Increased intestinal permeability in older subjects impacts the beneficial effects of dietary polyphenols by modulating their bioavailability

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Abstract

Polyphenols have great potential in regulating intestinal health and ameliorating pathological conditions related to increased intestinal permeability (IP). However, the efficacy of dietary interventions with these phytochemicals may significantly be influenced by inter-individual variability factors affecting their bioavailability and consequent biological activity. In the present study, urine samples collected from older subjects undergoing a crossover intervention trial with polyphenol-rich foods were subjected to metabolomics analysis for investigating the impact of increased IP on the bioavailability of polyphenols. Interestingly, urinary levels of phase II and microbiota-derived metabolites were significantly different between subjects with healthier intestinal barrier integrity and those with increased IP disruption. Our results support that this IP-dependent impaired bioavailability of polyphenols could be attributed to disturbances in the gut microbial metabolism and phase II methylation processes. Furthermore, we also observed that microbiota-derived metabolites could be largely responsible for the biological activity elicited by dietary polyphenols against age-related disrupted IP.

Keywords. Polyphenols; intestinal permeability; aging; metabolomics; microbiota; bioavailability

INTRODUCTION

The intestinal barrier is a complex functional structure that separates the gut luminal environment from the inner host, which is composed of a physical wall comprising epithelial cells and mucus layers, but also other elements such as the gut microbiota, immunological elements (e.g. immunoglobulin A, cytokines), as well as the intestinal endocrine, neuroenteric and vascular systems.[1] The integrity of this barrier is crucial in human health for maintaining normal intestinal permeability (IP), which regulates the transport and absorption of nutrients (e.g. sugars, vitamins, amino acids, fatty acids and other lipids) and other food-related compounds (e.g. polyphenols), and the translocation of bacterial components from the lumen to the bloodstream. The IP is controlled by a complex system of junctions, namely tight junctions (TJ), gap junctions and adherens junctions, comprising a myriad of transmembrane proteins (e.g. occludins, claudins) and junctional adhesion molecules that rule the flux between adjacent enterocytes.[2] However, the disruption of these intestinal junctions leads to increased IP, a pathological condition also known as leaky gut. This results in the diffusion of toxins, viruses and bacterial fragments from the intestinal environment to the circulating stream, which consequently activates the immune function and provokes systemic inflammation.[3] Increased IP has been proposed as a major contributor to multiple diseases, including gastrointestinal (e.g. irritable bowel syndrome, celiac disease),[4] metabolic (e.g. obesity, type II diabetes),[5] cardiovascular (e.g. atherosclerosis, chronic heart failure),[6] psychiatric (e.g. depression, autism)[7] and neurodegenerative (e.g. Parkinson's disease, Alzheimer's disease) disorders.[8] Furthermore, it is also noteworthy that leaky gut can frequently be observed during aging, contributing

to the characteristic low-grade systemic inflammation detected in older adults, i.e. the inflamm-aging process.[9] The most common causes behind this age-related increase of the IP include impairments in the intestinal epithelial and mucus barriers,[10] declined immune function (i.e. immune senescence)[9] and changes in the gut microbiota composition.[11]

Adequate nutritional status is crucial for maintaining normal gut barrier function. Adherence to the Western diet, characterized by high fat and sugar intake, is associated with increased IP,[12,13] whereas the Mediterranean diet, rich in fruits, vegetables and fiber, prevents the leaky gut.[13] In this vein, numerous studies have been conducted during the last years aimed to test the efficacy of dietary interventions for improving the IP and related conditions, with special focus on polyphenols.[14,15] These bioactive compounds are secondary metabolites widely distributed in plant-derived foods, including fruits, vegetables, legumes, cereals, beverages (e.g. tea, coffee) and many other foods, with recognized antioxidant and anti-inflammatory properties. Thus, it has previously been reported that polyphenols can ameliorate the leaky gut by directly regulating the TJ function, enhancing the synthesis and redistribution of TJ proteins, such as occludin, claudins and zonula occludens,[16,17] and by inhibiting different kinases involved in TJ expression.[2] Polyphenolic compounds are also able to block the production of inflammatory cytokines (e.g. necrosis factors, interleukins) and oxidative stress, thus protecting the intestinal barrier integrity.[2] Furthermore, polyphenols and the gut microbiota are interconnected through a bidirectional network, which plays a pivotal role in the intestinal health.[18] On one hand, the gut microbiota is involved in the biotransformation processes needed for the absorption and biological activity of these compounds. Indeed, various studies

have described that microbiota-derived metabolites could be responsible, at least in part, for the intrinsic biological effects traditionally attributed to polyphenols, especially taking into consideration the usual low bioavailability of the parent compounds.[19] Complementarily, the prebiotic activity of polyphenols and microbiota derivatives is also well known,[20] being the consumption of polyphenol-rich foods able to shape the microbiota composition towards the preservation of the intestinal barrier health by means of different mechanisms. For instance, the gut microbiota may directly influence the IP by contributing to the intestinal barrier integrity (e.g. affecting the turnover of intestinal epithelial cells, organization of TJs), but it is also involved in the modulation of inflammation.[21,22] Accordingly, the dietary-driven manipulation of the intestinal microbial ecosystem with polyphenols has previously demonstrated great efficacy for improving the IP and related inflammatory processes.[23-25] However, to the best of our knowledge, there is currently a total lack of studies focused on determining how increased IP and associated pathological conditions occurring during aging, such as inflammation and microbial dysbiosis, may affect the bioavailability of polyphenols, and consequently impact their biological activity. The aim of the present work is to investigate for the first time the impact of increased IP in older subjects on the bioavailability of dietary polyphenols, and therefore on their bioactivity and capacity to modulate the intestinal barrier integrity. To this end, a crossover intervention trial with a polyphenol rich diet was conducted in older adults, and serum zonulin was measured as a marker of the intestinal barrier integrity for stratifying the population in two sub-groups according to their IP (i.e. increased IP dysfunction and healthier subjects). Then, comprehensive quantitative metabolomics analyses were performed to

characterize the urinary food-related metabolome, comprising polyphenolic and other food-origin compounds, metabolites derived from phase I/II metabolism, and microbial-transformed derivatives.[26,27]

