 2020, Martin 2009). The prooxidant activity of polyphenols is also shown to occur when redox-active metals are present (Sulpizio et al. 2018) and can increase the formation of reactive oxygen species, leading to potential DNA damage (Anantharaju et al. 2016, Kyselova 2011). However, the prooxidant effect of polyphenols can in turn help activate and increase the production of enzymes and proteins that protect against the damaging effects of reactive oxygen species (Roos & Duthie 2015).

The traditional approach of human biomonitoring (HBM), i.e., measuring a dietary or environmental exposure in a biological fluid, is currently being extended by so-called exposomic approaches. Here, the intention is to go beyond single biomarkers and assess chemical exposures at the omic scale. Thus, exposomic research involves studying a vast number of biomarkers from human matrices to assess and better understand complex exposures and their impact on health and disease (Dennis et al. 2017). Importantly, newer definitions of the exposome, i.e., the totality of lifetime exposures, include the measurable biological response to these exposures. Hence, the metabolome (here defined as the totality of endogenous human metabolites) is a relevant part of the exposome for studying the various chemical pathways and processes as a response that

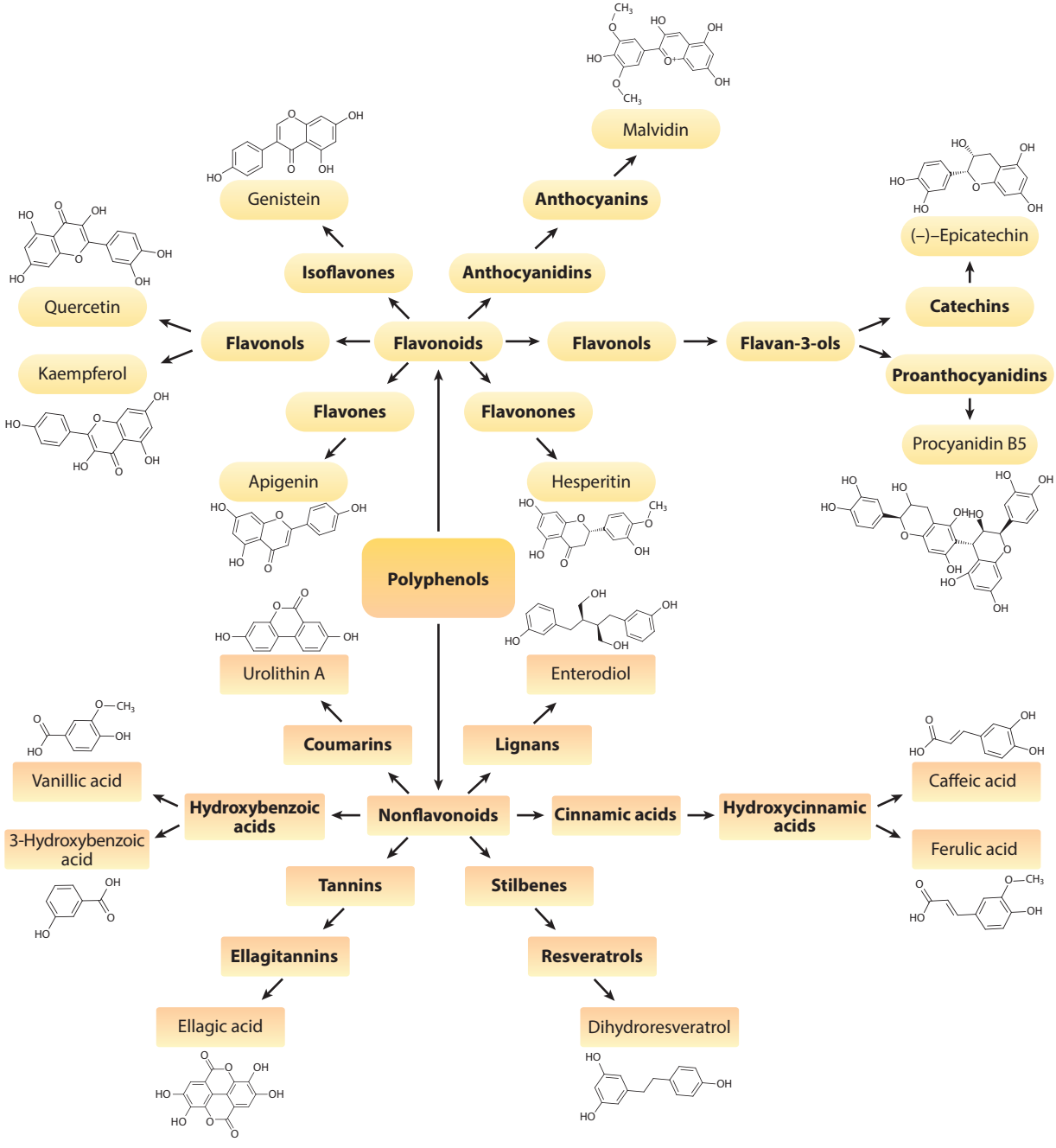


Figure 1

Flow chart depicting polyphenol classes and selected examples of each class.

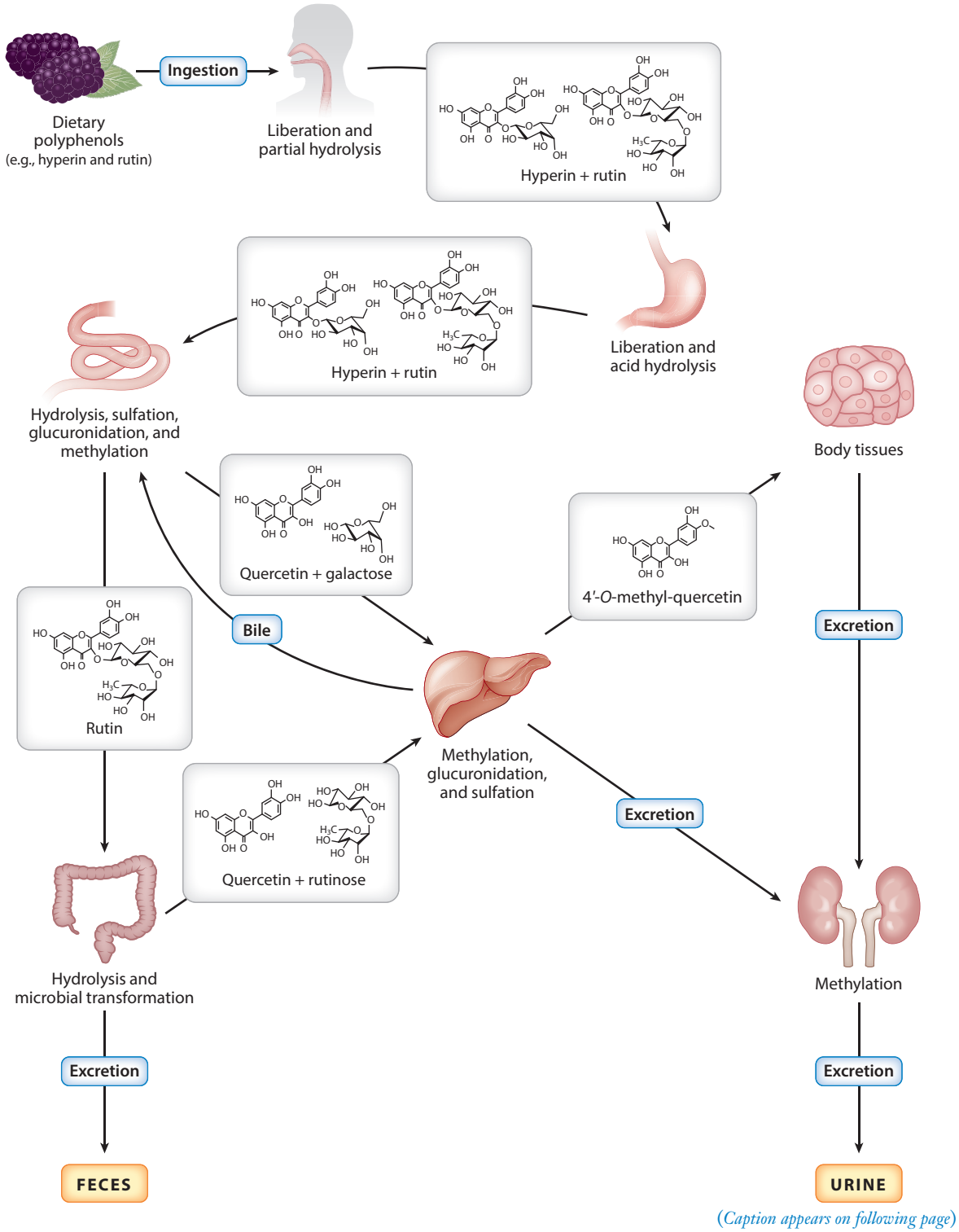
can help to understand bioactivity and toxicity. According to the Human Metabolome Database (HMDB), there are more than 110,000 different metabolites found in the human body, including those formed by the microbiota (Wishart et al. 2018). Therefore, due to the numerous potential effects of polyphenols, both beneficial and adverse, and the copious amount of at least 50,000 polyphenols present in plants, of which 8,000 were identified as dietary polyphenols (Ziaullah & Rupasinghe 2015), research initiatives for better characterization of exposure and health impact are warranted.

