Host: microbiome co-metabolic processing of dietary polyphenols – an acute, single blinded, cross-over study with different doses of apple polyphenols in healthy subjects.

Kajetan Trost[1,6,§#], Maria M. Ulaszewska[1§], Jan Stanstrup[1,#], Davide Albanese[2], Carlotta De Filippo[3], Kieran M. Tuohy[1*], Fausta Natella[4], Cristina Scaccini[4], Fulvio Mattivi[1,5*]

[1] Department of Food Quality and Nutrition, Research and Innovation Centre, Fondazione Edmund Mach, Via Mach 1, 38010 San Michele all'Adige (TN), Italy;

[2] Department of Computational Biology, Research and Innovation Centre, Fondazione Edmund Mach, Via Mach 1, 38010 San Michele all'Adige (TN), Italy;

[3] Institute of Biology and Agrarian Biotechnology, National Research Council, Pisa, Italy

[4] Research Center for Food and Nutrition (CREA-NUT), CREA Agricultural Research Council, Via Ardeatina 546 - 00178 Roma, Italy

[5]Center Agriculture Food Environment, University of Trento, San Michele all'Adige, Italy

[6]Systems Medicine, Steno Diabetes Centre Copenhagen, Denmark

*Corresponding addresses: fulvio.mattivi@unitn.it, kieran.tuohy@fmach.it

[§]These authors have contributed equally to this work

[#]Present address J.S: Department of Plant and Environmental Sciences Faculty of Science, University of Copenhagen, Copenhagen, Denmark

Key words: apple, polyphenols, nutrikinetics, metabolomics, microbiota, blood, urine, mass spectrometry

Declaration of interest: none.

ABSTRACT (200 words):

Apples are one of the most commonly consumed fruits and their high polyphenol content is considered one of the most important determinants of their health-promoting activities. Here we studied the nutrikinetics of apple polyphenols by UHPLC-HRMS metabolite fingerprinting, comparing bioavailability when consumed in a natural or a polyphenol-enriched cloudy apple juice. Twelve men and women participated in an acute single blind controlled crossover study in which they consumed 250 mL of cloudy apple juice (CAJ), Crispy Pink apple variety, or 250 mL of the same juice enriched with 750 mg of an apple polyphenol extract (PAJ). Plasma and whole blood were collected at time 0, 1, 2, 3 and 5 h. Urine was collected at time 0 and 0-2, 2-5, 5-8, and 8-24 h after juice consumption. Faecal samples were collected from each individual during the study for 16S rRNA gene profiling. As many as 110 metabolites were significantly elevated following intake of polyphenol enriched cloudy apple juice, with large inter-individual variations. The comparison of the average area under the curve of circulating metabolites in plasma and in urine of volunteers consuming either the CAJ or the PAJ demonstrated a stable metabotype, suggesting that an increase in polyphenol concentration in fruit does not limit their bioavailability upon ingestion. Faecal bacteria were correlated with specific microbial catabolites derived from apple polyphenols. Human metabolism of apple polyphenols is a co-metabolic process between human encoded activities and those of our resident microbiota. Here we have identified specific blood and urine metabolic biomarkers of apple polyphenol intake and identified putative associations with specific genera of faecal bacteria, associations which now need confirmation in specifically designed mechanistic studies.

KEYWORDS: apple, polyphenols, nutrikinetics, metabolomics, microbiota, blood, urine

1. INTRODUCTION

Apples are the most commonly consumed fruit in the world and are an important source of phytonutrients (Konopacka et al., 2010)(Herrick, Rossen, Nielsen, Branum, & Ogden, 2015). 2015). Together with sugars, organic acids and minerals, apples are rich in fibre, vitamins and polyphenols (J. Wu et al., 2007). Several epidemiological studies have reported protective health effects for apples (Theodoratou et al., 2007, Hansen et al., 2010, Feskanich et al., 2000, Gallus et al., 2005, Sesso, Gaziano, Liu, & Buring, 2003, Scalbert et al., 2014, Manach, Scalbert, Morand, Rémésy, & Jime, 2004). It appears that apples may play a significant role in reducing the risk of a wide variety of chronic disease and maintaining a healthy lifestyle in general. Their consumption has been most consistently associated with reduced risk of cancer, heart disease, asthma, and type II diabetes. Apple consumption was also positively associated with increased lung function and increased weight loss (Butland, 2000; Conceição de Oliveira, Sichieri, & Sanchez Moura, 2003; Knekt et al., 2002; Tabak, Arts, Smit, Heederik, & Kromhout, 2001). Some intervention studies involving feeding with apples (Muraki et al., 2013; Van Velzen et al., 2009) or apple extracts (Soriano-Maldonado, Hidalgo, Arteaga, de Pascual-Teresa, & Nova, 2014, Theuwissen & Mensink, 2008) correlated these health effects with apple phenolic content (Manach et al., 2004, Scalbert et al., 2014, Hyson, 2011). Moreover, many apple polyphenols showed interesting health promoting properties in cellular and animal models. Phloridzin, one of dihydrochalcones contained in apples and in apple-derived products, lowers blood sugar in diabetic rats (Masumoto, Akimoto, Oike, & Kobori, 2009, Najafian et al., 2012) through its inhibition of intestinal glucose absorption via SGLT1 and SGLT2, the sodium/glucose cotransporters in the intestine and kidney respectively. Microbial metabolites of apple procyanidins have been shown to inhibit proliferation of intestinal cancer cells (Caco2) *in vitro* (Gossé et al., 2005); this anti-cancerogenic activity has been suggested to contribute to the reduced risk of colorectal cancer reported for people consuming more than 2 apples per day (Rossi et al., 2012). Additionally, there are also reports suggesting apple phenols may prevent lipid peroxidation and suppress metalloprotein activity (Gerhauser, 2008).

Apple phenolics comprise of four main classes of polyphenol: cinnamic acids, flavanols, dihydrochalcones and flavonols, many of which are antioxidants. In addition to these, apple also contains small amounts of anthocyanins, stilbenes and triterpenoid acids (Farneti et al., 2015). Amongst the flavanols, the most abundant compounds are epicatechin and oligomeric procyanidins (Vrhovsek, Rigo, Tonon, & Mattivi, 2004), the latter present in large quantities. Chlorogenic acid is the main cinnamic acid, and phloridzin is the main dihydrochalcone. Finally, in the flavonols and anthocyanins, a mixture of various mono-glycosylated forms of quercetin and cyanidin are present.

A huge variability among varieties and species is present for both their concentration and pattern leaving room for a potential increase of the content of apple polyphenols in the fruit via innovative breeding strategies (Farneti et al., 2015). This raises an important question: is the bioavailability polyphenols be affected by their higher concentration upon ingestion?

One of the main limitations of existing data on bioavailability is that studies are often restricted to a single class of polyphenols (Rago, Gurdeniz, Gitte, & Dragsted, 2014, Kahle et al., 2007, Lee, Ebeler, Zweigenbaum, & Mitchell, 2012) and there are still very few human studies describing comprehensively the kinetics and transformation of apple polyphenols. This is confounded by the fact that, at least for more complex molecules or large oligomers, such as the proanthocyanidins, metabolism involves the human gut microbiota. The gut microbiota is a complex collection of many hundreds of different microbial species which reside within the human intestine. Indeed, co-metabolic processing by gut microbiota and host metabolic pathways are responsible for polyphenol catabolism and as consequence of their biological activities and impact on human health. Therefore, gut microbiota composition (species and relative abundances of microorganism) may affect the profile of polyphenol catabolites. However, we still know very little about which species are involved in these microbial catabolic processes, especially for any given class of polyphenols or indeed, whether presence/absence or differences in bacterial relative abundance determine overall microbiota catabolic output and the fate of plant derived bioactive nutrients (G. D. Wu et al., 2016, Guadamuro et al., 2015 Tamura et al., 2015).

Considering the complex transformations and overlapping metabolic pathways thus far described for different classes of apple polyphenols, there is no single conventional targeted analytical method which can provide accurate and precise measurement of the common apple polyphenols or their derivatives. Untargeted MS based metabolomics together with multivariate statistical analysis partially solves this problem providing the possibility to explore wide ranges of metabolites in a semi-quantitative manner.

The aim of the study was to identify the metabolic products of various classes of apple polyphenols upon ingestion by healthy subjects using an untargeted metabolomics approach and to describe the nutrikinetics of these metabolites in plasma and urine over respectively a 5 h and 24 h period. A second aim was to evaluate whether a higher concentration of polyphenols in the apple matrix, (i.e. the consumption from the same volunteers either of the cloudy apple juice (CAJ) or the polyphenol enriched apple juice (PAJ)) would lead to a corresponding increased metabolic output or would rather result in altered metabotype. An additional aim was to assess whether the variability of the pattern and circulating levels of apple derived plasma/urine microbial catabolites among the participants to the study could be related to a specific composition of the gut microbiota.

For this purpose, we designed a cross-over single blind trial at two dose levels of apple polyphenols. Twelve healthy subjects in a fasted state consumed either 250 mL of natural cloudy apple juice (CAJ) or the same apple juice enriched with an apple extract containing all four groups

of apple polyphenols (PAJ), followed by regular blood and urine sampling up the 24 hours and profiling of faecal microbiota.