MATERIALS AND METHODS

Study design

A randomized, controlled, crossover intervention trial with polyphenol-rich foods was conducted in older people living in a residential care setting (i.e. the MaPLE study, Microbiome mAnipulation through Polyphenols for managing Leakiness in the Elderly), as described elsewhere.[28] The study was performed in accordance with the principles contained in the Declaration of Helsinki. The Ethics Committee of the University of Milan approved the study protocol, and all the participants provided written informed consent. The trial was registered under ISRCTN.com (ISRCTN10214981).

Briefly, 51 older subjects (≥ 60 y) completed a crossover trial consisting of a polyphenol-rich diet (PR-diet) and a control diet (C-diet), each one of the arms lasting for 8 weeks and being separated by an 8-week wash-out period. Serum zonulin levels were measured as a marker of IP (Immunodiagnostik® ELISA kit, Bensheim, Germany),[29] and the median value within the study population (median = 40 ng/mL) was employed to stratify subjects in two sub-groups: the lower serum zonulin at baseline (LSZ) group (serum zonulin at baseline ≤ the median value) and the higher serum zonulin at baseline (HSZ) group (serum zonulin at baseline > the median value). Accordingly, zonulin levels were 33.2 ± 5.6 ng/mL and 51.5 ± 8.9 ng/mL (expressed as the mean ± standard deviation) for the LSZ and HSZ individuals, respectively. Subjects in these two groups were matched for sex (men/women: 11/15 vs 11/14), age (79.2 ± 10.4 vs 76.4 ±

10.2 y) and BMI (26.4 ± 6.4 vs 27.2 ± 4.5 kg/m^2). During the C-diet period, subjects consumed the regular menu provided by the nursing home, whereas the PR-diet was designed by substituting three portions per day of low-polyphenol products from the C-diet with food items with higher polyphenol content, but maintaining comparable levels of energy and nutrients. Specifically, PR-foods employed in this intervention study were berries (raw fruits and puree), blood orange (raw fruits and juice), pomegranate juice, green tea, Renetta apple (raw fruits and puree) and cocoa (chocolate callets and cocoa powder drink). At baseline and after each intervention period, subjects were asked to fast overnight for collecting serum and first morning void urine samples. Detailed description about the inclusion and exclusion criteria, the intervention trial, and the collection of biological samples has been previously reported by Guglielmetti et al.[28]

Metabolomics analysis of urine samples

Multi-targeted quantitative metabolomics analysis of the urinary food metabolome was accomplished by ultra-high-performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS), following the methodology optimized by González-Domínguez et al.[26,27] To this end, urine samples were subjected to solid-phase extraction (SPE) using Oasis® HLB extraction plates (Waters, Milford, MA, USA) with the aim of simultaneously extracting and pre-concentrating polyphenols and other food-related compounds, and their biotransformed metabolites (i.e. phase I/II and microbiota derivatives). Complementarily, urine samples were also analyzed after tenfold dilution to determine highly concentrated metabolites and polar compounds, these latter not extracted when using SPE. A set of internal

standards (taxifolin and caffeine-$^{13}C_3$, 100 µg/L) was added to all the samples for quantification and quality control (QC) assessment, as previously described.[26,27] Subsequent UHPLC-MS/MS metabolomics fingerprinting was performed by using the chromatographic and MS conditions described elsewhere for the simultaneous detection and quantitation of almost 350 dietary compounds and their host and microbial metabolites.[26] Metabolomics results were normalized in reference to the urinary refractive index (OPTi Digital Handheld Refractometer, Bellingham+Stanley, UK) to account for inter-individual differences in the hydration status and micturition frequency.

Quality control assessment

Quality control (QC) assessment of the metabolomics data was carried out by using a standardized protocol developed in-house. For this purpose, data were first pre-processed for removing metabolites with more than 20% missing values in all the study groups.[30] The remaining missing values were imputed by using the root square of the limit of detection for each metabolite,[26] and data were then log transformed and Pareto scaled. Afterwards, distances to the group centroid were computed based on Euclidean distances to remove outliers from the data matrix. Metabolites known to be influenced by pre-analytical factors (e.g. hippurate) were checked for the absence of abnormal values (±1.5×IQR), which could be indicative of improper handling/storage of urine samples.[31] Finally, the coefficient of variation was computed for areas, retention times and peak widths of the internal standards added to samples with the aim of evaluating the analytical reproducibility along the sequence run.