HBM typically applies targeted analytical approaches utilizing authentic reference standards for accurate quantification, whereas in exposomics, mostly untargeted workflows, often referred to as nontargeted analysis/screening (NTA/NTS), are developed and applied. A targeted approach involves analyzing specific biomarkers, which are often metabolites that represent exposure and/or effect. Contrarily, the untargeted approach involves studying all measurable metabolites present in a sample (Dennis et al. 2017). Both analytical approaches are currently applied in polyphenol research, and clearly both come with prospects and challenges that are discussed in this article. Moreover, a variety of analytical techniques can be utilized for both these approaches. The techniques typically involve using either liquid or gas chromatography for separation, followed by measuring the analytes by a connected detector. Diode array or ultraviolet/visible-light detectors were previously used extensively, despite challenges in metabolite identification (Scalbert et al. 2009). However, with the advancement of mass spectrometry (MS) and nuclear magnetic resonance (NMR), these detectors have become more common. Even though NMR detectors have the advantages of being nondestructive, highly reproducible, and require almost no sample preparation, NMR is less selective and sensitive and can detect a lower number of metabolites simultaneously compared to MS detectors (Emwas 2015). Thus, the focus in this article is on MS-based detectors, mainly those with liquid chromatography (LC) separation because of its high sensitivity and ability to analyze a variety of compounds (Shao et al. 2019). In addition, the application of new analytical methods and the impact of polyphenols on the gut microbiome are reviewed. Finally, future perspectives for polyphenol research, especially in the context of infant health and early-life exposure, are highlighted.

BIOMONITORING OF POLYPHENOLS IN HUMAN SPECIMENS

The metabolism of polyphenols is an intricate process, as an array of dietary polyphenols are found in various food sources, and, once ingested, they undergo a range of metabolic processes. This is, for example, reflected in the Phenol-Explorer database, where 375 different polyphenol metabolites found in urine and plasma from humans and animals are currently listed (Rothwell et al. 2013). Therefore, choosing the right biomarkers, if possible representing absorption and the effect of different polyphenols in the human body, is a critical endeavor.

As depicted in **Figure 2**, ingested dietary polyphenols can undergo two main types of biotransformations: phase I, which includes functionalization reactions such as hydrolysis or oxidation, and phase II, which includes detoxifying conjugation reactions such as glucuronidation. These transformations occur mainly in organs such as the liver or small intestine either before or after their tissue absorption or are caused by the gut microbiota found in the large intestine, yielding an assortment of microbial metabolites (Scalbert et al. 2014). For instance, ellagitannins are first partially metabolized by acid hydrolysis in the stomach or proximal small intestine, forming ellagic acid. Then, in the small intestine or colon, the gut microbiota further metabolizes the ellagitannins and ellagic acid into urolithins, which are absorbed and can undergo phase II transformations by glucuronosyltransferases and/or methyltransferases (Crozier et al. 2009). Other biotransformation reactions that may modify phenolic acids, such as cinnamic acids, are methylation in the small



(*Caption appears on following page*)

Figure 2 (*Figure appears on preceding page*)

Scheme of human polyphenol metabolism and biotransformation reactions, such as sulfation and methylation, that can occur, exemplified by the deconjugation and transformation of the flavonoid quercetin (Almeida et al. 2018, Heleno et al. 2015, Marín et al. 2015).

intestine and sulfation in the liver (Heleno et al. 2015). Generally, deciphering the full degree of polyphenol metabolism in the body is a complex task.

Targeted Analytical Approaches

There are two main ways targeted approaches have been used with polyphenols in HBM. First, because each foodstuff has its own polyphenolic profile, the exposure to a certain food source can be studied by looking at the biotransformation products of the polyphenols that are present in higher concentrations in that specific food source. Second, certain biotransformation products or unmetabolized polyphenols can be targeted to analyze how different types of exposures may change their concentrations in the body. An example of a targeted polyphenol metabolomics approach would be to measure levels of certain phenolic acid metabolites, such as isoferulic acid, to quantify the intake of tea or coffee (Hodgson et al. 2004). This approach can be used to better evaluate the validity of self-reported food intake data from study participants via food frequency questionnaires or food diaries, as these are often erroneous and do not provide quantitative data. Typically, for targeted approaches, reference standards are used for the unambiguous identification and quantification of either the parent molecule (typically after enzymatic deconjugation) or the respective metabolic products (frequently glucuronide and/or sulfate conjugates). However, because of a lack of commercially available reference standards for the conjugated forms, often only the aglycones are measured or used as reference standards for (semi)quantification in urine, although it is known that many compounds are fully conjugated. Also, if enzymatic deconjugation is applied, certain limitations apply. Frequently, the enzymatic conversion is incomplete, and the efficiency of deconjugation may differ between different polyphenols or even for the same polyphenol if it is conjugated at different positions. This can result in inaccurate quantification (Ottaviani et al. 2018). Furthermore, because of the choice of biomarkers, certain metabolites might not be detected by certain targeted methods, making the calculation of factors such as the bioavailability of polyphenols more difficult (Manach et al. 2005). Estimation errors can occur and certain metabolites can be missed because, even though polyphenols exist in a range of food sources, they are sometimes present at low concentrations, and as a consequence, their respective concentrations in human plasma or urine are even lower, typically below 1 nmol/L (Gleichenhagen & Schieber 2016).

Many methods for investigating polyphenol metabolites in human matrices have been published and are reported in **Table 1** (targeted) and **Table 2** (untargeted). Most of these apply a targeted approach with 2–50 different analytes included, and only a limited number of studies investigated more than 30 polyphenol metabolites, with the current highest number investigated being 54 metabolites (Zhong et al. 2017). However, this method also highlights some typical limitations that exist in quantification and can occur in targeted approaches. It employs neither deconjugation nor any reference standards for conjugated metabolites. Therefore, as mentioned above, it is very likely to severely underestimate exposure because most polyphenol metabolites are present in their conjugated forms following ingestion and biotransformation. Currently, the method with the highest number of quantifiable metabolites determines 46 analytes, including several glucuronide and sulfate conjugates; however, these metabolites were selected, as they are potential marker molecules for orange juice consumption (Ordóñez et al. 2018). Thus, there is still

Table 1 Selected MS-based methods for the targeted analysis of polyphenols and their metabolites in human-derived matrices

Matrix	Number of polyphenol analytes	Number of polyphenols quantified	Sample preparation	Enzyme treatment	Chromatography	Detection	Time (min)	LOQ (ng/mL)	LOQ (nM)	Reference
Plasma	10	5	Protein precipitation	None	UHPLC	ESI (negative)–Q-Orbitrap–MS/MS (PRM)	19	1.38–5.48	6–24	Svilar et al. 2019
Plasma	21	18	SPE	None	UHPLC	ESI (positive and negative)–QqQ–MS/MS (MRM)	22 (anthocyanin metabolites and urolithins) and 16 (phenolic acid metabolites)	NA	NA	Sandhu et al. 2018
Plasma	38	38	Enzymatic hydrolysis, followed by solvent extraction and ^{13}C and ^{12}C dansylation	β-Glucuronidase type H-1 (EC 3.2.1.31) from *Helix pomatia*	UHPLC	ESI (positive)–QTrap–MS/MS (MRM)	20.5	0.03–13.9	0.11–44	Achaintre et al. 2018
Plasma	54	20	SPE then centrifugation	None	UHPLC	ESI (positive and negative)–QTOF–MS/MS	52 (anthocyanin metabolites) and 16 (phenolic acid metabolites)	NA	NA	Zhong et al. 2017
Urine	11	0	Enzymatic hydrolysis, SPE, and silylation	β-Glucuronidase type H-3 (EC 3.2.1.31) and sulfatase type V (EC 3.1.6.1)	GC	EI–MS	70	NA	NA	Rios et al. 2003
Urine	11	11	Enzymatic hydrolysis, solvent extraction, and centrifugation	β-Glucuronidase and sulfatase from *H. pomatia*	HPLC	ESI (negative)–QqQ–MS/MS (MRM) and DAD (for hippuric acid)	15	NA	NA	Rios et al. 2003
Urine	15	15	Enzymatic hydrolysis, solvent extraction, and centrifugation	β-Glucuronidase type H-2 from *H. pomatia*	HPLC	ESI (negative)–QqQ–MS/MS (MRM)	6	5.97–1,450	20–5 × 10^3	Ito et al. 2005

(*Continued*)

Table 1 (*Continued*)

Matrix	Number of polyphenol analytes	Number of polyphenols quantified	Sample preparation	Enzyme treatment	Chromatography	Detection	Time (min)	LOQ (ng/mL)	LOQ (nM)	Reference
Urine	21	21	Enzymatic hydrolysis, SPE	β-Glucuronidase and sulfatase from *H. pomatia*	HPLC	ESI (negative)–QqQ–MS/MS (MRM)	10	0.5–100	1.68–558	Urpi-Sarda et al. 2009
Urine	28	28	SPE (with SDB-L or HLB cartridges) and silylation	None	Split GC	EI–MS	44	400–15,600	2,210–79,900	Ordóñez et al. 2018
Urine	41	18	SPE	None	HPLC	ESI (positive)–QTrap–MS/MS (MRM)	52	NA	NA	Kalt et al. 2017
Urine	43	24	Protein precipitation, enzymatic hydrolysis, and membrane filtration	β-Glucuronidase and sulfatase type H-2 from *H. pomatia*	UHPLC	ESI (positive and negative)–QTrap–MS/MS (SRM)	10	0.05–40.0	0.13–263	Magiera et al. 2012
Urine	46	46	SPE with SDB-L or HLB cartridges, or direct injection	None	UHPLC	ESI (negative)–Q-Orbitrap	45	9.87–807	27–5,920	Ordóñez et al. 2018
In vitro fecal fermentation (using pig samples)	6	6	Centrifugation and cellulose syringe filtration	None	UHPLC	ESI (positive)–Q-Orbitrap	13	NA	NA	Rocchetti et al. 2019
In vitro fermentation by human gut microbiota	18	18	Solvent extraction by centrifugation	None	UHPLC	ESI (negative)–QqQ–MS/MS (dMRM)	18	0.69–8.11	4.2–60	Zhao et al. 2018
In vitro fecal fermentation	18	18	Solvent extraction and silylation	None	Split GC	QqQ–MS/MS (MRM)	38	0.48–12.4	3.48–69.0	Carry et al. 2018
In vitro fecal fermentation	27	3	Solvent extraction by centrifugation	None	HPLC	ESI (negative)–QTrap–MS/MS (MRM)	38	NA	NA	de Santiago et al. 2019
Batch-culture fecal fermentation	30	6	Centrifugation and PVDF filtration	None	UHPLC	DAD–ESI (negative)–QqQ–MS (MRM)	18	9–200	15.6–231	Sánchez-Patán et al. 2012