2. MATERIALS AND METHODS

2.1 Chemical and reagents

HPLC-grade methanol, acetonitrile, 2-propanol and formic acid were obtained from Sigma Aldrich. The ultrapure water was obtained by purifying demineralized water in a Milli-Q system from Millipore (Bedford, MA, USA). Internal standard creatinine labeled ^{13}C was purchased from Sigma Aldrich and d_5 labeled *trans*-cinnamic acid, chenodeoxycholic acid-d_4 and taurocholic acid-d_5 were obtained from CDN ISOTOPES, Inc. (Pointe-Claire, Quebec, Canada). PVDF syringe filters 0.45 µm were obtained from Millipore, while Sirocco protein precipitation plate from Waters, (USA). Internal standards for urine samples were prepared in pure methanol with ^{13}C creatinine at 9.33 ppm and *trans*-cinnamic acid-d_5 at 5.5 ppm. For plasma and blood methanol extracts, internal standards additionally contained 0.5 ppm of chenodeoxycholic acid-d_4 and 0.5 ppm of taurocholic acid-d_5.

2.2 Experimental juices

Experimental juices were kindly provided by the company Macè Srl (http://www.macefruit.com). The cloudy apple juice (CAJ) was made from the Crispy Pink variety, product code SME1/6AP. In order to maintain its full nutritional properties it was cloudy and preserved by high-pressure pasteurisation. Enriched juices (PAJ) were prepared by adding 0.75 g of an apple extract into 250 mL of cloudy juice. The apple extract provided by Denk Ingredients GmbH (http://denkingredients.de; art. no: 968267) increased the content of polyphenols without changing the matrix. Juices were packaged and stored at -20°C until use.

2.2.1 Juice preparation and analysis

Samples of juice were analysed in triplicate as previously described (Vrhovsek et al 2012). Briefly, 0.5 mL of juice or PAJ was diluted with 0.5 mL of methanol, including 4 ppm of rosmarinic acid as an internal standard. The sample was centrifuged at 4 °C and 15,000 rpm for 10 min, and the supernatant was filtered with 0.22 µm, 13 mm Millex-GV PDVF filters (Millipore, USA). Multiclass polyphenols were analysed with Xevo TQMS (Waters, USA) faced to Acquity UPLC. The content of proanthocyanidins and their mean degree of polymerization (mDP) was evaluated by LC-DAD-MS before and after phloroglucinolysis, according to validated protocols (Gris et al., 2011). The results of polyphenols concentration in the CAJ and PAJs are listed in Supplementary Material 1.

2.3 Study design

Twelve, non-smoking, healthy volunteers (8 males and 4 females), aged 21 to 42 years, with a BMI between 18.5 and 25 kg/m^2 (normal weight), participated in the randomized crossover study.

Volunteers were instructed to refrain from consuming phenol-rich foods and beverages (wine, coffee, tea, fruits and vegetables), dietary supplements and medications in the three days prior to the experiments. To ensure adherence to the dietary instruction, we asked the subjects to keep a 3-day dietary record prior the study participation. The subjects reported to the laboratory on two separate occasions, two weeks apart, after fasting overnight (10-12 h). Three types of biological fluids were taken: urine, plasma and venous blood. A venous blood sample was taken at time 0. Immediately after the first blood collection, participants were provided with a glass of CAJ (250 mL) (CAJ Treatment) or a glass of PAJ (250 mL) (PAJ Treatment). The order of treatment allocation was randomly assigned: 6 subjects started with CAJ and the other 6 with PAJ. Further blood samples were taken at 1, 2, 3 and 5 hours after juice consumption. After sampling, the blood was transferred into ice-cold 95 % aqueous methanol and processed according to Vanzo et al. (Vanzo et al., 2013); additionally plasma was separated by centrifugation for 20 min at 1,500 g and stored at -80 °C. Urine was collected at time 0 and between 0 and 2, 2 and 5, 5 and 8, and 8 and 24 h after juice consumption. Urine samples from 0 to 8 hours were stored at 4 °C immediately after voiding, while 8-24 hour urine samples were collected in 2.5 L plastic bottles containing 9 mL hydrochloridric acid 20 % as a preservative. Urine samples were aliquoted and stored at -80 °C until analysis. Before the supplementation, a single faecal sample was collected from each subject and stored at -80 °C. All the subjects gave written informed consent before joining the study, and all procedures were approved by the Ethical Committee of the National Research Institute for Food and Nutrition ("Apple fruit quality in the post-genomic era from breeding new genotypes to post-harvest: nutrition and health" 0003288/01.11).

2.4 Biological sample preparation and analysis

The method for preparation of plasma samples was described by (Gürdeniz, Kristensen, Skov, & Dragsted, 2012) and slightly modified. Briefly, 100 μL of heparin plasma was thawed on ice and placed in a Sirocco protein precipitation plate (Waters, USA) with 200 μL of internal standards dissolved in methanol and 200 μL of solvent consisting of 0.1 % formic acid in methanol: water (4:1 V/V). Samples were filtered using a positive pressure-96 manifold (Waters, USA). Additionally, the filtering plate was eluted with 400 μL of solvent consisting of acetonitrile: acetone (4:1 V/V). Samples were evaporated with a gentle stream of nitrogen to dryness using a Techne Dr-block DB 3D heater at room temperature and redissolved with 200 mL of water:methanol (1:1 V/V).

For the urine samples 200 μL of sample was added to 200 μL of internal standards dissolved in methanol. Samples were vortexed and centrifuged at 4 °C and 15,000 rpm followed by filtration with 4 mm Millex DURAPORE PVDF filters with 0.45 μm pore size (Millipore, USA) and diluted with 600 μL of deionized water (Zhang, Creek, Barrett, Blackburn, & Watson, 2012).

1 mL of blood methanol extract was added to 200 μL of internal standard dissolved in methanol (Vanzo et al 2013). Sirocco protein precipitation plate and positive pressure manifold (Waters,

USA) were used for filtration. After filtration the samples were evaporated under nitrogen and redissolved with 200 μL of methanol:water (1:1 V:V). Plasma glucose was measured by commercial kits purchased from SIGMA Chem Co (St Louis, USA).

2.5 Chromatographic and mass spectrometry conditions

Samples were analysed by a hybrid linear ion trap Fourier Transform (LTQ FT) Orbitrap mass spectrometer (Thermo Fisher, Bremen, Germany) interfaced to a Dionex HPLC system, consisting of an auto-sampler and quaternary gradient HPLC-pump. Chromatographic separation of compounds was performed using a Kinetex C18 column (150 mm × 2.1 mm I.D., particle size 3.5 μm) with pre-column 4.0 mm x 2.0 mm I.D (Phenomenex Torrance, CA, USA). The flow rate was 300 μL/min and column temperature was maintained at 30 °C. Mobile phases used were: Milli-Q water (Solvent A), acetonitrile (Solvent B), and 2-propanol (Solvent C) all with 0.1 % formic acid. For urine, 95 % of solvent A and 5 % of solvent B were maintained for one minute, followed by an increase of solvent B to 45 % in 12 min and to 80% in 2 min and maintained for 2 min. The initial composition was restored in 3 min. Plasma and blood methanol extracts were analysed with the following gradient: 95 % of solvent A and 5 % of solvent B maintained for 1 min followed by an increase of solvent B to 100 % in 19 min. Then the phase C volumetric ratio was increased to 50 % in 5 min, while solvent A was maintained at 0 %. In two minutes the initial conditions were restored and held for 3 min.

The Orbitrap LTQ was equipped with an Electrospray Ionization (ESI) probe and operated in both positive and negative ionization modes. The conditions have been described elsewhere (Ulaszewska et al., 2016). Briefly the mass spectrometer operated under data-dependent-acquisition (DDA) mode during the complete chromatographic run. The resolving power for MS scan was 30,000 and for MS2 scans 7,500. The static exclusion list was made up of the 300 most abundant ions created by the injection of solvents, which followed the same preparation procedures as the samples. Dynamic exclusion allowed 3 repeated counts of the same ion in 15 s, while the exclusion duration was 45 s. The sequences were randomised with regard to participants and treatments, while samples from the same individual were kept together. Every 20 samples a quality control block was analysed consisting of solvents, internal standards and quality control samples.

2.6 Biomarker Identification

Markers contributing to the discrimination between two treatments were identified through a multiple-step procedure. Molecular ion and in-source molecular fragments were assigned as one compound based on a mass accuracy approach and peak shape. Discriminative markers were then compared with the monoisotopic molecular weight, chemical structures and LC-MS/MS spectra of metabolites proposed by freely available databases: m/z Cloud (www.mzcloud.org); the

Human Metabolome Database (Wishart et al., 2013), the METLIN (Smith et al., 2005), the MassBank (Horai et al., 2010) and the LIPID MAPS (Sud et al., 2007) databases. Mass accuracy was set to 2 mDa while searching on-line. Additionally, information from MS^n experiments were introduced to MetFusion to get candidate structures (Gerlich & Neumann, 2013). Final identification was achieved after a combination of LC-$HRMS^2$, LC-$HRMS^3$ experiments, on-line database information and literature verification. Levels of identification reported in Supplementary Materials 2 are as follow: Level I corresponds to compounds identified by matching masses and retention times with authentic standards in the laboratory; Level II corresponds to compounds identified by matching with LC-HR-MS, LC-HR MS/MS and LC-HR-MS^n of standards reported in the literature or to spectra from databases and literature. Compounds identified only by spectral similarities to a similar compound class and literature knowledge are reported as level III. Unknown compounds are reported as level IV.