Statistical analysis

Metabolomics data were pre-processed as detailed in the previous section, and were then subjected to statistical analysis by using R 3.6.2 software packages (http://www.r-project.org) to look for altered metabolites because of the intervention trial and to associate these metabolic alterations with changes in the IP. For this purpose, data normality was first checked by inspecting probability plots. Then, a linear mixed model was built to evaluate the impact of the PR-dietary intervention on urinary metabolites compared with the C-diet, taking into account the repeated measures by subject, the period (pre- and post-intervention) and the arm within the crossover design (i.e. first C-diet and then PR-diet, or vice versa). For each arm of the crossover trial, the effect of the intervention was estimated as the difference between the final and baseline metabolite concentrations. Finally, Pearson's correlations were computed between serum zonulin levels and significant urinary metabolites according to the previous linear model. All these analyses were conducted in the entire study population (i.e. the MaPLE study), as well as separately in participants stratified according to their baseline zonulin levels (i.e. the LSZ and HSZ sub-groups), as reported in section 2.1. All the statistical analyses were adjusted for the age, sex, BMI and the allocation order in the crossover trial as covariates, and were adjusted for multiple comparisons using the Benjamini-Hochberg false discovery rate (FDR). FDR-corrected p-values below 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Differential bioavailability of dietary polyphenols depending on the IP status

Metabolomics analysis of urine samples was accomplished to investigate the metabolism and bioavailability of polyphenols supplied through a PR-dietary intervention in older adults. For evaluating the impact of increased IP on metabolomics results, serum zonulin was measured as a surrogate marker of the intestinal barrier integrity, because the high rate of incontinence amongst the elderly participants participating in the intervention trial impeded the lactulose-mannitol urinary test to be performed. In this vein, although there is growing debate about the reliability of using zonulin as a marker of IP,[32] it has been previously demonstrated a high correlation between serum zonulin and the urinary lactulose/mannitol ratio.[33] On this basis, we stratified the study population according to the baseline zonulin levels with the aim of separately assessing the effect of the PR intervention in subjects with healthier intestinal barrier integrity (i.e. the LSZ group) and in those with increased IP dysfunction (i.e. the HSZ group). This is in line with previous works reporting that serum zonulin concentrations are normally raised during aging,[34] but especially in older adults with gastrointestinal symptoms compared to the general older population.[35]

The PR-diet supplied an average of 724 mg of total polyphenols per day, thus almost doubling the estimated polyphenol intake compared with the C-diet.[28] We observed that this PR-dietary intervention induced a slight decrease of serum zonulin levels in the MaPLE population.[36] This finding is supported by numerous scientific evidence that highlight the great potential of polyphenols in regulating the intestinal barrier function and preventing leaky gut, both *in vitro* and *in vivo*.[2,16] However, different behaviors were interestingly observed when stratifying subjects according to the serum zonulin levels at baseline, since only

the subjects with higher IP (i.e. HSZ group) experienced a significant decrease of serum zonulin, whereas those with LSZ were unaffected. Overall, these results underline the potential existence of different phenotypic groups in the older subjects characterized by the degree of IP, which significantly influences the efficacy of the PR-dietary intervention. This therefore demonstrates the crucial need of investigating the inter-individual variability in the bioavailability of polyphenols driving these discrepancies.

To this end, we employed a multi-targeted metabolomics platform with integrated QC assessment, which provided a comprehensive, accurate and quantitative characterization of the urinary food metabolome based on the simultaneous analysis of around 350 diet-related metabolites, including polyphenols and other food-origin compounds, metabolites derived from the host metabolism (i.e. phase I and II transformation processes), and microbiota derivatives.[26,27] Among all the metabolites measured, the intervention with PR-foods in the MaPLE trial induced a significant increase of the urinary levels of numerous food and microbiota-related metabolites compared with the C-diet (ca 70), as shown in Table 1. The concentrations within the four study groups (i.e. C-diet baseline, C-diet post-intervention, PR-diet baseline, PR-diet post-intervention) for the metabolites significantly altered because of the PR dietary intervention are listed in Tables S1-S3, for the entire MaPLE population, the LSZ and the HSZ sub-groups, respectively. Many of these metabolites are well known food-intake markers, as defined in the Food Biomarker Ontology,[37] thus accurately mirroring the consumption of the specific PR-foods employed in this intervention study. The most remarkable finding was the increased urinary content of phase II metabolites of flavan-3-ols (i.e. (epi)catechins and

methyl(epi)catechins) and their microbiota derived hydroxyphenyl-valeric acids and hydroxyphenyl-γ-valerolactones, associated with the consumption of procyanidin-rich foods (e.g. tea, berries, apple, cocoa). The intake of tea, cocoa and berries during the PR period was also reflected in the urinary excretion of methylgallic acid derivatives, theobromine and cyanidin 3-glucoside, respectively. The production of urolithins, derived from the microbial transformation of ellagitannins, could be attributed to pomegranate and berries. Furthermore, other numerous non-specific metabolites derived from the microbial metabolism of a wide range of polyphenol classes were also accumulated in urine samples, including phenolic acids (e.g. hydroxybenzoic acids, hydroxycinnamic acids) and enterolignans (e.g. enterolactone). Nonetheless, the most remarkable results were obtained when subjects were stratified according to the baseline zonulin levels. For LSZ individuals, the PR dietary intervention induced similar metabolomics changes to those previously described for the entire MaPLE population (Table 1). However, the number of metabolites that were significantly increased as a consequence of the PR-diet in HSZ subjects was considerably lower with respect to the LSZ group, especially regarding microbiota derivatives. The HSZ group of subjects only showed urinary alterations in the levels of flavan-3-ol phase II metabolites, hydroxycinnamic acids and a few other microbiota compounds compared with the C-diet. Interestingly, the fold of increase after the PR-diet for most of these metabolites was more pronounced in LSZ subjects compared with HSZ ones, except for methyl(epi)catechin derivatives that were excreted in larger amounts in this latter group. All this therefore suggests that the baseline IP status could be an important factor affecting the bioavailability of dietary polyphenols,

considering that only subjects with a healthier intestinal integrity were able to properly metabolize them. Particularly, metabolic discrepancies between the LSZ and HSZ groups were mainly observed in microbial metabolites, as shown in Table 1, which could support that alterations in the gut microbiota composition might play a central role in this hypothesized IP-driven reduced bioavailability.