(*Continued*)

Table 1 (*Continued*)

Matrix	Number of polyphenol analytes	Number of polyphenols quantified	Sample preparation	Enzyme treatment	Chromatography	Detection	Time (min)	LOQ (ng/mL)	LOQ (nM)	Reference
Plasma and urine	6	3	SPE and lipid removal for plasma samples	None	HPLC	ESI (positive)–QTrap–MS	68	NA	NA	Wu et al. 2002
Plasma and urine	8	1	SPE and enzymatic hydrolysis for quantification	β-Glucuronidase type VII-A and sulfatases type VIII and H-5	HPLC	ESI (negative)–QqQ–MS/MS (MRM)	9	13.3	45.8	Roura et al. 2005
Plasma and urine	14	5	SPE	None	HPLC	ESI (positive and negative)–QTrap–MS/MS (full scan) and ESI (negative)–QTOF–MS (full scan)	45	NA	NA	Teixeira et al. 2017
Plasma and urine	15	9 (8 standards were synthesized in-house)	Protein and phospholipid removal and filtration; half of the samples then went through enzymatic hydrolysis	β-Glucuronidase type H-1 and sulfatases from *H. pomatia*	UHPLC	ESI (negative)–QqQ–MS/MS (MRM)	8	5.6–14 (plasma) and 222–420 pg (urine)	12–30 (plasma) and 0.6–0.9 pmol (urine)	Actis-Goretta et al. 2012
Plasma, urine, and in vitro fecal fermentation	11 (urine), 6 (plasma), and 15 (feces)	0	Enzymatic hydrolysis (for all samples but feces) and silylation	β-D-glucuronidase type H-5 from *H. pomatia*	Split GC	EI–TOF–MS	35	NA	NA	Grün et al. 2008
Plasma, urine, and feces	45	45 (10 standards were made in-house)	SPE	None	HPLC	ESI (positive and negative)–QTrap–MS/MS (MRM)	28	NA	NA	de Ferrars et al. 2014
Normal and malignant breast tissue	15	15	Homogenization, centrifugation, filtration through a PVDF filter, enzymatic hydrolysis, and solvent extraction	β-Glucuronidase and sulfatase from *H. pomatia* for breast tissue samples	UHPLC	ESI (positive and negative)–QTOF – MS/MS (full scan)	22	0.18–29.7	0.6–130	Ávila-Gálvez et al. 2019

Abbreviations: EC, Enzyme Commission; DAD, diode array detector; dMRM, dynamic multiple reaction monitoring; EI, electron impact; ESI, electrospray ionization; GC, gas chromatography; HLB, hydrophilic-lipophilic-balanced; HPLC, high-performance liquid chromatography; LOQ, limit of quantification; MRM, multiple reaction monitoring; MS, mass spectrometer; NA, not applicable; PRM, parallel reaction monitoring; PVDF, polyvinylidene fluoride; Q-Orbitrap, hybrid quadrupole Orbitrap mass spectrometer; QqQ, triple quadrupole mass spectrometer; QTOF, quadrupole time-of-flight mass spectrometer; QTrap, triple quadrupole ion trap mass spectrometer; SDB-L, styrene divinylbenzene; SPE, solid phase extraction; SRM, single reaction monitoring; TOF, time-of-flight mass spectrometer; UHPLC, ultra-high-performance liquid chromatography.

Table 2 Selected MS-based methods for the untargeted analysis of polyphenols and their metabolites in human-derived matrices

Matrix	Number of annotated polyphenol metabolites	Number of identified polyphenol metabolites	Identification strategy and confidence level	Sample preparation	Enzyme treatment	Chromatography	Detection	Time (min)	Reference
Urine	NA	35 (23 not including isomers)	MS-based molecular networking using a cosine-similarity score between 0.7 and 1	SPE and PTFE HPLC filtration	None	UHPLC	ESI (positive and negative)–QTOF–MS/MS (auto mode)	55	Hakeem Said et al. 2020
Urine	NA	31	By comparing the exact mass (±5 mDa) to the values in free databases such as the Human Metabolome Database	Centrifugation and dilution in Milli-Q water	None	HPLC	ESI (positive and negative)–QTOF–MS/MS (full scan)	12	Garcia-Aloy et al. 2015
Urine[a]	65	46	With reference standards; MSI level 1	SPE with SDB-L or HLB cartridges, or direct injection	None	UHPLC	ESI (negative)–Q-Orbitrap	45	Ordóñez et al. 2018
Urine	629	83	Monoisotopic and standard MS–MS matching with databases or literature	Dilution until all urine samples had the same specific gravity (measured by a digital refractometer) and centrifugation	None	UHPLC	ESI (negative)–QTOF–MS/MS	12	Edmands et al. 2015

(*Continued*)

Table 2 (*Continued*)

Matrix	Number of annotated polyphenol metabolites	Number of identified polyphenol metabolites	Identification strategy and confidence level	Sample preparation	Enzyme treatment	Chromatography	Detection	Time (min)	Reference
In vitro fecal fermentation (using pig samples)[b]	75	NA	Annotation using data from Phenol-Explorer; COSMOS MSI level 2	Centrifugation followed by cellulose syringe filtration	None	UHPLC	ESI (positive)–QTOF–MS (scan mode)	35	Rocchetti et al. 2019
Plasma and urine	61 (urine) and 9 (plasma)	6 (urine) and 3 (plasma)	Matching masses and retention times with authentic standards using the same analytical method	Millipore PVDF SPE extraction	None	HPLC	ESI (positive and negative)–Q-FT-Orbitrap–MS/MS (DDA)	13	Ulaszewska et al. 2020
Plasma, urine, and normal and malignant breast tissue	90 (urine), 60 (plasma), 31 (normal breast tissue), and 27 (malignant breast tissue)	33	With reference standards; MSI level 1	Homogenization for breast tissue samples; centrifugation and filtration through a PVDF filter for all samples	None	UHPLC	ESI (positive and negative)–QTOF–MS/MS	22	Ávila-Gálvez et al. 2019

[a]A targeted screening method was also developed and applied (see **Table 1**).

[b]A targeted method was also developed and used (see **Table 1**).

Abbreviations: ESI, electrospray ionization; HLB, hydrophilic-lipophilic-balanced; HPLC, high-performance liquid chromatography; MS, mass spectrometry; MSI, Metabolomics Standards Initiative; NA, not applicable; PTFE, polytetrafluorethylene; PVDF, polyvinylidene fluoride; Q-FT-Orbitrap, hybrid linear ion trap Fourier transform Orbitrap mass spectrometer; Q-Orbitrap, hybrid quadrupole Orbitrap mass spectrometer; QTOF, quadrupole time-of-flight mass spectrometer; SDB-L, styrene divinylbenzene; SPE, solid phase extraction; UHPLC, ultra-high-performance liquid chromatography.

a vast potential and need to develop broader and more comprehensive methods for the accurate quantification of polyphenols and their human metabolites.

Most studies tend to develop methods for a limited number of related subclasses of polyphenols to address a specific biological question. Thus, the methods are optimized to have favorable separation for one chemical class or similar subclasses. This narrow focus can be a necessity to detect polyphenol metabolites where concentrations are very low. However, a method that was optimized for a subclass of polyphenols may not be optimal for another, thus making it difficult to study chemically diverse polyphenols simultaneously. Therefore, such a method is appropriate only for looking at a few desired subclasses of polyphenols but is not ideal to gain a broader and more complete picture, which would be required to assess the real impact of food bioactives on human health in a systems-wide manner. Creating such a quantitative multiclass assay would allow for studying exposure via a variety of food sources in-depth for more holistic cohort studies. Moreover, having such an extensive and broad multi-analyte LC-MS/MS method could support benchmarking and standardization initiatives for NTA. For such a method, both targeted and untargeted LC-MS-based methods can be applied, with the untargeted approach having the advantage of involving potential unknown molecules (Bocato et al. 2019).