2.7 DNA extraction, PCR amplification of the V3-V5 region of bacterial 16S rDNA

DNA extraction was performed using the FastDNA™ SPIN Kit for Feces (MP Biomedicals, Santa Ana, CA, USA) following manufacturer's instructions. DNA integrity and quality were checked on 1 % agarose gel TAE 1X and quantified with a NanoDrop® spectrophotometer. For each DNA sample, 16S rRNA gene was amplified using fusion primer set specific for V3-V5 hypervariable regions (F333: 5'-TCCTACGGGAGGCAGCAG-3' and R934: 5'-TGTGCGGGCCCCCGTCAATT-3') containing adaptors, key sequence and barcode (Multiple IDentifier) sequences as described by the 454 Sequencing System Guidelines for Amplicon Experimental Design (Roche, Basel, Switzerland). PCR reactions were performed using the FastStart High Fidelity PCR system (Roche, Basel, Switzerland) according to the following protocol: 5 min at 95 °C, 25 cycles of 30 sec at 95 °C, 30 sec at 58 °C and 1 min at 72 °C, followed by a final extension of 8 minutes at 72°C. The PCR reaction mix contained 1X FastStart High Fidelity PCR buffer 1.8 mM MgCl2, 200 µM of dNTPs, 0.4 µM of each primer (Eurofins, PRIMM, Milano, Italy), 2.5 U of FastStart High Fidelity Polymerase Blend and 10 ng of gDNA as template.

2.8 Library construction and pyrosequencing

The PCR products were analyzed by gel electrophoresis and cleaned using the AMPure XP beads kit (Beckman Coulter, Brea, CA, USA) following the manufacturer's instructions, quantified via quantitative PCR using the Library quantification kit – Roche 454 titanium (KAPA Biosystems, Boston, MA) and pooled in equimolar way in a final amplicon library. The 454 pyrosequencing was carried out on the GS FLX+ system using the XL+ chemistry following the manufacturer's recommendations (Roche, Basel, Switzerland).

2.9 Metagenomics data analysis

Pyrosequencing resulted in a total of 138,280 reads for 16S rDNA with a mean of 11.523 sequences per sample. Raw 454 files were demultiplexed using the Roche's sff file software. Reads were preprocessed using the MICCA pipeline (version 0.1, http://compmetagen.github.io/micca/) (Albanese et al 2015). Forward and reverse primers trimming and quality filtering were performed using micca-preproc (parameters -f TCCTACGGGAGGCAGCAG - r TGTGCGGGCCCCCGTCAATT -O 15 -l 300 -q 22) truncating reads shorter than 300 nt.

De-novo sequence clustering, chimera filtering and taxonomy assignment were performed by micca-otu-denovo (parameters -s 0.97 -c): operational taxonomic units (OTUs) were assigned by clustering the sequences with a threshold of 97 % pair-wise identity, and their representative sequences were classified using the RDP (Wang et al 2007) software version 2.7. Template-guided multiple sequence alignment (MSA) was performed using PyNAST (Caporaso et al., 2010) (version 0.1) against the multiple alignment of the Greengenes database (DeSantis et al., 2006) (release 13_05) filtered at 97 % similarity. Finally, a phylogenetic tree was inferred using FastTree55 and micca-phylogeny (parameters: -a template --template-min-perc 5). Sampling heterogeneity was reduced by rarefaction (6732 sequences per sample). Alpha (within-sample richness) and beta-diversity (between-sample dissimilarity) estimates were computed using the phyloseq R package (McMurdie & Holmes, 2013). Permutational MANOVA (PERMANOVA) statistical tests were performed using the R package vegan (adonis()function) with 999 permutations. To compare the relative abundances of OTUs between the two forests, two-sided, unpaired Welch t-statistics were computed using the function mt() in the phyloseq library and the p-values were adjusted for multiple comparison controlling the family-wise Type I error rate (minP procedure) (Westfall & Young, 1993).

2.10 Metabolomic data treatment and statistics

Data from untargeted assays were processed using Sieve 2.0 (Thermo Scientific, USA), which aligned, picked peaks and compared deconvoluted data with internal and external libraries. Framing was set to a 10 ppm window and 1 min time window ranging from 70 to 700 Da for urine and 100-1000 Da for plasma and methanol extracts. The maximum number of frames was set to 5,000.

Peak lists created by Sieve were submitted to statistical evaluation in R (R Core Team, 2013) (http://www.r-project.org). The urine dataset was normalised by urine volume, and time point based cumulative values were calculated and used throughout the statistical evaluation. Plasma and blood methanol extracts datasets were adjusted for baseline (time 0) values. Two linear mixed models were fitted for each feature and compared. The first model included treatment (two doses of polyphenols, i.e. CAJ and PAJ intake) and time main effect and interactions, whereas the second model only contained a time effect. In both models, a subject-specific random effect was

also included. Subsequently, the collection of p values for all features was corrected for multiple testing, according to the two-stage Benjamini and Hochberg step-up false discovery rate (FDR) controlling procedure (Benjamini et al 2006). Features with a corrected p value (q value) lower than 1 % were selected for further evaluation.

Nutrikinetic curves at the population level for each biomarker were plotted using Statistica 9.0 (StatSoft, USA), based on extracted integrated intensities, and approximated via non-compartmental pharmacokinetic (PK) analysis. With the aim to determine the average degree of exposure to the several circulating apple metabolites following apple juice consumption (in particular, area under the curve - AUC, and associated nutrikinetics) (van Duynhoven, van Velzen, & Jacobs, 2017).

In order to correlate operational taxonomic units (OTUs) provided by pyrosequencing with metabolomics data, urine sampling timeframe depending integrated intensities were multiplied by urine volume in mL and summed together. In this case they represent an equivalent of total amount excreted in 24 hours. Equivalents of total absorbed amount of biomarkers in plasma were calculated using area under curve method. As most of the time nutrikinetic curves did not reach appropriate shape, partial area under curves were calculated using trapezoid rule with equation: $y = \Sigma\ (((II_n+II_{n-1})/2)*(t_n-t_{n-1}))$. Ratio was calculated using area under curves between PAJ treatment and CAJ treatment. Results are shown in Table 1, Figure Z and Supplementary Material 3 and 4.

2.11 Metabolomic data-sharing.

The study participants have given written consent to pseudonymised data-sharing. All untargeted data in mzXML format and metadata are available for download from the MetaboLights public repository http://www.ebi.ac.uk/metabolights/ (Haug et al., 2013; Salek et al., 2013). The Ager data are deposited in MetaboLights with the persistent unique public identifier MTBLS473..Permanent link https://www.ebi.ac.uk/metabolights/MTBLS473.

3. RESULTS AND DISCUSSION

Compliance with the dietary instruction was good (all the subjects followed the 3-days polyphenol-poor diet before the experiment day. Blood, plasma and urines of subjects supplemented with cloudy apple juice (CAJ) or polyphenol-enriched apple juice (PAJ) were analysed, working in positive and negative ionization modes. The total number of statistically significant features discovered (also called putative biomarkers) was varied between matrices, plasma: 6 ESI^+ and 154 ESI^-, methanol blood: 45 ESI^+ and 186 ESI^- and urine: 221 ESI^+ and 320 ESI^-.

The list of features discriminating the composition of urine and plasma of subjects in the CAJ treatment group from the PAJ treatment group are shown in Table 1. We classified these

putative biomarkers into several groups, each containing closely related derivatives. Each group was build combining the information on the chemical class of their precursors (metabolites) or their common pathways of origin (for breakdown catabolites): the derivatives of dihydrochalcones, naringenin, (epi)catechin, valerolactone, catechol, chlorogenic acid, quercetin, hippuric acids, tyrosine and tryptophan, fatty acids, and several classes of phenolic acids derivatives, namely: vanillic acid, cinnamic acid, propionic and acetic acids.

3.1 Apple polyphenol metabolites

As shown in Figure 1, 2 and Supplementary Materials 3, apple polyphenol metabolites were found after intake of both juices. Most of these compounds represent the glucuronide, methyl and sulphate conjugates of native apple polyphenols. These have been previously reported in urine and/or plasma after cider, fruits, tea and nuts consumption ((Borges, Lean, Roberts, & Crozier, 2013; Borges, Mullen, Mullan, Lean, & Roberts, 2010; Hooft, Mihaleva, Vos, Bino, & Vervoort, 2012; Ito, Gonthier, Manach, Morand, & Mennen, 2005; Manach, Williamson, Morand, & Scalbert, 2005; Marks, Mullen, Borges, & Crozier, 2009; Pimpão et al., 2014; A. R. Rechner, Pannala, & Rice-Evans, 2001; Andreas R. Rechner, Spencer, Kuhnle, Hahn, & Rice-Evans, 2001; Urpi-Sarda, Monagas, et al., 2009; Urpi-Sarda, Garrido, et al., 2009)). In agreement with previous results (Olthof, Hollman, Buijsman, Van Amelsvoort, & Katan, 2003), very few polyphenols were found in their parental forms. In fact, only small amounts of chlorogenic acids were detected in urine and no (epi)catechins, quercetin or phloridzin was found.