In this context, the gut microbiota has been proposed as one of the most important factors influencing the bioavailability of polyphenols and, consequently, their bioactivity.[19] The microbial metabolism of polyphenols usually comprises an initial hydrolysis step of the conjugated species present in foods to release the corresponding aglycones, which can subsequently be transformed by a range of reactions, including ring fissions, dehydroxylations, decarboxylations, demethylations, reductions, and many others.[18,38] While numerous enterobacterial species from the four most abundant phyla can be involved in the deconjugation of polyphenols (i.e. Firmicutes, Bacteroidetes, Actinobacteria and Proteobacteria), only two phyla have been associated with further metabolism of the aglycones (Firmicutes and Actinobacteria), as illustrated in Figure 1. Among them, *Clostridium* and *Eubacterium* species from the Firmicutes phylum are essential for the bioavailability of most polyphenols by driving C-ring cleavage reactions, which lead to the production of simpler phenolic acids and other intermediates that may undergo subsequent conversions to generate more complex microbiota derivatives (e.g. hydroxyphenyl-γ-valerolactones, urolithins, enterolignans). In contrast, hydroxycinnamic acids are mainly released in the colon by the action of microbial species from the *Bifidobacterium* and *Lactobacillus* genera (Figure

1).[38] Within this complex interplay between the gut microbiota and dietary polyphenols, it should be also noted that aging-related impairments in the intestinal health have closely been associated with significant gut dysbiosis. In general, the microbiota composition in older adults is characterized by an overall decrease of the bacterial diversity and stability, with a shift in the proportion of Bacteroidetes (increased) and Firmicutes (decreased) species,[39,40] and increased abundance of potentially pathogenic and pro-inflammatory bacteria.[40-42] Among the Firmicutes, numerous studies have demonstrated that older subjects with impaired intestinal health have decreased content of *Clostridium* and *Eubacterium* species,[42-44] which are directly involved in the microbial biotransformations of polyphenols as described above. On the other hand, various authors have recently described that aging has not a significant impact on the *Bifidobacterium* genus,[41,44] refuting earlier studies;[45,46] whereas contradictory results have been published regarding the influence of aging in *Lactobacillus* bacteria.[47,48] Therefore, these previous metagenomics findings totally support the metabolomic discrepancies observed in the present study between the LSZ and HSZ groups, since older subjects with increased IP (i.e. HSZ) are expected to have lower Firmicutes diversity, thus negatively affecting the bioavailability of most polyphenols and consequently reducing the urinary excretion of their microbiota derivatives, while showing only a minor impact on the content of hydroxycinnamates produced by *Bifidobacterium* and *Lactobacillus* species.

On the other hand, increased methylation of dietary (epi)catechins was also observed in the HSZ group, which was paralleled by decreased rate of glucuronidation and sulfation processes (Table 1). In this vein, it has been

previously described that the *in vitro* bioavailability and intestinal absorption of methylated polyphenols is considerably higher than that elicited by the corresponding glucuronide and sulfate species.[49] These results could therefore suggest that a shift towards increased methylation is induced in HSZ individuals to partially compensate the impairments in the microbial metabolism of polyphenols described above. This sharpened excretion of phase II methyl(epi)catechin metabolites in the HSZ group could be attributed to altered expression of catechol-O-methyltransferase, the enzyme responsible for the conversion of dietary polyphenols into their methylated analogues.[50] The proper regulation of this catechol-metabolizing system has been demonstrated to be crucial in human health due to its potential pathophysiological and pathogenic role in neurodegenerative diseases, cancers and cardiovascular disorders.[51] However, this is the first time to our knowledge that an IP-dependent regulation of this methylation system is described in older adults.

Association between dietary polyphenols, microbial metabolites and intestinal barrier health

To further investigate the possible impact of the hypothesized IP-driven reduced bioavailability on the beneficial effects of polyphenols supplied through the PR-diet, linear correlations were computed between urinary metabolite concentrations and serum zonulin levels. For the LSZ sub-group, two conjugated phenolic acids were strongly and negatively correlated with zonulin levels, namely 3,4-dihydroxybenzoic acid 3-glucuronide ($r = -0.47$, FDR-corrected $p = 0.042$) and m-coumaric acid glucuronide ($r = -0.50$, FDR-corrected $p = 0.061$), but no significant associations were found with parent polyphenol compounds (Table S4). In contrast, no statistically significant

correlations were observed between zonulin and food-derived metabolites when considering the HSZ group (FDR-corrected $p > 0.2$, Table S4). Phenolic acids are common microbial metabolites derived from the intestinal degradation of multiple polyphenol classes, although they can also be present in original foods.[18] Thus, these results reinforce that the gut microbiota is responsible to a large extent for the bioavailability and subsequent biological activity elicited by dietary polyphenols. In this context, multiple *in vitro* and *in vivo* studies have previously reported that polyphenols (e.g. quercetin, kaempferol, myricetin, genistein, catechin, curcumin) can modulate the intestinal barrier function by promoting TJ integrity, protecting against inflammatory and oxidative disruptions, and consequently decreasing intestinal permeability.[16] However, the results presented here allows hypothesizing that (i) microbial phenolic acids could be the major contributors to the IP improvement induced by the PR-dietary intervention in older subjects, and (ii) that the efficacy of dietary polyphenols is considerably impaired in subjects with increased IP dysfunction. In conclusion, we have demonstrated in the present study a connection between the degree of IP at baseline and the bioavailability of dietary polyphenols in older adults. On the basis of our findings and previous literature, we hypothesize that disturbances in the gut microbiota composition and IP-associated regulation of the phase II methylation of polyphenols could explain, at least in part, the metabolomics results presented here. Furthermore, we also found that microbial metabolites could be the major contributors to the biological activity elicited by dietary polyphenols, being this bioactivity significantly impaired in older subjects with increased IP. To validate these hypotheses, future metagenomics studies are needed to associate polyphenol-driven

changes in the IP (i.e. serum zonulin) and the food metabolome with the gut microbiota composition. Therefore, this work highlights the crucial need of developing personalized nutritional strategies for managing the IP in older adults, and the pivotal role of gut microbiota in modulating the beneficial effects of the diet on human health.