Untargeted Liquid Chromatography–High-Resolution Mass Spectrometry–Based Metabolomics

With the advent of more affordable and easier to use high-resolution mass spectrometric instrumentation during the past two decades, untargeted approaches have emerged as an alternative to strictly targeted assays in many fields. This includes the screening (and, partly, quantification) of known and unknown chemical exposures in the food and exposure sciences. An untargeted approach requires high-resolution MS (HRMS) for full scan capacities, which thus creates massive data files that consequently require advanced bioinformatic solutions to extract relevant information from the obtained metabolic features (Bocato et al. 2019). For polyphenol metabolites in human matrices, relatively little research utilizing the power of untargeted methods has been performed to date because of the complexity of this methodology. However, there is considerable usefulness in this approach, illustrated in **Figure 3**, as it screens numerous polyphenol metabolites, allowing the identification of as yet unknown metabolites and finding metabolites that can act as better biomarkers in subsequent targeted approaches. For example, a study demonstrated that following consumption of six different polyphenol-rich foods, 83 different metabolites were annotated and used as potential dietary biomarkers (Edmands et al. 2015). Similarly, in another experiment, out of 187 potential polyphenol metabolites, 90 were annotated, of which 33 were identified with reference standards (Ávila-Gálvez et al. 2019). These are two of the methods with the highest number of annotated metabolites. However, confident identification of polyphenols and metabolites is still a major challenge. According to the Metabolomics Standards Initiative (MSI), authentic reference standards are required for confidently identifying a certain molecule (Sumner et al. 2007). This again underlines the importance of reference standard availability, notably of the conjugated metabolites. Currently, the method with the highest number of identified polyphenol metabolites that have a reference standard for each metabolite quantitatively assesses 46 polyphenols and metabolites (Ordóñez et al. 2018). Clearly, there is ample potential in further expanding the untargeted toolbox, whether it involves annotating or identifying more metabolites, looking at exposure and its correlation with environmental and food factors, developing bioinformatic pipelines, or observing other human sample matrices. **Table 2** presents an overview of published untargeted MS-based methods for the analysis of polyphenols and their metabolites in human samples.

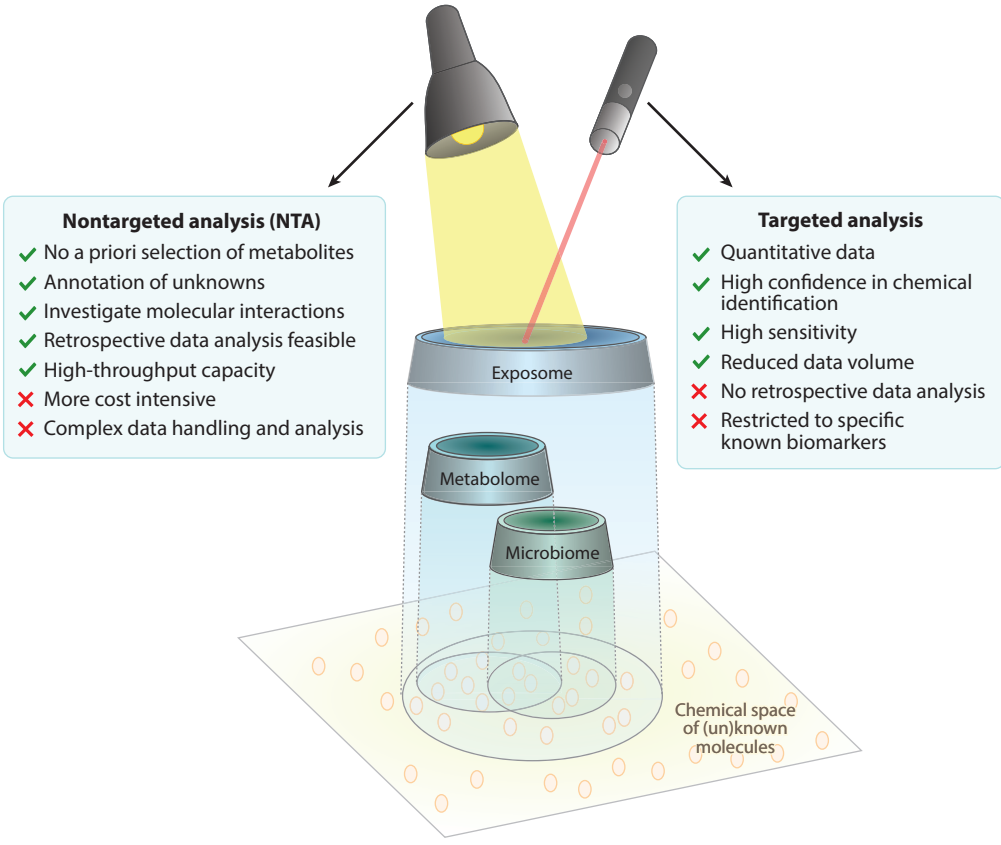


Figure 3

The exposome comprises xenobiotic exposures, including food bioactives such as polyphenols, and associated biological responses that may be reflected in the endogenous metabolome. Contrary to the selective targeted analysis of food/environmental exposures for biomonitoring purposes (symbolized by the laser), nontargeted analysis (symbolized by the floodlight) allows the more holistic investigation of chemical exposure and endogenous as well as microbial metabolites.

Polyphenol Metabolites in Human Matrices

As stated above, polyphenols tend to be metabolized by biotransformation, and various metabolites can be determined in different biological samples. As reported in **Tables 1** and **2**, the main matrices investigated for HBM purposes were plasma and urine. Plasma is often available in HBM and clinical studies and has an advantage in that it interacts with all living cells in the body; thus, a broad range of metabolites may be present (Wallace et al. 2016). Urine is frequently used in HBM studies, as the majority of absorbed metabolites are excreted via the kidneys, it is noninvasive, and large volumes can be collected. Moreover, it can easily be used in routine sample collection, and longitudinal experimental designs are easier to perform than with other matrices (Smolders et al. 2009). However, polyphenol metabolites can also be present in other sample types, e.g., 39 different metabolites were found in breast tissue (Ávila-Gálvez et al. 2019). Studying rather uncommon sample matrices holds special potential for novel insights. For instance, few studies use saliva as a matrix, even though various metabolites have been detected. One study, for example, examined the intake of red wine by studying associated metabolites found in saliva, although it only measured the levels of melatonin and 11 phenylpropanoids (Varoni et al. 2013).

Even less research has been performed on polyphenol metabolism in matrices other than biofluids or stool. An interesting option might be the investigation of biomarkers in bones, as it has recently been shown that various polyphenols, such as urolithins, from pigs fed with plant-based diets were detectable in their bones. This may be applied to humans' postmortem for assessing life-long exposure (Alldritt et al. 2019). Another possible matrix could involve teeth, as one study has found that it is possible to measure a variety of compounds, from metals to organic molecules such as pollutants like bisphenol A, in primary teeth, indicating prenatal exposure (Andra et al. 2015). The advantage of such an approach for exposure assessment is derived from the fact that organic compounds are likely more stable in the inorganic tooth matrix than in organic matrices, thus allowing better storage and cumulative exposure over a period of time. Hair is another potentially interesting matrix for the analysis of polyphenol metabolomics, as it is readily available, can be sampled noninvasively, and has the potential to deliver longitudinal exposure monitoring.

MICROBIAL POLYPHENOL METABOLITES AND MICROBIOME STUDIES

The microbiome is a vital, yet still incompletely understood, component of the human body. The composition and the metabolic activities of the microbiome can be modified by diverse factors including diet, drug treatment, and lifestyle (Moco et al. 2012). Each person harbors a unique microbiota, and the variation in microbiota composition can have dramatic effects on the extent of polyphenol metabolism. For example, soy isoflavones can be transformed into equol, an estrogen receptor agonist, by a restricted number of low-abundance intestinal bacteria, most notably *Adlercreutzia* species (Clavel et al. 2014). As not everyone has *Adlercreutzia* in their gut microbiota, there is dramatic interindividual variability in the production of equol (Setchell & Clerici 2010). It is likely that variation in the microbiota is a key underlying determinant in the observed variability between individuals in the metabolism of many polyphenols. Because of the high complexity of the microbiome, most studies exploring microbial polyphenol metabolism and its impact are performed either in vivo in mice, by in vitro models in which stool samples or derived fecal water is fermented, or in single-species bacterial culture.

Consumption of certain polyphenols has been shown to act as a prebiotic and promote the growth of specific microorganisms and thereby modulate the gut microbiome (Han & Xiao 2020), resulting in several reported effects. For example, in mice consuming a diet supplemented with bioactive dietary polyphenols, the gut microbiota was changed in a way that led to an improvement in sleep-deprived induced cognitive impairment (Frolinger et al. 2019). Conversely, polyphenols may be inhibitory to some microorganisms and reduce their activity or abundance and thereby produce a positive health effect. For example, an increase in consumption of procyanidins has been shown to reduce the amount of *Lachnospiraceae* species and thereby elicit an antiobesity effect (Gowd et al. 2019). Moreover, the effects of polyphenols, whether inhibiting or promoting, on different species in the gut microbiota depends on the polyphenols' backbone and its conjugation. For instance, the flavanol (+)-catechin promoted the growth of *Eubacterium rectale* (Etxeberria et al. 2013). For flavonols and flavanone aglycones to inhibit the activity of gut microbiota, they require a 4-carbonyl group attached to the C-ring in their backbone; this activity transpires only with aglycones and is not present in the glycosides (Duda-Chodak et al. 2015).