Although the urine metabolite pattern was quite similar (for all the chemical groups) after CAJ or PAJ supplementation, PAJ consumption determined a several order of magnitude increase in total polyphenol bioavailability, as evidenced by the total amount excreted in urine (Figure 2). This experiment demonstrated that an increase of polyphenols in apple, with domains of validity (Scannell & Bosley, 2016) within the concentration range compatible with the natural variability of polyphenols in apples (Farneti et al., 2015) (Supplementary Table 1) would lead to an increase of their concentration in biofluids.

Plasma integrated intensity-time (II-time) curves and urine cumulative excretion curves of selected metabolites from the main polyphenols families are shown in Figure 1. These curves clearly show two distinct nutrikinetics patterns.

Pattern 1: human metabolites. Metabolites of phloretin (M1-M4) and (epi)catechin (M9-M15), as well as small phenolic acids namely vanillic acid sulfate (M83), ferulic acid sulfate (M54) and feruloylquinic acid isomers (M49-M50) reach their maximum plasmatic concentrations within the first hour post-dose. This early absorption peak is followed by a rapid decrease plasma concentration (within next five hours) and a fast appearance in urine. These data suggest that this fraction of the native polyphenols is quickly absorbed and metabolised in the upper gut with little or no contribution from the human colonic microbiota and rapidly excreted in urine.

Pattern 2: microbial catabolites. On the contrary, the derivatives of valerolactones (M18, M19, M21-M23, M33, M34, M36-M39), catechol (M43, M46), hippuric (M74-M76), propionic and acetic acids (M60, M62, M66-M69, M72) did not reach their maximum concentrations in plasma within the five hours after juice ingestion. Moreover, they are characterized by a delayed appearance in urine (24 hours), suggesting a prolonged metabolism along the gut with a likely involvement of the gut microbiota.

The observation of coherent trends for both plasma concentration and urine excretion profiles for the same metabolic pathways provide experimental evidence of the presence of these two distinct nutrikinetics patterns. Cumulative excretion curves for the catabolites of valerolactones (M17-M39, M42), catechol (M43-M48), hippuric acid (M74-M82), chlorogenic (M55-M59), propionic and acetic acids (M60-M65, M68-M71, M73) were characterized by increasing concentrations over the 24 h test period, with no maximum concentration. Conversely, metabolites of phloretin and naringenin M1-M7, (epi)catechin (M9-M16), and chlorogenic acids (M49-M54) reached their maximum of excretion within 8 hours post-dose. See Figure 1 and 3, and Supplementary Materials 3 and 4 for details.

The apple flavanols are known to be the main class of apple flavonoids, and their circulating metabolites have a very important bioavailability, as shown by really high (totalling 261 µM) urinary concentrations after repeated green tea intake (Brindani et al., 2017). The catabolites of the flavanols are possibly involved in the improvement of endothelial function following the consumption of apple with the skin (Bondonno et al., 2018) as result coherent with health claims approved by European Food Safety Authority EFSA panel on Dietetic products, Nutrition and Allergies (EFSA) for the role of cocoa procyanidins in the maintenance of normal endothelium-dependent vasodilation. ("Scientific Opinion on the substantiation of a health claim related to cocoa flavanols and maintenance of normal endothelium-dependent vasodilation pursuant to Article 13(5) of Regulation (EC) No 1924/2006," 2012).

3.2 Dihydrochalcone metabolites (M1-M5)

The most characteristic apple polyphenol is phloridzin which belongs to family of chalcones. Although its concentration in apple fruit flesh is not very high, phloridzin is a unique compound characteristic for the Rosaceae family, and therefore also for apples. Among apple bioactives, dihydrochalcones are the most interesting targets for innovative breeding, since it has been observed that their concentration (Farneti et al, 2015) and pattern (Ibdah et al 2014) have been strongly and negatively affected by domestication.

During digestion, phloridzin is first deglycosylated to give phloretin, which then undergoes phase I and II metabolism. Indeed, sulfate and glucuronide conjugates of phloretin (5 metabolites in urine and one in plasma) were found to be statistically significant markers of apple juice intake, while phloretin was found only in urine after PAJ intake. The pattern of urine metabolites presented as AUC in Figure 2 shows that glucuronidation was the main route of conjugation; while sulfate and

glucuronide-sulfate metabolites were minor conjugates of phloretin. Metabolite intensities were several orders of magnitude higher after ingestion of the PAJ compared to the CAJ as shown in Figure 1 with the ratio of AUC_{PAJ} to AUC_{CAJ} for urine phloretin glucuronide and sulfate conjugates ranging between 125-733.

Apple and apple juice possess a low glycemic index (Makarova et al 2015) and this is probably due both to the high proportion of fructose in its carbohydrate fraction and to the presence of phloridzin, which appears to inhibit glucose absorption in the small intestine (Ehrenkranz et al 2005; Chan et al 2012). The apple juices utilized in this study contained about 11 g total carbohydrates per 100 mL, that correspond to 25 g of carbohydrates per ingested dose (250 mL) and the principal sugar was fructose (60%) (data not shown). The ingestion of a similar amount of readily digestible carbohydrate should induce an increase of plasma glucose, with a pick point at 30 min. Unfortunately, our experiment was designed to measure the nutrikinetics of apple polyphenols and we were limited in the number of blood samples collected for ethical considerations. Thus, no time point was collected at 30 min post juice consumption. The first time points, at 1, 2 and 3 hours post intake, did however show a small glycemic curve fitting with the typical postprandial plasma glucose response in healthy subjects. With higher levels at 1 h, a dip at 2 h and a return to basal values at 3 h. Figure 4 combines the kinetic curves of phloretin glucuronide(I) in urine (A), plasma (B), metabolites contribution to total phloretin metabolism (C), and the postprandial glucose response (D).

3.3 Naringenin metabolites (M6-M7)

It is known that naringin is present in *Malus domestica* at a concentration of 0.18-0.80 mg/100 g (Coseteng & Lee, 1987); however, in our experimental juices (screened for the presence, but not quantified of naringin and naringenin), we found only naringenin and only in PAJ. In agreement with juice composition data, just two glucuronide conjugates of naringenin were found to be statistically significant after PAJ consumption. See Figure 3 linking juices ingredients and its metabolites and Supplementary Material 3 for kinetic curves.

3.4 Quercetin catabolites (M8)

The sulphate conjugate of dihydroquercetin (taxifolin) was found to be statistically significant in urine. We assume that the presence of dihydroquercetin sulfate in urine is due to the microbial conversion of quercetin, followed by uptake from intestine and subsequent conjugation with the sulfate moiety in the liver. (Jaganath, Mullen, Lean, Edwards, & Crozier, 2009) reported two dihydroquercetin isomers of quercetin-3-O-rutinoside upon incubation of quercetin with human fecal bacteria. Similarly, (Braune, Gütschow, Engst, & Blaut, 2001) suggested dihydroquercetin as an intermediate metabolite in the conversion of quercetin into 3,4-dihydroxyphenylacetic acid

mediated by gut bacteria. See Figure 3 depicting metabolic pathways for juices ingredients and its metabolites and Supplementary Material 3 for kinetic curves.

3.5 (Epi)catechin metabolites (M9-M16)

(Epi)catechin sulfate and methylsulfate isomers were the most abundant metabolites among (epi)catechin's family accounting for about 88 % of the total MS signal These metabolites were 5 to 11 times higher in urine following the PAJ compared to the CAJ. Figure 2 shows the contribution of urine biomarkers to the metabolite pattern, and ratio of AUC_{PAJ} to AUC_{CAJ} in urine. The level of MS annotation confidence was set to II based on comparison with laboratory standards, and to the high resolution MS spectra obtained with Orbitrap mass spectrometer by van der Hooft et al (Hooft, Vos, et al., 2012), and Liu et al (Liu, Garrett, Su, Khoo, & Gu, 2017) (See Table 1 and Supplementary Material 2). Several investigations have previously reported the presence of (epi)catechin conjugates in urine after cocoa (Urpi-Sarda, Garrido, et al., 2009), grape juice (Stalmach, Edwards, Wightman, & Crozier, 2011) or tea consumption (Hooft, Vos, et al., 2012)(Williamson, Dionisi, & Renouf, 2011), and both in urine and plasma after almond consumption (Urpi-Sarda, Monagas, et al., 2009). Our findings are in good agreement with these studies. Indeed, apples share a family of flavanols, specifically monomers of (epi)catechins, with cocoa and almonds. Moreover, the amount of catechin and epicatechin in PAJ was about eleven times higher than in the CAJ (See Supplementary Material 1) thus the statistical significance of its metabolites were expected.