Abbreviations. C, control; HSZ, higher serum zonulin at baseline; IP, intestinal permeability; LSZ, lower serum zonulin at baseline; PR, polyphenol-rich;TJ, tight junction

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Supporting information. Concentrations within the four study groups (C-diet baseline, C-diet post-intervention, PR-diet baseline, PR-diet post-intervention) of the metabolites significantly altered because of the PR dietary intervention, considering the entire MaPLE population, LSZ and HSZ sub-groups; and Pearson's correlation coefficients between these metabolites and serum zonulin levels.

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Figure Captions

Figure 1. The interplay between the gut microbiota and the metabolism of polyphenols.

Table 1. Urinary Food and Microbiota-related Metabolites Significantly Altered after the PR-diet for the Entire MaPLE Population, the LSZ and HSZ Sub-groups. Results are Expressed as the Percentage of Change, with FDR-corrected p-values in Brackets (NS, Non-Significant).

metabolite	**MaPLE (N=51)**	**LSZ (N=26)**	**HSZ (N=25)**
phenolic acids, hydroxybenzenes & hydroxybenzaldehydes (microbiota)			
2-hydoxybenzoic acid glucuronide	186.7 ($3.3 \cdot 10^{-2}$)	317.5 ($1.1 \cdot 10^{-2}$)	44.4 (NS)
3-hydoxybenzoic acid glucuronide	208.5 ($1.8 \cdot 10^{-3}$)	87.6 ($1.1 \cdot 10^{-2}$)	81.7 (NS)
4-hydoxybenzoic acid glucuronide	85.1 ($4.5 \cdot 10^{-2}$)	92.9 (NS)	76.1 (NS)
3-hydoxybenzoic acid sulfate	634.9 ($1.1 \cdot 10^{-4}$)	332.6 ($8.4 \cdot 10^{-3}$)	454.4 (NS)
3,4-dihydoxybenzoic acid 3-glucuronide	217.6 ($2.3 \cdot 10^{-4}$)	329.9 ($9.1 \cdot 10^{-3}$)	95.0 (NS)
3,4-dihydoxybenzoic acid 4-glucuronide	99.0 ($2.3 \cdot 10^{-4}$)	118.1 ($2.0 \cdot 10^{-2}$)	74.8 ($3.2 \cdot 10^{-2}$)
3,4-dihydoxybenzoic acid 3-sulfate	134.0 ($4.3 \cdot 10^{-2}$)	156.0 (NS)	111.0 (NS)
hippuric acid	864.6 ($3.7 \cdot 10^{-2}$)	1062.3 (NS)	658.8 (NS)
3-hydroxyhippuric acid	1715.4 ($4.3 \cdot 10^{-2}$)	1009.7 (NS)	2450.5 (NS)
vanillic acid glucuronide	174.2 ($6.8 \cdot 10^{-4}$)	129.3 ($2.8 \cdot 10^{-2}$)	221.1 (NS)

isovanillic acid glucuronide	193.9 ($2.0 \cdot 10^{-3}$)	281.9 ($2.3 \cdot 10^{-2}$)	94.0 (NS)
syringic acid	155.1 ($2.2 \cdot 10^{-3}$)	104.5 ($1.0 \cdot 10^{-2}$)	61.7 (NS)
4-methylgallic acid	548.8 ($2.6 \cdot 10^{-2}$)	824.4 ($1.4 \cdot 10^{-2}$)	287.1 (NS)
methylgallic acid glucuronide	75.3 ($5.5 \cdot 10^{-4}$)	85.6 ($1.8 \cdot 10^{-2}$)	64.5 (NS)
methylgallic acid sulfate	235.3 ($2.5 \cdot 10^{-2}$)	248.9 (NS)	221.1 (NS)
3-hydoxyphenylacetic acid	187.3 ($2.3 \cdot 10^{-4}$)	150.7 ($4.0 \cdot 10^{-3}$)	185.7 (NS)
4-hydoxyphenylacetic acid glucuronide	91.6 ($4.1 \cdot 10^{-2}$)	77.4 (NS)	78.2 (NS)
3,4-dihydoxyphenylacetic acid glucuronide	870.3 ($1.8 \cdot 10^{-3}$)	107.3 ($1.1 \cdot 10^{-2}$)	56.9 (NS)
homovanillic acid glucuronide	200.2 ($1.9 \cdot 10^{-2}$)	263.3 (NS)	131.6 (NS)
homovanillyl alcohol	104.5 ($1.7 \cdot 10^{-2}$)	65.8 (NS)	77.9 (NS)
o-coumaric acid	133.6 ($9.9 \cdot 10^{-4}$)	215.2 ($1.3 \cdot 10^{-2}$)	76.4 (NS)
o-coumaric acid glucuronide	158.9 (NS)	223.6 ($4.5 \cdot 10^{-2}$)	88.3 (NS)
m-coumaric acid glucuronide	222.5 ($1.2 \cdot 10^{-5}$)	292.3 ($2.8 \cdot 10^{-3}$)	169.3 ($2.4 \cdot 10^{-2}$)
p-coumaric acid glucuronide	224.9 ($1.2 \cdot 10^{-3}$)	303.3 ($4.7 \cdot 10^{-2}$)	143.2 ($3.2 \cdot 10^{-2}$)
m-coumaric acid sulfate	257.3 ($2.7 \cdot 10^{-2}$)	293.8 ($1.5 \cdot 10^{-2}$)	219.2 (NS)