Examples of potential metabolic pathways for the biotransformation of polyphenols are outlined in **Table 3**. Some main mechanisms for polyphenol metabolism by the microbiota involve cleavage of various bonds in their heterocyclic carbon ring mediated by dioxygenases, hydrogenations of alkene moieties, or dehydroxylation (Stevens & Maier 2016). As an example, proanthocyanidins are cleaved into smaller phenolic acids such as 2-(*p*-hydroxyphenyl)-propionic acid

Table 3 Examples of microbial metabolites formed from polyphenols in the human body and details on the respective studies

Microbial metabolite	Matrix where metabolite was found[a]	CAS number	Microbes responsible for metabolite[a]	Parent polyphenol (and example of a food source)	Chromatography	Detection	Min, max, and mean concentrations (μg/mL)	Example of biological activity of the metabolite	Reference
Dihydroresveratrol	NA	58436-28-5	NA	Resveratrol (peanuts)	UHPLC	ESI–QTOF–MS	Min: 0 Max: 0.7 ± 0.4 Mean: NA (from in vitro fecal fermentation)	Reduces lung injury from acute pancreatitis in mice (Lin et al. 2016)	Jarosova et al. 2019
5-(3′,4′-Dihydroxyphenyl)-γ-valerolactone	Urine, blood, and feces	191666-22-5	NA	Flavan-3-ol (grape seed)	UHPLC	DAD–ESI–QqQ–MS	Min: 0 Max: 62 Mean: 8.5 (from in vitro fecal fermentation)	Inhibits TNF-α-stimulated proinflammatory responses (Lee et al. 2017)	Sánchez-Patán et al. 2012
3-(3-Hydroxyphenyl)-propionic	Urine, blood, and feces	621-54-5	*Escherichia coli* and *Eubacterium oxidoreducens*	Proanthocyanidin (legume seeds)	UHPLC	ESI–QqQ–MS/MS	Min: 0.012 Max: 18.3 Mean: 1.50 ± 3.50 (from fecal sample)	Antioxidant properties (Rios et al. 2003)	Gutiérrez-Díaz et al. 2017
3-Hydroxybenzoic acid	Urine, blood, and feces	99-06-9	*Pseudomonas*	Hydroxybenzoic acid (pineapple)	UHPLC	ESI–QqQ–MS/MS	Min: 0.009 Max: 0.174 Mean: 0.047 ± 0.042 (from fecal sample)	Analgesic and stress response desensitizing activities; overall has similar activities as aspirin (Khan et al. 2016)	Gutiérrez-Díaz et al. 2017
Gallic acid	Urine, blood, and feces	149-91-7	NA	Anthocyanin (red wine)	UHPLC	ESI–QqQ–MS/MS	Min: NA Max: NA Mean: 2.19 ± 0.24 μg/g feces (from fecal sample)	Numerous effects against, e.g., cardiovascular, gastrointestinal, and neuropsychological diseases (such as improving passive avoidance memory) (Kahkeshani et al. 2019)	Muñoz-González et al. 2013
3,4-Dihydroxy-hydrocinnamic acid	Urine, blood, feces, and cytoplasm	1078-61-1	*Lactobacillus gasseri*, *Bifidiobacterium lactis*, and *E. coli*	Flavone (chamomile)	UHPLC	DAD–ESI–QqQ–MS	Min: 0 Max: 0.33 Mean: 0.084 (from in vitro fecal fermentation)	Reduces ferric ions in plasma (DeGraft-Johnson et al. 2007)	Sánchez-Patán et al. 2012

(*Continued*)

Table 3 (*Continued*)

Microbial metabolite	Matrix where metabolite was found[a]	CAS number	Microbes responsible for metabolite[a]	Parent polyphenol (and example of a food source)	Chromatography	Detection	Min, max, and mean concentrations (μg/mL)	Example of biological activity of the metabolite	Reference
Vanillic acid	Urine, blood, feces, cytoplasm, and saliva	121-34-6	*Pseudomonas putida*, *Pseudomonas* sp. AZ10 UPM, and *Delftia acidovorans*	Anthocyanin (grape)	UHPLC	ESI–QqQ–MS/MS	Min: 0.385 Max: 0.808 Mean: 0.484 ± 0.105 (from fecal sample)	Inhibits anticoagulation effect of *Naja naja* venom by inhibiting various enzymes such as 5′ nucleotidase (Dhananjaya et al. 2006)	Gutiérrez-Díaz et al. 2017
Protocatechuic acid	Urine, blood, feces, fibroblast, and testicle	99-50-3	*Azomonas macrocytogenes*	Flavanol (apple)	UHPLC	ESI–QqQ–MS/MS	Min: 0.002 Max: 0.927 Mean: 0.150 ± 0.177 (from fecal sample)	Antioxidant neuroprotective properties by, e.g., stimulating cell proliferation (Guan et al. 2009)	Gutiérrez-Díaz et al. 2017
Urolithin A	Urine, feces, membrane, and cell membrane	1143-70-0	NA	Ellagic acid and ellagitannin (pomegranate)	UHPLC	DAD–ESI–QTOF–MS	NA	Inhibits inflammation in the bowel by improving gut barrier health (Singh et al. 2019)	García-Villalba et al. 2019
Ferulic acid	Urine, blood, feces, cytoplasm, fibroblast, and epidermis	537-98-4	*Pseudomonas acidovorans* and *Pseudomonas fluorescens*	Cinnamic acid (oats)	UHPLC	ESI–QqQ–MS/MS	Min: 0.001 Max: 0.975 Mean: 0.066 ± 0.158 (from fecal sample)	Antioxidant effect by protecting neuronal cells and synaptosomal membrane from oxidation (Mancuso et al. 2007)	Gutiérrez-Díaz et al. 2017
Hippuric acid	Urine, blood, feces, breast milk, saliva, cerebrospinal fluid, cytoplasm, kidney, liver, prostate, and placenta	495-69-2	*Actinomycetaceae*, *Clostridium*, *Eubacterium*, *Ruminococcus*, and *Faecalibacterium prausnitzii*	Phenolic acid (blueberry)	UHPLC	ESI–QqQ–MS/MS	Min: 0.008 Max: 0.123 Mean: 0.042 ± 0.040 (from fecal sample)	Potential biomarker for obesity and hypertension (Vernocchi et al. 2016)	Gutiérrez-Díaz et al. 2017

[a]These columns have data extracted from the Human Metabolome Database (Wishart et al. 2018).

Abbreviations: CAS, Chemical Abstracts Service; DAD, diode array detector; ESI, electrospray ionization; MS, mass spectrometry; NA, not applicable; QqQ, triple quadrupole mass spectrometer; QTOF, quadrupole time-of-flight mass spectrometer; TNF, tumor necrosis factor; UHPLC, ultra-high-performance liquid chromatography.

(Déprez et al. 2000). Quercetin, as another example, was shown to be transformed into homoprocatechuic acid or 4-hydroxybenzoic acid by species such as *Bifidiobacterium* B-9 and *Streptococcus* S-2 (Santangelo et al. 2019).

Because of the extreme chemical diversity of polyphenol metabolites formed by the gut microbiota, untargeted HRMS techniques offer a unique chance for the discovery of microbial metabolites in a similar manner as described above for human metabolic products. However, distinguishing metabolites derived from human or microbial transformation activity is not straightforward. Dissecting the origin of a specific microbial metabolite to a bacterial species is even more challenging. However, it is expected that novel technologies based on stable isotopes will, in the near future, provide new avenues for approaching this topic (Flasch et al. 2020).

As mentioned above, the common technique to better understand the processes of polyphenol metabolism by the gut microbiota is to mimic the large intestine in vitro. A specific method was developed to analyze 19 different metabolites from the biotransformation of grape polyphenols by the gut microbiota (Zhao et al. 2018). Another study used an untargeted approach to annotate 75 polyphenol metabolites during fecal fermentation (Rocchetti et al. 2019). However, certain biotransformation reactions that occur in other organs or during digestion can be missed when using this in vitro model. A polyphenol could be absorbed and transformed in the liver and then released back to the small intestine with the bile and finally reach the gut microbes for further transformation. To date, a limited number of studies have used untargeted LC-HRMS for polyphenol metabolomics in gut microbiome research. This results in knowledge gaps that could be highly relevant when systematically investigating the impact of polyphenol exposure on health parameters in clinical trials.

EARLY-LIFE EXPOSURE AND IMPACT ON HUMAN HEALTH

Following the developmental origin of health and disease hypothesis (Wadhwa et al. 2009), studying early-life exposure is vital, and studying polyphenols more holistically by untargeted LC-HRMS workflows would certainly be of high interest because of their reported bioactive properties. Exposure to environmental factors, including bioactives, contaminants, and toxins, especially during gestation and the first 1,000 days of life, is believed to often result in chronic health issues later in life. However, because of limitations in measuring the thousands of (low-dose) exposures and the complexity of toxicological interactions, hard evidence is currently lacking for many pathologies. In the case of undernourished human fetuses, changes in metabolism and decreased growth rates were demonstrated to ultimately lead to health issues such as hypertension or heart disease (Osmond & Barker 2000). In addition, during embryonic and fetal development there are critical windows of susceptibility during which exposure is particularly detrimental (Selevan et al. 2000); for example, pregnant mothers that were exposed to air pollution during the second trimester gave birth to children who later in life had a higher risk of developing asthma (Hsu et al. 2015). Because polyphenols are able to reduce certain toxic effects, there is a clear rationale to coinvestigate them in future exposomic-scale environmental health studies.

Current research in early-life exposomics is usually done in animal models, as it is challenging to assess fetal exposure to xenobiotics because various biomarkers in plasma and urine have a short half-life. Numerous studies in animal models have shown the benefits of polyphenols in protecting against chronic diseases. Silva et al. (2019) showed that consuming resveratrol from gestation until postnatal day 21 helped reduce hypertension development in rats; however, as a side effect, postnatal growth was restricted (Care et al. 2016, Silva et al. 2019). Studies investigating the impact of polyphenol exposure in human early-life models are rare, and those that have been done tend to look at short-term effects (Silva et al. 2019). Furthermore, some studies have also shown negative

effects of consuming polyphenols during pregnancy, e.g., consumption of polyphenol-rich foods during the third trimester could have a negative impact on the fetus by causing ductal constriction, which can increase the risk of neonatal pulmonary hypertension (Zielinsky et al. 2010, 2013). However, in this work, polyphenol exposure was estimated based on food frequency questionnaires rather than biomonitoring. Polyphenols can also be used as chemosensors for various ions that can have negative or positive impacts on health, such as using curcumin as the complexing agent against ions like Hg^{2+} or F^{-} (Khorasani et al. 2019). Similarly, polyphenol oxidases can be used as chemosensors to detect polyphenols in, for example, urine; however, issues have arisen because of their lack of specificity (Gul et al. 2017). Clearly, investigating polyphenol exposure and metabolism during early life using novel high-end technology offers unique opportunities for attaining a better picture of the combinatory impact of diverse environmental exposures.