3.6 Valerolactone and valeric acid catabolites (M17-M42)

As many as twenty-eight phase II metabolites of valerolactone, colon-derived polyphenol catabolites, were tentatively identified in plasma and urine after both treatments. Amongst these (hydroxyphenyl)-; (dihydroxyphenyl)-; and methoxy(hydroxyphenyl)-γ-valerolactones were conjugated to (methyl)glucuronide, (methyl)sulfate moieties and similar conjugates were found for valeric acid derivatives. (Dihydroxyphenyl)-γ-valerolactone glucuronide isomers were the most abundant compounds within the group, accounting for 47 % of the total signal. Figure 2 shows the contribution of each valerolactone/valeric acid metabolite to the pattern, and metabolite ratio of AUC_{PAJ} to AUC_{CAJ} in urine. Profiles of phenyl-γ-valerolactones and phenyl-γ-valeric acids are in good agreement with those previously reported after apples, almonds and tea consumption ((Brindani et al., 2017b; Hooft, Vos, et al., 2012; Llorach et al., 2010; Rago et al., 2014; Urpi-Sarda, Garrido, et al., 2009; Wiese et al., 2015)). Valerolactone and valeric acid metabolites can arise from procyanidins, catechin and epicatechin, which were all present in both experimental juices, via microbial activities involving reductive cleavage of the heterocyclic C-ring forming diphenylpropan-2-ols and its lactonization leading to the formation of hydroxyphenylvalerolactones. Further fission of the valerolactone ring leads to hydroxyphenylvaleric acids (Groenewoud & Hundt,

519 1986; Meselhy, Nakamura, & Hattori, 1966; Stoupi, Williamson, Drynan, Barron, & Clifford, 2010a,
520 2010b), followed by β-oxidation resulting in the production of hydroxyphenylpropionic and
521 hydroxybenzoic acids (Meselhy et al., 1966). Finally, the α-oxidation of hydroxyphenylpropionic
522 acid gives rise to phenylacetic acids (Gonthier et al., 2003).. The latter metabolites however, in a
523 recent study by Appeldoorm et al (Appeldoorn, Vincken, Aura, Hollman, & Gruppen, 2009), were
524 suggested to arise exclusively from procyanidins. It seems therefore that distinct pathways may
525 coexist possibly depending on interindividual differences in gut microbiota composition. After
526 absorption from the intestine, microbial-derived valerolactones and valeric acid microbial
527 catabolites may be conjugated with glucuronide and sulphate moieties in the liver and kidney
528 before excretion in the urine, as shown in a simplified scheme of metabolomics pathway (Figure 3).
529 See Figure 2 for individual contribution of each metabolite to valerolactone and valeric acid family,
530 as well as Supplementary Material 3 and Figure 1 for kinetic curves.
531 *3.7 Catechol catabolites (M43-M48)*
532 Seven methylcatechol derivatives, mainly glucuronide and sulphate conjugates, (M43-M48) were
533 found to be statistically higher in PAJ urine and two in plasma. These metabolites have been
534 reported in the literature after consumption of berries puree, green tea and coffee (Hooft, Vos, et
535 al., 2012; Nieman et al., 2013; Pimpão, Ventura, Ferreira, Williamson, & Santos, 2015). The origin
536 of catechol is probably related to the microbial transformation of dihydroxyphenylacetic acid
537 ((Gonzalez-Barrio, Edwards, & Crozier, 2011; Pimpão et al., 2015; Scheline, 1967). Once collected
538 from intestine, catechol can be further conjugated leading to the formation of methyl, glucuronide
539 and sulphate metabolites. However, catechol can be derived also from the catabolism of vanillin,
540 which was present in the PAJ but not in the CAJ. Study of Strand & Scheline, 1975 reported the
541 appearance of dihydroxybenzoic acid, catechol and methylcatechol in rat urine after feeding with
542 vanillin. To our knowledge, such a wide range of methylcatechol conjugates has never reported
543 before in biological fluids after polyphenol intake. The nutrikinetic of methylcatechol conjugates
544 show similarities to those of hydroxy- and dihydroxyphenyl propionic acid metabolites and reflect
545 the general trend observed in of the studies of (Garrido et al., 2010; Gonthier et al., 2003; van
546 Duynhoven et al., 2014) , where excretion in urine and circulation in plasma did not reach
547 maximum concentrations within the first five hours. Thus indicating a possible major involvement of
548 the gut microbiota in catechol catabolism. See Figure 3 depicting metabolic pathways for juices
549 ingredients and its metabolites and Supplementary Material 3 for kinetic curves.
550
551 *3.8 Chlorogenic acids metabolites (M49-M53)*
552 Five chlorogenic acids were found to be among the statistically significant features in urine and
553 plasma, including two isomers of feruloylquinic acid and three isomers of coumaroylquinic acid.
554 Isomers of coumaroylquinic acids have been reported in urine in several previous studies related to
555 the metabolism of chlorogenic acid and coffee (Clifford, 2000; A. R. Rechner et al., 2001; Andreas

R. Rechner et al., 2001; Stalmach, Mullen, et al., 2010; Stalmach, Steiling, Williamson, & Crozier, 2010)(See Table 1 and Supplementary Table 2 for details). They have also been reported in experiments incubating apple polyphenols with saliva, simulated gastric or duodenal juice and rat hepatocytes (Kahle et al., 2011). Stalmach, Steiling, et al., 2010 reported the excretion of two isomers of coumaroylquinic quinic acid and three isomers of feruloylquinic acid in ileal fluid of ileostomy subjects after coffee consumption, (the latter was also found in urine), between 0-24 h. The same authors reported three feruloylquinic acid isomers in urine (maximum concentration within two hours) and plasma (maximum concentration within one hour). In this study, the quantity of un-metabolized feruloylquinic acid corresponded to approximately 5 % of feruloylquinic acid intake from coffee beverage. Our observations are in good agreement with this study confirming that a certain amount of un-metabolized chlorogenic acids may circulate in the body (See Figure 1,and Supplementary Material 3 for metabolite kinetics). The nutrikinetic curve for feruloylquinic and coumaroylquinic acids in urine plateaued within 8 hour, while in plasma their concentrations decreased quickly (within one hour). Differently, the glucuronide and sulfate forms of dihydroferulic acid (M67-M71) did not reach their maximum concentrations within 24 hours in urine, while in plasma T_{max} was achieved in the 5 hours after intake. Our results regarding both chlorogenic acids and dihydroferulic acid conjugates are in good agreement with study of (Stalmach, Mullen, et al., 2010) and suggest chlorogenic acid is metabolized quickly, probably in the upper gut without much involvement of the gut microbiota.

3.9 Cinnamic acids metabolites (M54-M59)

The bioavailability of this class of compounds, which are among the major polyphenols accounting for the health properties of coffee (Natella, Nardini, Belelli, & Scaccini, 2007), has been the subject of several detailed studies (Erk, Williamson, Renouf, Marmet, & Steiling, 2012; Stalmach et al., 2009; Stalmach, Mullen, et al., 2010; Stalmach, Steiling, et al., 2010). Several cinnamic acid metabolites (including glucuronide and sulfate conjugates of ferulic, caffeic and hydroxycinnamic acid) were found to vary their concentration in a statistically significant way according to the amount present in the apple juice (see supplementary table 1 for juice composition). Cinnamates (M54-M59) excretion in urine did not reach the maximum concentration within first 24 hours, while in plasma T_{max} was about 1 hours, which is in good agreement with available literature data, suggesting rapid absorption and metabolism in the upper gut (Borges et al., 2010; R M De Ferrars, Czank, Zhang, Botting, & Kroon, 2014; Stalmach et al., 2009; Stalmach, Steiling, et al., 2010). See Figure 3 depicting metabolic pathways for juices ingredients and its metabolites and Supplementary Material 3 for kinetic curves

3.10 Phenylpropionic and phenylacetic acids catabolites (M60-M73)

Fourteen derivatives of phenylpropionic and phenylacetic acid with different hydroxylation patterns were found to be significantly higher in PAJ urine and plasma. Several studies have reported an increase of these phenolic acid catabolites after intake of coffee, grape juice (Stalmach, Edwards, Wightman, & Crozier, 2013) or almonds (Urpi-Sarda, Monagas, et al., 2009). As shown in the simplified scheme of metabolic pathways (Figure 3), phenylpropionic and phenylacetic acids are common intermediates and/or end-products of the microbial catabolism of several polyphenol families (Appeldoorn et al., 2009; Bazzocco, Mattila, Guyot, Renard, & Aura, 2008; Del Rio et al., 2013; Garrido et al., 2010; Henning et al., 2013; Hooft, Vos, et al., 2012; Olthof et al., 2003; Rios et al., 2003; Tuohy, Conterno, Gasperotti, & Viola, 2012). Therefore, the phenylpropionic and phenylacetic acids metabolites observed here may have been derived from a number of different polyphenol families present in the test juices, and lack the specificity to be useful biomarkers of apple intake. Also their nutrikinetics in urine and plasma confirms the role of the gut microbiota in their biosynthesis, as maximum concentrations were not reached in plasma or urine over the 24 h period (See Figure 1, and Supplementary Materials 3 and 4 for details).