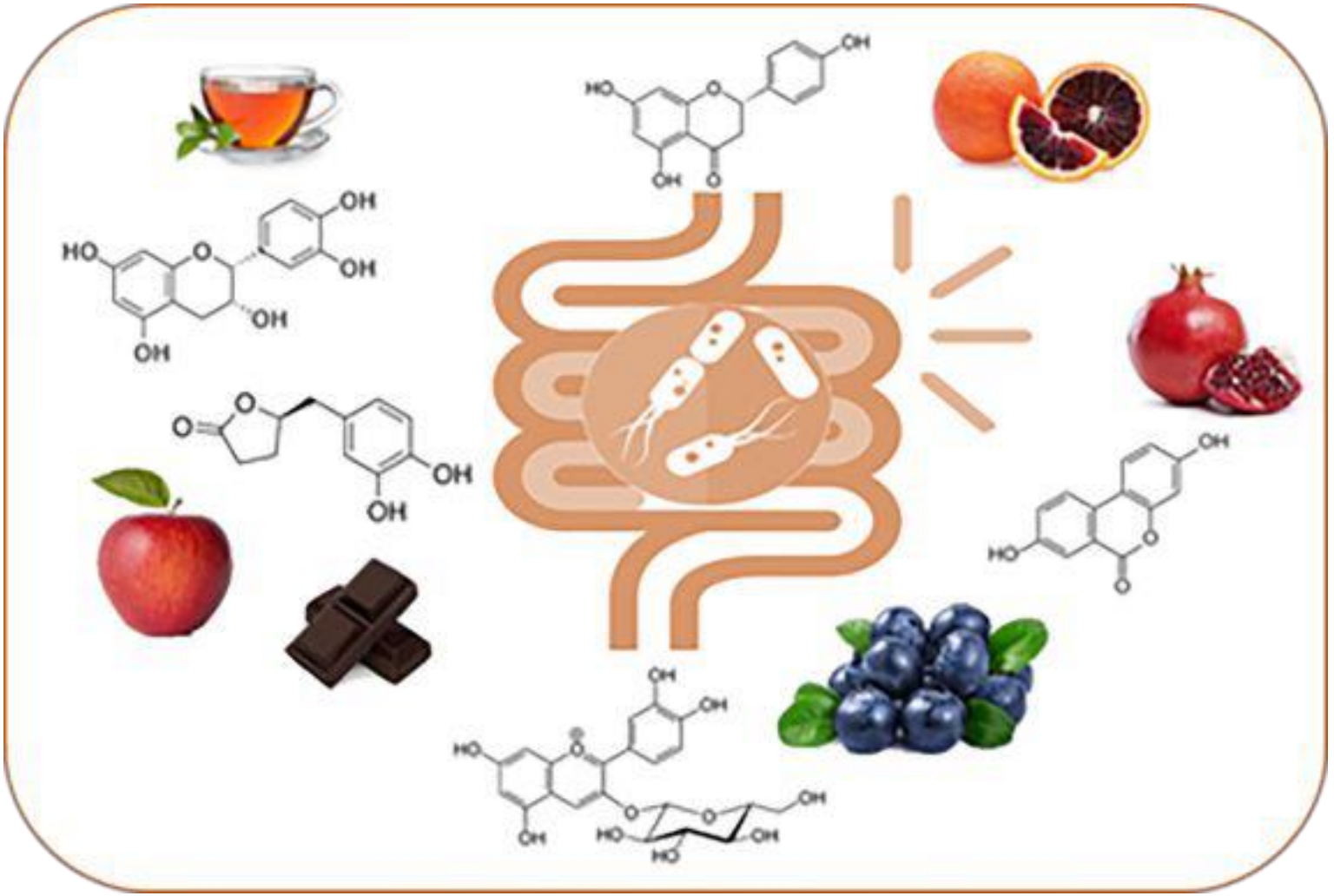
caffeic acid 3-glucuronide	84.4 ($2.2 \cdot 10^{-3}$)	118.9 ($2.4 \cdot 10^{-2}$)	48.5 ($3.2 \cdot 10^{-2}$)
caffeic acid 4-glucuronide	167.4 ($7.3 \cdot 10^{-4}$)	206.8 (NS)	126.3 ($2.2 \cdot 10^{-2}$)
ferulic acid glucuronide	582.2 ($2.1 \cdot 10^{-3}$)	57.5 (NS)	100.2 ($3.2 \cdot 10^{-2}$)
isoferulic acid glucuronide	1158.7 ($9.4 \cdot 10^{-3}$)	168.5 (NS)	80.9 (NS)
ferulic acid sulfate	44.9 ($4.8 \cdot 10^{-2}$)	55.3 (NS)	34.0 (NS)
isoferulic acid sulfate	109.1 ($3.6 \cdot 10^{-2}$)	157.1 (NS)	57.0 (NS)
methylpyrogallol sulfate	312.0 ($2.6 \cdot 10^{-3}$)	492.4 ($1.8 \cdot 10^{-2}$)	124.1 (NS)
4-methylcatechol glucuronide (isomer 1)	166.7 ($1.3 \cdot 10^{-3}$)	190.1 ($1.7 \cdot 10^{-3}$)	141.0 (NS)
4-methylcatechol glucuronide (isomer 2)	333.5 ($3.8 \cdot 10^{-2}$)	495.2 ($3.5 \cdot 10^{-2}$)	157.1 (NS)
vanillin	119.0 ($3.3 \cdot 10^{-2}$)	183.8 ($3.3 \cdot 10^{-2}$)	51.4 (NS)
flavan-3-ols			
(epi)catechin glucuronide (isomer 1)	169.7 ($1.2 \cdot 10^{-4}$)	281.4 ($7.6 \cdot 10^{-3}$)	72.9 (NS)
(epi)catechin glucuronide (isomer 2)	433.2 ($1.9 \cdot 10^{-5}$)	602.2 ($6.1 \cdot 10^{-3}$)	348.7 ($1.1 \cdot 10^{-2}$)
(epi)catechin glucuronide (isomer 3)	679.6 ($5.5 \cdot 10^{-4}$)	1053.2 ($2.1 \cdot 10^{-2}$)	341.6 (NS)
(epi)catechin glucuronide (isomer 4)	4462.2 ($6.1 \cdot 10^{-2}$)	7009.2 ($2.9 \cdot 10^{-3}$)	2157.8 ($1.1 \cdot 10^{-2}$)