Importantly, the composition of the early-life gut microbiome is thought to be of relevance for chronic disease development, especially with regard to inflammatory and autoimmune disorders (Kelly et al. 2007, Stiemsma & Michels 2018). Because polyphenols have been shown to influence the gut microbiome, it would be beneficial to study the associated effect in larger and more systematic multi-omic studies during early life. Research in Asian women consuming a high soy diet indicated a reduced risk of breast cancer (Wu et al. 2008). These protective properties are believed to follow exposure to soy-related isoflavones and their effect on the gut microbiome occurs during early life (Warri et al. 2008). The protective effects of genistein could be due to a change in the composition of the gut microbiota (Paul et al. 2017) or the microbial metabolites formed (Hullar et al. 2014).

CONCLUSION AND OUTLOOK

The field of polyphenol research and analysis is clearly of high relevance in the context of novel omic-scale exposure assessment and systems toxicology approaches. Recent advances in analytical instrumentation, namely HRMS, bioinformatic capacities, and tailored structural and biological databases allow for a more holistic and in-depth investigation of polyphenols and their abundant human and microbial metabolites.

Future research efforts should include the development of a very broad (targeted) reference method to analyze >100 polyphenols and key metabolites simultaneously to get a more complete understanding of the various food bioactives present. Investigating only a limited number of subclasses is not sufficient, as mixture effects are likely to occur. Developing methods to analyze polyphenols in matrices other than blood, urine, or feces, and continuing the development of untargeted methods along with tailored bioinformatic solutions for the resulting big data will pave the way for identifying potential new biomarkers of exposure and effect. In addition, possibly new correlations between metabolites from the consumption of specific polyphenol-rich foods or different influential factors may be derived.

Other research areas of interest may include the investigation of polyphenol-related enzymes, e.g., the polyphenol oxidases from various sources such as apples (Kampatsikas et al. 2019), walnuts (Panis et al. 2020), and mushrooms (Pretzler et al. 2017). These enzymes can exhibit bioactivity, as quinones can be derived via polyphenol transformation by these enzymes (Queiroz et al. 2008) or lignans such as nordihydroguaiaretic acid, which is a powerful antioxidant, can show antiviral properties, and is derived from larreatricin with the help of polyphenol oxidases (Martin et al. 2018). The bioactivity of these enzymes may also aid the development of polyphenol chemosensors with higher specificity. Finally, we encourage better investigation of polyphenol exposure during different stages of development from early to late life and the potential correlations with

the etiology and/or prevention of chronic disease in the perspective of the expanding exposome paradigm.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

The authors would like to acknowledge Augustine Scalbert (IARC, Lyon, France), Doris Marko and Gudrun Pahlke (University of Vienna, Austria), and Gary Siuzdak (Scripps Research, La Jolla, CA) for critical feedback and fruitful discussions. All members of the Warth lab are acknowledged for providing feedback on content and figure style. This work was partly supported by the University of Vienna, and the European Research Council (D.B., Starting Grant FunKeyGut 741623).

LITERATURE CITED

Achaintre D, Gicquiau A, Li L, Rinaldi S, Scalbert A. 2018. Quantification of 38 dietary polyphenols in plasma by differential isotope labelling and liquid chromatography electrospray ionization tandem mass spectrometry. *J. Chromatogr. A.* 1558:50–58

Actis-Goretta L, Lévèques A, Giuffrida F, Romanov-Michailidis F, Viton F, et al. 2012. Elucidation of (−)-epicatechin metabolites after ingestion of chocolate by healthy humans. *Free Radic. Biol. Med.* 53(4):787–95

Alldritt I, Whitham-Agut B, Sipin M, Studholme J, Trentacoste A, et al. 2019. Metabolomics reveals diet-derived plant polyphenols accumulate in physiological bone. *Sci. Rep.* 9:8047

Almeida AF, Borge GIA, Piskula M, Tudose A, Tudoreanu L, et al. 2018. Bioavailability of quercetin in humans with a focus on interindividual variation. *Compr. Rev. Food Sci. Food Saf.* 17(3):714–31

Anantharaju PG, Gowda PC, Vimalambike MG, Madhunapantula SV. 2016. An overview on the role of dietary phenolics for the treatment of cancers. *Nutr. J.* 15(1):99

Andra SS, Austin C, Wright RO, Arora M. 2015. Reconstructing pre-natal and early childhood exposure to multi-class organic chemicals using teeth: towards a retrospective temporal exposome. *Environ. Int.* 83:137–45

Ávila-Gálvez MÁ, García-Villalba R, Martínez-Díaz F, Ocaña-Castillo B, Monedero-Saiz T, et al. 2019. Metabolic profiling of dietary polyphenols and methylxanthines in normal and malignant mammary tissues from breast cancer patients. *Mol. Nutr. Food Res.* 63(9):1801239

Bocato MZ, Ximenez JPB, Hoffmann C, Barbosa F. 2019. An overview of the current progress, challenges, and prospects of human biomonitoring and exposome studies. *J. Toxicol. Environ. Health B* 22(5–6):131–56

Care AS, Sung MM, Panahi S, Gragasin FS, Dyck JRB, et al. 2016. Perinatal resveratrol supplementation to spontaneously hypertensive rat dams mitigates the development of hypertension in adult offspring. *Hypertension* 67(5):1038–44

Carry E, Zhao D, Mogno I, Faith J, Ho L, et al. 2018. Targeted analysis of microbial-generated phenolic acid metabolites derived from grape flavanols by gas chromatography–triple quadrupole mass spectrometry. *J. Pharm. Biomed. Anal.* 159:374–83

Clavel T, Lepage P, Charrier C. 2014. The Family Coriobacteriaceae. In *The Prokaryotes: Actinobacteria*, ed. E Rosenberg, EF DeLong, S Lory, E Stackebrandt, F Thompson, pp. 201–38. Berlin: Springer

Crozier A, Jaganath IB, Clifford MN. 2009. Dietary phenolics: chemistry, bioavailability and effects on health. *Nat. Prod. Rep.* 26(8):1001–43

de Ferrars RM, Czank C, Saha S, Needs PW, Zhang Q, et al. 2014. Methods for isolating, identifying, and quantifying anthocyanin metabolites in clinical samples. *Anal. Chem.* 86(20):10052–58

de Roos B, Duthie GG. 2015. Role of dietary pro-oxidants in the maintenance of health and resilience to oxidative stress. *Mol. Nutr. Food Res.* 59(7):1229–48

de Santiago E, Gill CIR, Carafa I, Tuohy KM, De Peña M-P, Cid C. 2019. Digestion and colonic fermentation of raw and cooked *Opuntia ficus-indica cladodes* impacts bioaccessibility and bioactivity. *J. Agric. Food Chem.* 67(9):2490–99

DeGraft-Johnson J, Kolodziejczyk K, Krol M, Nowak P, Krol B, Nowak D. 2007. Ferric-reducing ability power of selected plant polyphenols and their metabolites: implications for clinical studies on the antioxidant effects of fruits and vegetable consumption. *Basic Clin. Pharmacol. Toxicol.* 100(5):345–52

del Rio D, Rodriguez-Mateos A, Spencer JPE, Tognolini M, Borges G, Crozier A. 2012. Dietary (poly)phenolics in human health: structures, bioavailability, and evidence of protective effects against chronic diseases. *Antioxid. Redox Signal.* 18(14):1818–92

Dennis KK, Marder E, Balshaw DM, Cui Y, Lynes MA, et al. 2017. Biomonitoring in the era of the exposome. *Environ. Health Perspect.* 125(4):502–10

Déprez S, Brezillon C, Rabot S, Philippe C, Mila I, et al. 2000. Polymeric proanthocyanidins are catabolized by human colonic microflora into low-molecular-weight phenolic acids. *J. Nutr.* 130(11):2733–38

Devi KP, Malar DS, Nabavi SF, Sureda A, Xiao J, et al. 2015. Kaempferol and inflammation: from chemistry to medicine. *Pharmacol. Res.* 99:1–10

Dhananjaya BL, Nataraju A, Rajesh R, Gowda RCD, Sharath BK, et al. 2006. Anticoagulant effect of *Naja naja* venom 5′nucleotidase: demonstration through the use of novel specific inhibitor, vanillic acid. *Toxicon* 48(4):411–21

Duda-Chodak A, Tarko T, Satora P, Sroka P. 2015. Interaction of dietary compounds, especially polyphenols, with the intestinal microbiota: a review. *Eur. J. Nutr.* 54(3):325–41

Edmands WM, Ferrari P, Rothwell JA, Rinaldi S, Slimani N, et al. 2015. Polyphenol metabolome in human urine and its association with intake of polyphenol-rich foods across European countries. *Am. J. Clin. Nutr.* 102(4):905–13

Emwas A-HM. 2015. The strengths and weaknesses of NMR spectroscopy and mass spectrometry with particular focus on metabolomics research. In *Metabonomics: Methods and Protocols*, ed. JT Bjerrum, pp. 161–93. New York: Springer

Etxeberria U, Fernández-Quintela A, Milagro FI, Aguirre L, Martínez JA, Portillo MP. 2013. Impact of polyphenols and polyphenol-rich dietary sources on gut microbiota composition. *J. Agric. Food Chem.* 61(40):9517–33

Fahrioğlu U, Dodurga Y, Elmas L, Seçme M. 2016. Ferulic acid decreases cell viability and colony formation while inhibiting migration of MIA PaCa-2 human pancreatic cancer cells in vitro. *Gene* 576(1):476–82