3.11 Hippuric acid catabolites (M74-M82)

In urine we found intermediate and final catabolites of hippurate, such as cyclohexene-, cyclohexadiene carboxylic acid glycine, hippuric, methylhippuric and hydroxyhippuric acids, while in plasma only hippuric and hydroxyhippuric acids were observed. Studies of (Olthof et al., 2003) and (Ulaszewska et al., 2016) reported several intermediate metabolites of hippurate after extensive feeding with black tea or low flavonoid fruits and vegetables diet. Hydroxyhippurates and methylhippurates have also been reported in literature (Ulaszewska et al., 2016; Nørskov, Hedemann, Lærke, Erik, & Knudsen, 2013) after extensive intake of polyphenols. The hippurate metabolic pathway crosses with those of other polyphenolic compounds and thus hippuric acid is considered one of the end products of the catabolism of several classes of polyphenols present in our apple juices (see Figure 3). All hippurate urine nutrikinetic curves were characterized by an increasing trend without achieving maximum concentrations in urine within 24 h (Figure 1), again indicating a microbiota involvement in this metabolic pathway.

3.12 Vanillic acid metabolites (M83-M88)

Six metabolites associated with vanillic acid (M85-M90) were found to be statistically significant in urine. Vanilpyruvic acid and homovanillic acid are known metabolites of catecholamine (Figure 2). There is only scant evidence supporting the hypothesis that fruit polyphenols affect catecholamine metabolism, (van Dorsten et al., 2009), however, considering the statistically significant increase in homovanillic acid in urine after PAJ intake, the possible overlap of vanillic acid and catecholamine metabolic pathways warrants further investigation. Vanillic acid conjugates have been reported previously as urinary metabolites after tea (Hooft, Vos, et al., 2012), almonds (Llorach et al., 2010),

mixed berry fruits puree (Pimpão et al., 2015), vanillin (Strand & Scheline, 1975) and after anthocyanins intake (R M De Ferrars et al., 2014). Presence of vanilloylglycine conjugate in urine might be due to the presence of vanillin in the PAJ, as vanilloylglycine has been detected in rat urine after feeding with vanillin by (Strand & Scheline, 1975). In the studies of Ferrars et al (Rachel M. de Ferrars, Cassidy, Curtis, & Kay, 2014; R M De Ferrars et al., 2014) vanillic acid conjugates were found to reach the maximum concentration in urine at 4-5 h, and in plasma at 1-2 h. The plasma kinetic curves observed in our study are in good agreement with these previous studies and suggest a rapid absorption and metabolism in the upper gut, while in urine concentration of metabolites did not reach the maximum within 24 hours (Supplementary Material 3).

3.13 Other metabolites

Tryptophan and tyrosine metabolites (M89-M94)

Bacterial metabolites of tyrosine and tryptophan (including indoxyl sulfate, phenol sulfate, dihydroxyindole glucuronide, and toluene sulfonate) were found mainly in urine. Their amount increased in urine and decreased in plasma, suggesting a clearance similar to that of toxic compounds. Diet:microbe interactions in regulating human tryptophan and tyrosine metabolic pathways is receiving much attention given their suggested importance in the gut:brain axis and systemic immune function (see supplementary material 2) (Romani et al., 2014, (O'Mahony, Clarke, Borre, Dinan, & Cryan, 2015). The unconjugated forms of these compounds have also been identified as uremic toxins (Vanholder, Glorieux, De Smet, & Lameire, 2003), and associated with cardiovascular disease risk (Raff et al. 2008). It seems, therefore, that apple may potentially aid the clearance of toxic compounds from the body, as already suggested by Rago et al., 2014. See Supplementary Material 2 and 3 for details.

3.14 Fatty acids (M95-106)

The last group of compounds found to be biomarkers, and occurring only in plasma were the fatty acids. There is scant information concerning the biological role and indeed, chemical nature of dietary oxidized medium and long chain fatty acids in the human body and we can only assume they were involved in the energy cycle and metabolic disturbances where free fatty acids play a crucial role. The occurrence of dicarboxylic fatty acid suggests the expression of alternative fatty acid oxidation mechanisms triggered by polyphenols (Guillot, 1993; Hoek-van den Hil et al., 2013; Nørskov et al., 2013; Papamandjaris, Macdougall, & Jones, 1998). Lack of commercially available standards only allowed annotation based on isotopic distribution of molecular ions and adducts in positive and negative ionization modes.

3.15 Comparison of CAJ versus PAJ.

As summarized in Figure 2, the fortification of the cloudy apple juice with additional apple polyphenols in the PAJ (Supplementary Table 1) lead to an increase of their total absorption by human volunteers, estimated from the total amount excreted in urine, for each of the eleven chemical groups. Within each of these groups, we did not observe any major change of the metabotype (the pattern of metabolites at population level) in the urine of volunteers who consumed either the CAJ or the PAJ. This experiment demonstrated that an increase of polyphenols in apple, with domains of validity (Scannell & Bosley, 2016) within the concentration range compatible with the natural variability of polyphenols in apples (Farneti et al., 2015) would lead to an increase in their concentration in biofluids, their pattern remains remaining conserved. In light of the relatively small number of volunteers involved in this study, such finding warrants further investigation, involving a larger population.

3.16 Kinetic evaluation of plasma and urine metabolites and impact of individual microbiota variability

Several authors have investigated the transformation kinetics of (epi)catechin, valerolactone and procyanidins by the gut microbiota and measured the appearance of their metabolites in plasma and urine. van Duynhoven et al., 2014 reported that valerolactones and valeric acid metabolites reached their maximum concentration in plasma between 5-8 h after black tea consumption, which is in good agreement with our study, where maximum concentrations were not reached until after 5 hours. Unno, Tamemoto, Yayabe, & Kakuda, 2003 investigated the kinetics of epicatechin and valerolactone metabolites in rat urine after intake of (-)-epicatechin, and concluded that microbial metabolism in the intestine could be accomplished within 24 h. A study of Van der Hooft (2012) showed that T_{max} of valerolactone metabolites can be achieved within 2h, 3h, 4h, 5h, 6h, or 8h after tea intake, suggesting strong interindividual differences in the metabolism of these compounds, possibly as the result of microbiome differences. Stoupi and coworkers (Stoupi et al., 2010a, Stoupi et al., 2010b), compared biotransformation of epicatechin and procyanidin B2 by human faecal microbiota and showed that procyanidins were catabolized twice as rapidly as epicatechin, even if the same catabolites were formed. Brindani et al., 2017 filled the gap of knowledge due to the lack of commercial standard, developing an efficient synthesis of several of these microbial metabolites. In their study, the urinary concentrations recorded for some phenyl-γ-valerolactones were quite high, reaching 132 µM for 5-(3^{i},4^{i}-dihydroxyphenyl)-γ-valerolactone-3^{i}-*O*-sulphate after one week of green tea supplementation. Kinetic curves from our study in plasma and urine are presented in Figure 1 and Supplementart Material 3.

From these considerations we can argue that at least two factors may affect T_{max} and C_{max} of polyphenol metabolites: 1) the degree of flavanol polymerization, 2) the variation in metabolic potential between individuals, a factor which combines differences in both human genome encoded metabolic pathways and co-metabolic pathways involving the gut microbiota.

3.17 Correlations between annotated metabolites and microbiota

The gut microbiota is known to play an important role in the transformation of many complex plant polyphenols, thus we wanted to explore whether the variation in the composition of gut microbiota could explain, at least partially, the observed large inter-individual catabolic differences. With this aim, we correlated the composition of the gut microbiota of participants with the profiles of microbial catabolites observed in urine and plasma after consuming the different apple juices. We selected urinary and plasma metabolites which displaying delayed nutrikinetic curves, indicating a possible microbiota intervention, see Supplementary Material 3. The area under the curve of each metabolite was correlated, separately for plasma and urine, and for each dose of apple polyphenols, with the relative abundance of different bacterial genera using Spearman correlation analysis.