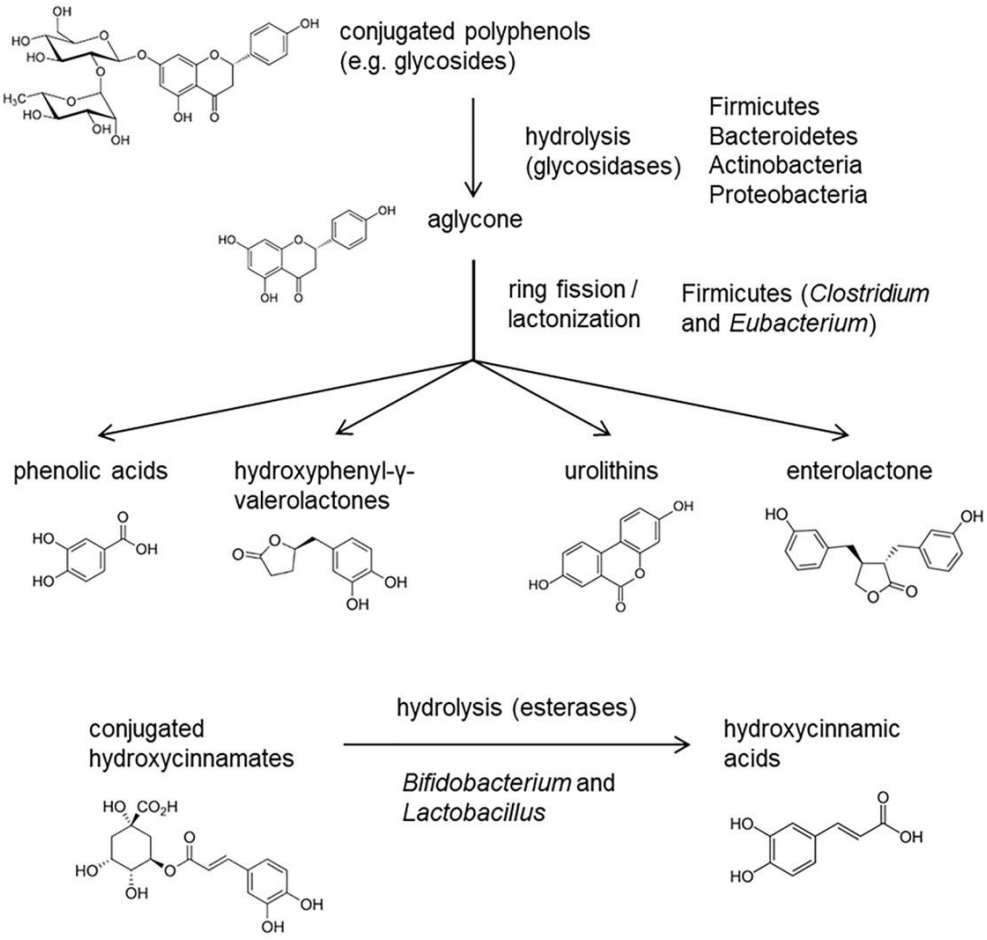
(epi)catechin sulfate (isomer 1)	1678.6 ($2.0 \cdot 10^{-2}$)	3790.6 ($3.2 \cdot 10^{-2}$)	1150.9 (NS)
(epi)catechin sulfate (isomer 2)	4687.8 ($5.5 \cdot 10^{-3}$)	5271.0 ($1.7 \cdot 10^{-2}$)	2157.8 (NS)
methyl(epi)catechin glucuronide (isomer 1)	294.7 ($1.3 \cdot 10^{-3}$)	324.1 (NS)	181.4 (NS)
methyl(epi)catechin glucuronide (isomer 2)	334.7 ($2.0 \cdot 10^{-3}$)	428.9 (NS)	168.6 (NS)
methyl(epi)catechin glucuronide (isomer 3)	1034.0 ($2.6 \cdot 10^{-8}$)	1232.1 ($3.5 \cdot 10^{-4}$)	813.9 ($2.7 \cdot 10^{-3}$)
methyl(epi)catechin glucuronide (isomer 4)	391.6 ($8.1 \cdot 10^{-8}$)	556.0 ($6.5 \cdot 10^{-4}$)	207.7 ($2.7 \cdot 10^{-3}$)
methyl(epi)catechin sulfate (isomer 1)	711.0 ($2.5 \cdot 10^{-3}$)	700.6 (NS)	721.8 (NS)
methyl(epi)catechin sulfate (isomer 2)	1194.0 ($1.9 \cdot 10^{-5}$)	725.3 ($1.9 \cdot 10^{-2}$)	1662.6 ($6.4 \cdot 10^{-3}$)
methyl(epi)catechin sulfate (isomer 3)	4601.4 ($1.2 \cdot 10^{-6}$)	1833.2 ($8.9 \cdot 10^{-4}$)	7485.0 ($4.6 \cdot 10^{-3}$)
methyl(epi)catechin sulfate (isomer 4)	1619.9 ($3.5 \cdot 10^{-8}$)	1291.8 ($1.3 \cdot 10^{-4}$)	1962.2 ($4.6 \cdot 10^{-3}$)
methyl(epi)catechin sulfate (isomer 5)	2701.9 ($6.0 \cdot 10^{-6}$)	1964.3 ($2.3 \cdot 10^{-3}$)	3471.5 ($1.1 \cdot 10^{-2}$)
methyl(epi)catechin sulfate (isomer 6)	817.5 ($1.2 \cdot 10^{-6}$)	861.1 ($1.6 \cdot 10^{-3}$)	767.7 ($4.4 \cdot 10^{-3}$)
hydroxyphenyl-γ-valeric acids & hydroxyphenyl-γ-valerolactones (microbiota)			
5-(3',4'-dihydroxyphenyl)-4-hydroxyvaleric acid 3'-glucuronide	367.7 ($9.5 \cdot 10^{-4}$)	681.5 ($2.3 \cdot 10^{-2}$)	116.6 (NS)
5-(3',4'-dihydroxyphenyl)-4-hydroxyvaleric acid 4'-glucuronide	884.5 ($8.5 \cdot 10^{-4}$)	1038.5 (NS)	723.5 (NS)