Figueira I, Menezes R, Macedo D, Costa I, Nunes dos Santos C. 2017. Polyphenols beyond barriers: a glimpse into the brain. *Curr. Neuropharmacol.* 15(4):562–94

Flasch M, Bueschl C, Woelflingseder L, Schwartz-Zimmermann HE, Adam G, et al. 2020. Stable isotope-assisted metabolomics for deciphering xenobiotic metabolism in mammalian cell culture. *ACS Chem. Biol.* 15(4):970–81

Frolinger T, Sims S, Smith C, Wang J, Cheng H, et al. 2019. The gut microbiota composition affects dietary polyphenols-mediated cognitive resilience in mice by modulating the bioavailability of phenolic acids. *Sci. Rep.* 9:3546

Garcia-Aloy M, Llorach R, Urpi-Sarda M, Jáuregui O, Corella D, et al. 2015. A metabolomics-driven approach to predict cocoa product consumption by designing a multimetabolite biomarker model in free-living subjects from the PREDIMED study. *Mol. Nutr. Food Res.* 59(2):212–20

García-Villalba R, Selma MV, Espín JC, Tomás-Barberán FA. 2019. Identification of novel urolithin metabolites in human feces and urine after the intake of a pomegranate extract. *J. Agric. Food Chem.* 67(40):11099–107

Gleichenhagen M, Schieber A. 2016. Current challenges in polyphenol analytical chemistry. *Curr. Opin. Food Sci.* 7:43–49

Gowd V, Karim N, Shishir MRI, Xie L, Chen W. 2019. Dietary polyphenols to combat the metabolic diseases via altering gut microbiota. *Trends Food Sci. Technol.* 93:81–93

Granato D, Mocan A, Câmara JS. 2020. Is a higher ingestion of phenolic compounds the best dietary strategy? A scientific opinion on the deleterious effects of polyphenols in vivo. *Trends Food Sci. Technol.* 98:162–66

Grün CH, van Dorsten FA, Jacobs DM, Le Belleguic M, van Velzen EJJ, et al. 2008. GC-MS methods for metabolic profiling of microbial fermentation products of dietary polyphenols in human and in vitro intervention studies. *J. Chromatogr. B* 871(2):212–19

Guan S, Ge D, Liu T-Q, Ma X-H, Cui Z-F. 2009. Protocatechuic acid promotes cell proliferation and reduces basal apoptosis in cultured neural stem cells. *Toxicol. In Vitro* 23(2):201–8

Gul I, Ahmad MS, Naqvi SMS, Hussain A, Wali R, et al. 2017. Polyphenol oxidase (PPO) based biosensors for detection of phenolic compounds: a review. *J. Appl. Biol. Biotech.* 5(3):72–85

Gutiérrez-Díaz I, Fernández-Navarro T, Salazar N, Bartolomé B, Moreno-Arribas MV, et al. 2017. Adherence to a Mediterranean diet influences the fecal metabolic profile of microbial-derived phenolics in a Spanish Cohort of middle-age and older people. *J. Agric. Food Chem.* 65(3):586–95

Hakeem Said I, Truex JD, Heidorn C, Retta MB, Petrov DD, et al. 2020. LC-MS/MS based molecular networking approach for the identification of cocoa phenolic metabolites in human urine. *Food Res. Int.* 132:109119

Han Y, Xiao H. 2020. Whole food-based approaches to modulating gut microbiota and associated diseases. *Annu. Rev. Food. Sci. Technol.* 11:119–43

Heleno SA, Martins A, Queiroz MJRP, Ferreira ICFR. 2015. Bioactivity of phenolic acids: metabolites versus parent compounds: a review. *Food Chem.* 173:501–13

Hodgson JM, Chan SY, Puddey IB, Devine A, Wattanapenpaiboon N, et al. 2004. Phenolic acid metabolites as biomarkers for tea- and coffee-derived polyphenol exposure in human subjects. *Br. J. Nutr.* 91(2):301–5

Hsu H-HL, Chiu Y-HM, Coull BA, Kloog I, Schwartz J, et al. 2015. Prenatal particulate air pollution and asthma onset in urban children. Identifying sensitive windows and sex differences. *Am. J. Respir. Crit. Care Med.* 192(9):1052–59

Hullar MAJ, Burnett-Hartman AN, Lampe JW. 2014. Gut microbes, diet, and cancer. *Cancer Treat. Res.* 159:377–99

Ito H, Gonthier M-P, Manach C, Morand C, Mennen L, et al. 2005. Polyphenol levels in human urine after intake of six different polyphenol-rich beverages. *Br. J. Nutr.* 94(4):500–9

Jarosova V, Vesely O, Marsik P, Jaimes JD, Smejkal K, et al. 2019. Metabolism of stilbenoids by human faecal microbiota. *Molecules* 24(6):1155

Kahkeshani N, Farzaei F, Fotouhi M, Alavi SS, Bahramsoltani R, et al. 2019. Pharmacological effects of gallic acid in health and diseases: a mechanistic review. *Iran. J. Basic Med. Sci.* 22(3):225–37

Kalt W, McDonald JE, Liu Y, Fillmore SAE. 2017. Flavonoid metabolites in human urine during blueberry anthocyanin intake. *J. Agric. Food Chem.* 65(8):1582–91

Kampatsikas I, Bijelic A, Pretzler M, Rompel A. 2019. A peptide-induced self-cleavage reaction initiates the activation of tyrosinase. *Angew. Chem. Int. Ed.* 58(22):7475–79

Kelly D, King T, Aminov R. 2007. Importance of microbial colonization of the gut in early life to the development of immunity. *Mutat. Res.* 622(1):58–69

Khan SA, Chatterjee SS, Kumar V. 2016. Low dose aspirin like analgesic and anti-inflammatory activities of mono-hydroxybenzoic acids in stressed rodents. *Life Sci.* 148:53–62

Khorasani MY, Langari H, Sany SBT, Rezayi M, Sahebkar A. 2019. The role of curcumin and its derivatives in sensory applications. *Mater. Sci. Eng. C* 103:109792

Kyselova Z. 2011. Toxicological aspects of the use of phenolic compounds in disease prevention. *Interdiscip. Toxicol.* 4(4):173–83

Lee CC, Kim JH, Kim JS, Oh YS, Han SM, et al. 2017. 5-(3′,4′-Dihydroxyphenyl-γ-valerolactone), a major microbial metabolite of proanthocyanidin, attenuates THP-1 monocyte-endothelial adhesion. *Int. J. Mol. Sci.* 18(7):1363

Lin Z-S, Ku CF, Guan Y-F, Xiao H-T, Shi X-K, et al. 2016. Dihydro-resveratrol ameliorates lung injury in rats with cerulein-induced acute pancreatitis. *Phytother. Res.* 30(4):663–70

Magiera S, Baranowska I, Kusa J. 2012. Development and validation of UHPLC-ESI-MS/MS method for the determination of selected cardiovascular drugs, polyphenols and their metabolites in human urine. *Talanta* 89:47–56

Manach C, Williamson G, Morand C, Scalbert A, Rémésy C. 2005. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *Am. J. Clin. Nutr.* 81(1):230S–42

Mancuso C, Scapagnini G, Currò D, Giuffrida Stella AM, de Marco C, et al. 2007. Mitochondrial dysfunction, free radical generation and cellular stress response in neurodegenerative disorders. *Front. Biosci.* 12(1):1107–23

Marín L, Miguélez EM, Villar CJ, Lombó F. 2015. Bioavailability of dietary polyphenols and gut microbiota metabolism: antimicrobial properties. *BioMed Res. Int.* 2015:905215

Martin HJ, Kampatsikas I, Oost R, Pretzler M, Al-Sayed E, et al. 2018. Total synthesis stereochemical assignment, and divergent enantioselective enzymatic recognition of larreatricin. *Chem. Eur. J.* 24:15756–60

Martin KR. 2009. Polyphenols as dietary supplements: a double-edged sword. *Nutr. Diet. Suppl.* 2:1–12

Moco S, Martin F-PJ, Rezzi S. 2012. Metabolomics view on gut microbiome modulation by polyphenol-rich foods. *J. Proteome Res.* 11(10):4781–90

Muñoz-González I, Jiménez-Girón A, Martín-Álvarez PJ, Bartolomé B, Moreno-Arribas MV. 2013. Profiling of microbial-derived phenolic metabolites in human feces after moderate red wine intake. *J. Agric. Food Chem.* 61(39):9470–79

Neveu V, Perez-Jiménez J, Vos F, Crespy V, du Chaffaut L, et al. 2010. Phenol-Explorer: an online comprehensive database on polyphenol contents in foods. *Database* 2010:bap024

Ordóñez JL, Pereira-Caro G, Ludwig I, Muñoz-Redondo JM, Ruiz-Moreno MJ, et al. 2018. A critical evaluation of the use of gas chromatography- and high performance liquid chromatography-mass spectrometry techniques for the analysis of microbial metabolites in human urine after consumption of orange juice. *J. Chromatogr. A.* 1575:100–12

Osmond C, Barker DJ. 2000. Fetal, infant, and childhood growth are predictors of coronary heart disease, diabetes, and hypertension in adult men and women. *Environ. Health Perspect.* 108(Suppl. 3):545–53

Ottaviani JI, Fong RY, Borges G, Schroeter H, Crozier A. 2018. Use of LC-MS for the quantitative analysis of (poly)phenol metabolites does not necessarily yield accurate results: implications for assessing existing data and conducting future research. *Free Radic. Biol. Med.* 124:97–103

Panis F, Kampatsikas I, Bijelic A, Rompel A. 2020. Conversion of walnut tyrosinase into a catechol oxidase by site directed mutagenesis. *Sci. Rep.* 10:1659