Figure 5 and 6 show heatmaps (for urine and plasma, respectively) correlating the selected metabolites with 16S rRNA profiles faecal bacteria at the genus level. In both matrices correlations between microbiota and metabolites were found, predominantly after PAJ intake indicating the dose of polyphenol ingested impacted on our ability to measure statistically significant microbiota correlations. Many correlations were however not statistically significant, probably due to small number of participants in this pilot study, experimental design and high variability of metabolic responses between individuals. Table 2 reports statistically significant correlations found between urine and plasma metabolites and bacterial genera. There are very few data describing correlations between gut microbiota and plant polyphenol metabolites in biological fluids; existing studies are carried out *in vitro* incubating polyphenol with faeces. Therefore, to interpret the observed correlations, we focused on the metabolic enzymatic reactions leading to the formation of polyphenol metabolites (Aura et al., 2008; Dueñas et al., 2015; Selma, Espín, & Tomás-Barberán, 2009)

Procyanidins and flavan-3-ols present in the experimental juices act as substrates for the formation of valeric acid, valerolactone and (epi)catechin metabolites through common reactions such as dimer cleavage, ring fission, C&A-rings cleavage (lactone formation) and β-oxidation, followed by phase II metabolism (Mongas et al 2010). Several statistically significant correlations were found in plasma and urine between valeric acid, valerolactone and (epi)catechin metabolites and faecal bacteria (see Table 2). High production of these metabolites was positively associated with *Dialister, Prevotella and Escherichia,* while the presence of these compounds were negatively associated with *Anaerostipes, Turicibacter, Lachnospiracea incertae sedis, Coprococcus* and *Blautia.*

Chlorogenic acids, polyphenols of coffee and tea, through hydrolysis, dihydroxylation and reduction followed by phase II metabolism give a rise to cyclohexadiene carboxylic acid glycine, homovanillic acid and finally to hippurate metabolites (hydroxyhippuric acids, methylhippuric acids). The latter

metabolites are also the final products of several plant polyphenols. We found positive correlations of chlorogenic acids, and its metabolites with *Clostridium sensu stricto, Ruminococcus, Bacteroides, Butyriococcus,* and *Turycibacter,* while negative with *Roseburia, Faecalibacterium* and *Dorea.*

Only a few studies have examined the impact of dietary polyphenols on the human gut microbiota *in vivo*, and most focused on single polyphenol molecules and selected bacterial populations. The randomized-crossover trial of Queipo-Ortuño, 2012, in which subjects were supplemented with red wine, dealcoholized red wine, and gin, showed an increase of *Prevotella, Enterococcus, Bacteroides* and *Bifidobacterium* and a decrease of *Clostridium spp.* A study using a gastrointestinal simulators and red wine-grape or black tea extract reported the decrease of *Blautia,* and *Bacteroides* genus (Kemperman et al., 2013). Few studies have, as we did, measured the contrary interaction, the impact of microbiota composition on the profile of metabolites produced in mammalian biofluids over time.

Taken together, our findings support the theory that distantly related members of the gut microbiota share catabolic pathways for various polyphenol families and appear to work together to metabolize complex plant polyphenols. The results of these reactions are rather difficult to predict, also considering that a larger accumulation of a given metabolite in an individual could be due to increased synthesis, or to limited catabolism downstream, or both. We suggest that experiments using the approach described in this proof-of-concept study, when used in a larger population, should provide useful experimental data to elucidate the overall role of microbiota in nutrient metabolism.

4. Conclusions

To our knowledge this is first study which examines kinetics of such a wide range of apple polyphenol metabolites using an untargeted MS based metabolomics approach, and that correlates such data with gut microbiota composition. We observed the nutrikinetic at population level of a large number of microbial catabolites (valerolactones and valeric acids) of apple flavanols (catechins and procyanidins) confirming the key importance of these compounds. The presence of a wide range of methylcatechol metabolites, never reported before in biological fluids after apple juice ingestion, together with the presence of vanillactic, vanilpyruvic and homovanilic acid suggest a possible impact of apple polyphenols on catecholamine metabolism. However, the overlapping of these metabolic pathways and their inter-regulation awaits further specifically designed mechanistic studies to elucidate the exact metabolic relation and a possible physiological effects on the host.

New medium chained, di- and monocarboxylic fatty acids containing hydroxyl or methyl groups were identified in plasma. These lipid metabolites may potentially be novel biomarkers of apple polyphenol consumption. We have also confirmed the strong involvement of the intestinal

microbiota in the metabolism of complex plant polyphenols and speculated that the appearance of some phenolic catabolites can be correlated to the relative abundance of different, phylogenetically distant bacterial genera. As such, this work takes a small initial step in linking systems level metabolic processing of dietary polyphenols with microbiome architecture, a necessary move away from limited taxonomic descriptions or measurement of metabolic potential and towards improved understanding of microbiome metabolic function and nutrikinetics.

Funding Sources.

This research was conducted with the project “Apple fruit quality in the post-genomic era, from breeding new genotypes to post-harvest: nutrition and health”, funded by the AGER (Agribusiness and research) with grant no. 2010-2119. The funding body had no involvement in the study design, collection, analysis and interpretation of the data, in the writing of the report, and in the decision to submit the article for publication.

Acknowledgement.

Thanks to Riccardo Velasco for the project management, to Dr. Anna Maria Giusti for fundamental support to the study design, to Daniele Perenzoni for the LC-MS analysis of the apple juices. Thanks to the company Macè Srl and in particular to Giovanni Gallerani for kindly providing the experimental juices, and for useful discussion.

Figure captions.

Figure 1. Selected metabolite kinetic curves in plasma and urine of features discriminating the composition of urine and plasma of CAJ treatment (volunteers who consumed the natural cloudy apple juice) from PAJ treatment (same volunteers who consumed the cloudy apple juice enriched with 750mg of mixed apple polyphenol extract). Plasma graphs: X axis: timepoints; Y axis: peak intensity (MS response). Urine graphs: X axis timepoints, Y axis: cumulative intensities of peak (MS response). *** p value <0.001; ** p value 0.01-0.001; * p value 0.05-0.01

Figure 2. Comparison of the pattern of metabolites, at the population level, in the urine of volunteers who consumed either the CAJ or the PAJ. The biomarkers found in the urine were attributed to eleven chemical groups, and for each biomarker it is shown in the histogram the percentage contribution to their respective metabolite pool in urine after the consumption of each juice (natural vs enriched). Also the value of the relative total bioavailability for each group is given in brackets, estimated as ratio of the total amount excreted in urine for each chemical treatment (i.e., the ratio of total area under the curve AUC_{PAJ} divided by AUC_{CAJ}).

Figure 3. A global, simplified scheme of the metabolic pathways for main classes of apple polyphenols and their crossing trajectories.

Figure 4. The figure shows a compilation of phloretin glucuronide curves, respectively in urine (A) and plasma (B), as well as the pattern of all urine phloretin metabolites with their individual ratio of total area under the curve AUC_{PAJ} divided by AUC_{CAJ} (C), and the glycemic index in plasma (D).

Figure 5. Heatmaps correlating AUC_{PAJ} of metabolites measured over 5 hours in blood and genus level 16S rRNA relative abundance of faecal microbiota present in each subject.

Figure 6. Heatmaps correlating the AUC_{PAJ} of metabolites excreted over 24 h in urine and genus level 16S rRNA relative abundance of faecal microbiota present in each subject.

Table captions.

Table 1. Urine and plasma metabolites found to be statistically significant between CAJ and PAJ treatment. For each metabolite a substrate is given as well as retention time (min); elemental composition with MS identification level; $T_{max(P)}$, $T_{max(U)}$ (hours); adjusted p value; matrix in which metabolite was found and direction ↑ higher, or ↓ lower; literature reference;

Table 2. Metabolites in plasma and urine after PAJ intake, found to be statistically significant correlated with microbiota at genus level. For each correlation metabolite:microbiota genus, a parent polyphenol was proposed together with chemical mechanism leading to it formation.

ASSOCIATED CONTENT

Supporting Information.

Supplementary Material 1. Table with polyphenols found in cloudy apple juice (CAJ) and polyphenols enriched apple juice (PAJ). Concentrations given in mg/L.

Supplementary Material 2. Urine and plasma metabolites found to be statistically significant at any time point between CAJ and PAJ treatment. For each metabolite a retention time is given as well as elemental composition, exact mass of molecule, MS identification level; relative intensities of in-source fragment ions found in full scan mode with annotation, MS/MS^2 and MS/MS^3 spectra; matrix in which metabolite was found and direction ↑ higher, or ↓ lower; literature reference.

Supplementary Material 3. Kinetic curves in plasma and urine for all metabolites found to be statistically significant between CAJ and PAJ treatment.

Supplementary Material 4. Table with unknown compounds found to be statistically significant in plasma and urine between CAJ and PAJ treatment.

This material is available free of charge via the Internet at http://pubs.acs.org.

References:

Appeldoorn, M. M., Vincken, J. P., Aura, A. M., Hollman, P. C. H., & Gruppen, H. (2009). Procyanidin dimers are metabolized by human microbiota with 2-(3,4-dihydroxyphenyl)acetic acid and 5-(3,4-dihydroxyphenyl)-??- valerolactone as the major metabolites. *Journal of Agricultural and Food Chemistry*, *57*(3), 1084–1092. https://doi.org/10.1021/jf803059z

Aura, A. M., Mattila, I., Seppänen-Laakso, T., Miettinen, J., Oksman-Caldentey, K. M., & Orešič, M. (2008). Microbial metabolism of catechin stereoisomers by human faecal microbiota: Comparison of targeted analysis and a non-targeted metabolomics method. *Phytochemistry Letters*, *1*(1), 18–22. https://doi.org/10.1016/j.phytol.2007.12.001

Bazzocco, S., Mattila, I., Guyot, S., Renard, C. M. G. C., & Aura, A. M. (2008). Factors affecting the conversion of apple polyphenols to phenolic acids and fruit matrix to short-chain fatty acids by human faecal microbiota in vitro. *European Journal of Nutrition*, *47*(8), 442–452. https://doi.org/10.1007/s00394-008-0747-2

Bondonno, N. P., Bondonno, C. P., Blekkenhorst, L. C., Considine, M. J., Maghzal, G., Stocker, R., … Croft, K. D. (2018). Flavonoid-Rich Apple Improves Endothelial Function in Individuals at Risk for Cardiovascular Disease: A Randomized Controlled Clinical Trial. *Molecular Nutrition & Food Research*, *62*(3), 1700674. https://doi.org/10.1002/mnfr.201700674

Borges, G., Lean, M. E. J., Roberts, S. A., & Crozier, A. (2013). Bioavailability of dietary (poly) phenols following acute ingestion of an enriched drink by ileostomists, 1–8.