5-(3’,4’-dihydroxyphenyl)-4-hydroxyvaleric acid 3’-sulfate	2579.2 ($7.9 \cdot 10^{-4}$)	4018.7 ($2.8 \cdot 10^{-2}$)	1079.7 (NS)
5-(3’,4’-dihydroxyphenyl)-γ-valerolactone 3’-glucuronide	6782.4 ($1.2 \cdot 10^{-5}$)	10020.4 ($3.1 \cdot 10^{-3}$)	3544.4 ($3.2 \cdot 10^{-2}$)
5-(3’,4’-dihydroxyphenyl)-γ-valerolactone 4’-glucuronide	1415.3 ($1.0 \cdot 10^{-4}$)	2534.3 ($4.8 \cdot 10^{-3}$)	245.4 (NS)
5-(3’,4’-dihydroxyphenyl)-γ-valerolactone 3’-sulfate	948.5 ($4.1 \cdot 10^{-5}$)	605.6 ($4.2 \cdot 10^{-3}$)	195.6 (NS)
5-(3’,4’-dihydroxyphenyl)-γ-valerolactone 4’-sulfate	7772.8 ($1.2 \cdot 10^{-3}$)	13594.6 ($3.5 \cdot 10^{-2}$)	1673.8 (NS)
5-(3’,4’,5’-trihydroxyphenyl)-γ-valerolactone 3’-sulfate	2393.4 ($7.6 \cdot 10^{-4}$)	331.6 ($8.9 \cdot 10^{-4}$)	320.6 (NS)
5-(3’,4’,5’-trihydroxyphenyl)-γ-valerolactone 4’-sulfate	12184.4 ($2.0 \cdot 10^{-2}$)	22850.4 (NS)	548.7 (NS)
5-(4’-hydroxy-3’-methoxyphenyl)-γ-valerolactone	377.3 ($9.3 \cdot 10^{-3}$)	507.2 ($3.2 \cdot 10^{-2}$)	247.5 (NS)
5-(4’-hydroxy-3’-methoxyphenyl)-γ-valerolactone glucuronide	4957.0 ($7.2 \cdot 10^{-5}$)	7987.1 ($4.5 \cdot 10^{-3}$)	1800.6 (NS)
5-(4’-hydroxy-3’-methoxyphenyl)-γ-valerolactone sulfate	1342.5 ($1.1 \cdot 10^{-4}$)	2065.2 ($4.5 \cdot 10^{-3}$)	589.8 (NS)
Urolithins (microbiota)			
urolithin A glucuronide	23040.5 ($7.6 \cdot 10^{-4}$)	38347.0 ($3.5 \cdot 10^{-4}$)	10649.5 (NS)
urolithin A sulfate	998.1 ($8.2 \cdot 10^{-4}$)	1397.2 ($1.3 \cdot 10^{-3}$)	660.4 (NS)
Enterolignans (microbiota)			
enterolactone glucuronide	2882.1 ($3.2 \cdot 10^{-2}$)	495.5 ($2.3 \cdot 10^{-2}$)	83.9 (NS)

Anthocyanins			
cyanidin 3-glucoside	523.6 ($8.5 \cdot 10^{-4}$)	649.3 ($1.0 \cdot 10^{-2}$)	421.4 (NS)
xanthine alkaloids			
theobromine	2138.1 ($3.2 \cdot 10^{-3}$)	3983.6 ($2.1 \cdot 10^{-2}$)	215.7 (NS)
other flavonoids			
naringenin glucuronide	520.7 (NS)	804.3 ($2.6 \cdot 10^{-2}$)	237.1 (NS)
luteolin 3’-glucuronide	19.6 ($4.3 \cdot 10^{-2}$)	34.6 ($9.2 \cdot 10^{-3}$)	3.3 (NS)

Graphic for Table of Contents





The interplay between the gut microbiota and the metabolism of polyphenols.