Paul B, Royston KJ, Li Y, Stoll ML, Skibola CF, et al. 2017. Impact of genistein on the gut microbiome of humanized mice and its role in breast tumor inhibition. *PLOS ONE* 12(12):e0189756

Pretzler M, Bijelic A, Rompel A. 2017. Heterologous expression and characterization of functional mushroom tyrosinase (*Ab*PPO4). *Sci. Rep.* 7:1810

Queiroz C, Lopes MLM, Fialho E, Valente-Mesquita VL. 2008. Polyphenol oxidase: characteristics and mechanisms of browning control. *Food Rev. Int.* 24(4):361–75

Rios LY, Gonthier M-P, Rémésy C, Mila I, Lapierre C, et al. 2003. Chocolate intake increases urinary excretion of polyphenol-derived phenolic acids in healthy human subjects. *Am. J. Clin. Nutr.* 77(4):912–18

Rocchetti G, Bhumireddy SR, Giuberti G, Mandal R, Lucini L, Wishart DS. 2019. Edible nuts deliver polyphenols and their transformation products to the large intestine: an in vitro fermentation model combining targeted/untargeted metabolomics. *Food Res. Int.* 116:786–94

Rocchetti G, Pagnossa JP, Blasi F, Cossignani L, Hilsdorf Piccoli R, et al. 2020. Phenolic profiling and in vitro bioactivity of *Moringa oleifera* leaves as affected by different extraction solvents. *Food Res. Int.* 127:108712

Rothwell JA, Perez-Jimenez J, Neveu V, Medina-Remón A, M'Hiri N, et al. 2013. 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* 2013:bat070

Roura E, Andrés-Lacueva C, Jáuregui O, Badia E, Estruch R, et al. 2005. Rapid liquid chromatography tandem mass spectrometry assay to quantify plasma (−)-epicatechin metabolites after ingestion of a standard portion of cocoa beverage in humans. *J. Agric. Food Chem.* 53(16):6190–94

Sánchez-Patán F, Cueva C, Monagas M, Walton GE, Gibson GR, et al. 2012. Gut microbial catabolism of grape seed flavan-3-ols by human faecal microbiota. Targetted analysis of precursor compounds, intermediate metabolites and end-products. *Food Chem.* 131(1):337–47

Sandhu AK, Miller MG, Thangthaeng N, Scott TM, Shukitt-Hale B, et al. 2018. Metabolic fate of strawberry polyphenols after chronic intake in healthy older adults. *Food Funct.* 9(1):96–106

Santangelo R, Silvestrini A, Mancuso C. 2019. Ginsenosides, catechins, quercetin and gut microbiota: current evidence of challenging interactions. *Food Chem. Toxicol.* 123:42–49

Scalbert A, Brennan L, Fiehn O, Hankemeier T, Kristal BS, et al. 2009. Mass-spectrometry-based metabolomics: limitations and recommendations for future progress with particular focus on nutrition research. *Metabolomics* 5(4):435

Scalbert A, Brennan L, Manach C, Andres-Lacueva C, Dragsted LO, et al. 2014. The food metabolome: a window over dietary exposure. *Am. J. Clin. Nutr.* 99(6):1286–308

Selevan SG, Kimmel CA, Mendola P. 2000. Identifying critical windows of exposure for children's health. *Environ. Health Perspect.* 108(suppl 3):451–55

Setchell KDR, Clerici C. 2010. Equol: history, chemistry, and formation. *J. Nutr.* 140(7):1355S–62S

Shahidi F, Yeo J. 2018. Bioactivities of phenolics by focusing on suppression of chronic diseases: a review. *Int. J. Mol. Sci.* 19(6):1573

Shao B, Li H, Shen J, Wu Y. 2019. Nontargeted detection methods for food safety and integrity. *Annu. Rev. Food Sci. Technol.* 10:429–55

Silva LBAR, Pinheiro-Castro N, Novaes GM, de Freitas Laiber Pascoal G, Ong TP. 2019. Bioactive food compounds, epigenetics and chronic disease prevention: focus on early-life interventions with polyphenols. *Food Res. Int.* 125:108646

Singh R, Chandrashekharappa S, Bodduluri SR, Baby BV, Hegde B, et al. 2019. Enhancement of the gut barrier integrity by a microbial metabolite through the Nrf2 pathway. *Nat. Commun.* 10(1):89

Smolders R, Schramm K-W, Nickmilder M, Schoeters G. 2009. Applicability of non-invasively collected matrices for human biomonitoring. *Environ. Health.* 8(1):8

Stevens JF, Maier CS. 2016. The chemistry of gut microbial metabolism of polyphenols. *Phytochem. Rev.* 15(3):425–44

Stiemsma LT, Michels KB. 2018. The role of the microbiome in the developmental origins of health and disease. *Pediatrics* 141(4):e20172437

Sulpizio C, Breibeck J, Rompel A. 2018. Recent progress in synthesis and characterization of metal chalcone complexes and their potential as bioactive agents. *Coord. Chem. Rev.* 374:497–524

Sumner LW, Amberg A, Barrett D, Beale MH, Beger R, et al. 2007. Proposed minimum reporting standards for chemical analysis Chemical Analysis Working Group (CAWG) Metabolomics Standards Initiative (MSI). *Metabolomics* 3(3):211–21

Svilar L, Martin J-C, Defoort C, Paut C, Tourniaire F, Brochot A. 2019. Quantification of trans-resveratrol and its metabolites in human plasma using ultra-high performance liquid chromatography tandem quadrupole-orbitrap mass spectrometry. *J. Chromatogr. B.* 1104:119–29

Teixeira LL, Costa GR, Dörr FA, Ong TP, Pinto E, et al. 2017. Potential antiproliferative activity of polyphenol metabolites against human breast cancer cells and their urine excretion pattern in healthy subjects following acute intake of a polyphenol-rich juice of grumixama (*Eugenia brasiliensis* Lam.). *Food Funct.* 8(6):2266–74

Ulaszewska MM, Koutsos A, Trošt K, Stanstrup J, Garcia-Aloy M, et al. 2020. Two apples a day modulate human:microbiome co-metabolic processing of polyphenols, tyrosine and tryptophan. *Eur. J. Nutr.* 59:3691–714

Urpi-Sarda M, Monagas M, Khan N, Lamuela-Raventos RM, Santos-Buelga C, et al. 2009. Epicatechin, procyanidins, and phenolic microbial metabolites after cocoa intake in humans and rats. *Anal. Bioanal. Chem.* 394(6):1545–56

Varoni EM, Vitalini S, Contino D, Lodi G, Simonetti P, et al. 2013. Effects of red wine intake on human salivary antiradical capacity and total polyphenol content. *Food Chem. Toxicol.* 58:289–94

Vernocchi P, del Chierico F, Putignani L. 2016. Gut microbiota profiling: metabolomics based approach to unravel compounds affecting human health. *Front. Microbiol.* 7:1144

Wadhwa PD, Buss C, Entringer S, Swanson JM. 2009. Developmental origins of health and disease: brief history of the approach and current focus on epigenetic mechanisms. *Semin. Reprod. Med.* 27(5):358–68

Wallace MAG, Kormos TM, Pleil JD. 2016. Blood-borne biomarkers and bioindicators for linking exposure to health effects in environmental health science. *J. Toxicol. Environ. Health B* 19(8):380–409

Warri A, Saarinen NM, Makela S, Hilakivi-Clarke L. 2008. The role of early life genistein exposures in modifying breast cancer risk. *Br. J. Cancer* 98(9):1485–93

Wishart DS, Feunang YD, Marcu A, Guo AC, Liang K, et al. 2018. HMDB 4.0: the human metabolome database for 2018. *Nucleic Acids Res.* 46(D1):D608–17

Wu AH, Yu MC, Tseng C-C, Pike MC. 2008. Epidemiology of soy exposures and breast cancer risk. *Br. J. Cancer* 98(1):9–14

Wu X, Cao G, Prior RL. 2002. Absorption and metabolism of anthocyanins in elderly women after consumption of elderberry or blueberry. *J. Nutr.* 132(7):1865–71

Zhao D, Yuan B, Carry E, Pasinetti GM, Ho L, et al. 2018. Development and validation of an ultra-high performance liquid chromatography/triple quadrupole mass spectrometry method for analyzing microbial-derived grape polyphenol metabolites. *J. Chromatogr. B.* 1099:34–45

Zhong S, Sandhu A, Edirisinghe I, Burton-Freeman B. 2017. Characterization of wild blueberry polyphenols bioavailability and kinetic profile in plasma over 24-h period in human subjects. *Mol. Nutr. Food Res.* 61(12):1700405

Zhu Y, Yin Q, Yang Y. 2020. Comprehensive investigation of *Moringa oleifera* from different regions by simultaneous determination of 11 polyphenols using UPLC-ESI-MS/MS. *Molecules* 25(3):676

Ziaullah, Vasantha Rupasinghe HP. 2015. Application of NMR spectroscopy in plant polyphenols associated with human health. In *Applications of NMR Spectroscopy*, Vol. 2, ed. A ur-Rahman, MI Choudhary, pp. 3–92. Sharah, UAE: Bentham Sci. Publ.

Zielinsky P, Piccoli AL Jr., Manica JL, Nicoloso LH, Menezes H, et al. 2010. Maternal consumption of polyphenol-rich foods in late pregnancy and fetal ductus arteriosus flow dynamics. *J. Perinatol.* 30(1):17–21

Zielinsky P, Piccoli AL Jr., Vian I, Zílio AM, Naujorks AA, et al. 2013. Maternal restriction of polyphenols and fetal ductal dynamics in normal pregnancy: an open clinical trial. *Arq. Bras. Cardiol.* 101(3):217–25