Borges, G., Mullen, W., Mullan, A., Lean, M. E. J., & Roberts, S. A. (2010). Bioavailability of multiple components following acute ingestion of a polyphenol-rich juice drink, 268–277. https://doi.org/10.1002/mnfr.200900611

Braune, A., Gütschow, M., Engst, W., & Blaut, M. (2001). Degradation of Quercetin and Luteolin by Eubacterium ramulus. *Applied and Environmental Microbiology*, *67*(12), 5558–5567. https://doi.org/10.1128/AEM.67.12.5558-5567.2001

Brindani, N., Mena, P., Calani, L., Benzie, I., Choi, S.-W., Brighenti, F., … Del Rio, D. (2017a). Synthetic and analytical strategies for the quantification of phenyl-γ-valerolactone conjugated metabolites in human urine. *Molecular Nutrition & Food Research*, *61*(9), 1700077. https://doi.org/10.1002/mnfr.201700077

Brindani, N., Mena, P., Calani, L., Benzie, I., Choi, S. W., Brighenti, F., … Del Rio, D. (2017b). Synthetic and analytical strategies for the quantification of phenyl-γ-valerolactone conjugated metabolites in human urine. *Molecular Nutrition and Food Research*, *61*(9), 6–10. https://doi.org/10.1002/mnfr.201700077

Butland, B. K. (2000). Diet, lung function, and lung function decline in a cohort of 2512 middle aged men. *Thorax*, *55*(2), 102–108. https://doi.org/10.1136/thorax.55.2.102

Caporaso, J. G., Bittinger, K., Bushman, F. D., Desantis, T. Z., Andersen, G. L., & Knight, R. (2010). PyNAST: A flexible tool for aligning sequences to a template alignment. *Bioinformatics*, *26*(2), 266–267. https://doi.org/10.1093/bioinformatics/btp636

Clifford, M. N. (2000). Review Chlorogenic acids and other cinnamates – nature , occurrence , dietary burden , absorption and metabolism, *1043*(October 1999), 1033–1043.

Conceição de Oliveira, M., Sichieri, R., & Sanchez Moura, A. (2003). Weight Loss Associated With a Daily Intake of Three Apples or Three Pears Among Overweight Women. *Nutrition*, *19*(3), 253–256. https://doi.org/10.1016/S0899-9007(02)00850-X

COSETENG, M. Y., & LEE, C. Y. (1987). Changes in Apple Polyphenoloxidase and Polyphenol Concentrations in Relation to Degree of Browning. *Journal of Food Science*, *52*(4), 985–989. https://doi.org/10.1111/j.1365-2621.1987.tb14257.x

de Ferrars, R. M., Cassidy, A., Curtis, P., & Kay, C. D. (2014). Phenolic metabolites of anthocyanins following a dietary intervention study in post-menopausal women. *Molecular Nutrition & Food Research*, *58*(3), 490–502. https://doi.org/10.1002/mnfr.201300322

Del Rio, D., Rodriguez-Mateos, A., Spencer, J. P. E., Tognolini, M., Borges, G., & Crozier, A. (2013). Dietary (Poly)phenolics in Human Health: Structures, Bioavailability, and Evidence of Protective Effects Against Chronic Diseases. *Antioxidants & Redox Signaling*,

18(14), 1818–1892. https://doi.org/10.1089/ars.2012.4581

DeSantis, T. Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E. L., Keller, K., … Andersen, G. L. (2006). Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Applied and Environmental Microbiology*, *72*(7), 5069–5072. https://doi.org/10.1128/AEM.03006-05

Dueñas, M., Muñoz-González, I., Cueva, C., Jiménez-Girón, A., Sánchez-Patán, F., Santos-Buelga, C., … Bartolomé, B. (2015). A Survey of Modulation of Gut Microbiota by Dietary Polyphenols. *BioMed Research International*, *2015*, 1–15. https://doi.org/10.1155/2015/850902

Erk, T., Williamson, G., Renouf, M., Marmet, C., & Steiling, H. (2012). Dose-dependent absorption of chlorogenic acids in the small intestine assessed by coffee consumption in ileostomists, 1488–1500. https://doi.org/10.1002/mnfr.201200222

Farneti, B., Masuero, D., Costa, F., Magnago, P., Malnoy, M., Costa, G., … Mattivi, F. (2015). Is there room for improving the nutraceutical composition of apple? *Journal of Agricultural and Food Chemistry*, *63*(10), 2750–2759. https://doi.org/10.1021/acs.jafc.5b00291

Ferrars, R. M. De, Czank, C., Zhang, Q., Botting, N. P., & Kroon, P. A. (2014). The pharmacokinetics of anthocyanins and their metabolites in humans. https://doi.org/10.1111/bph.12676

Feskanich, D., Ziegler, R. G., Michaud, D. S., Giovannucci, E. L., Speizer, F. E., Willett, W. C., & Colditz, G. a. (2000). Prospective study of fruit and vegetable consumption and risk of lung cancer among men and women. *Journal of the National Cancer Institute*, *92*(22), 1812–1823. https://doi.org/10.1093/jnci/92.22.1812

Gallus, S., Talamini, R., Giacosa, A., Montella, M., Ramazzotti, V., Franceschi, S., … La Vecchia, C. (2005). Does an apple a day keep the oncologist away? *Annals of Oncology*, *16*(11), 1841–1844. https://doi.org/10.1093/annonc/mdi361

Garrido, I., Urpi-sarda, M., Go, C., Martı, P. J., Llorach, R., & Andre, C. (2010). Targeted Analysis of Conjugated and Microbial-Derived Phenolic Metabolites in Human Urine After Consumption of an Almond Skin Phenolic Extract 1 – 3, (19). https://doi.org/10.3945/jn.110.124065.and

Gerhauser, C. (2008). Cancer chemopreventive potential of apples, apple juice, and apple components. *Planta Medica*, *74*(13), 1608–1624. https://doi.org/10.1055/s-0028-1088300

Gerlich, M., & Neumann, S. (2013). MetFusion : integration of compound identi fi cation strategies, (April 2012), 291–298. https://doi.org/10.1002/jms.3123

Gonthier, M. P., Donovan, J. L., Texier, O., Felgines, C., Remesy, C., & Scalbert, A. (2003). Metabolism of dietary procyanidins in rats. *Free Radical Biology and Medicine*, *35*(8), 837–844. https://doi.org/10.1016/S0891-5849(03)00394-0

Gonzalez-Barrio, R., Edwards, C. A., & Crozier, A. (2011). Colonic Catabolism of Ellagitannins, Ellagic Acid, and Raspberry Anthocyanins: In Vivo and In Vitro Studies. *Drug Metabolism and Disposition*, *39*(9), 1680–1688. https://doi.org/10.1124/dmd.111.039651

Gossé, F., Guyot, S., Roussi, S., Lobstein, A., Fischer, B., Seiler, N., & Raul, F. (2005). Chemopreventive properties of apple procyanidins on human colon cancer-derived metastatic SW620 cells and in a rat model of colon carcinogenesis. *Carcinogenesis*, *26*(7), 1291–1295. https://doi.org/10.1093/carcin/bgi074

Gris, E. F., Mattivi, F., Ferreira, E. A., Vrhovsek, U., Pedrosa, R. C., & Bordignon-Luiz, M. T. (2011). Proanthocyanidin profile and antioxidant capacity of Brazilian Vitis vinifera red wines. *Food Chemistry*, *126*(1), 213–220. https://doi.org/10.1016/j.foodchem.2010.10.102

Groenewoud, G., & Hundt, H. K. L. (1986). The microbial metabolism of condensed (+)-catechins by rat-caecal microflora, *16*(2), 99–107.

Guadamuro, L., Delgado, S., Redruello, B., Flórez, A. B., Suárez, A., Martínez-Camblor, P., & Mayo, B. (2015). Equol status and changes in fecal microbiota in menopausal women receiving long-term treatment for menopause symptoms with a soy-isoflavone concentrate. *Frontiers in Microbiology*, *6*(AUG), 1–10. https://doi.org/10.3389/fmicb.2015.00777

Guillot, B. Y. E. (1993). Intestinal absorption and liver uptake of medium-chain fatty acids in non-anaesthetized pigs.

Gürdeniz, G., Kristensen, M., Skov, T., & Dragsted, L. O. (2012). The Effect of LC-MS Data Preprocessing Methods on the Selection of Plasma Biomarkers in Fed vs. Fasted Rats. *Metabolites*, *2*(4), 77–99. https://doi.org/10.3390/metabo2010077

Hansen, L., Dragsted, L. O., Olsen, A., Christensen, J., Tjønneland, A., Schmidt, E. B., & Overvad, K. (2010). Fruit and vegetable intake and risk of acute coronary syndrome. *British Journal of Nutrition*, *104*(2), 248–255. https://doi.org/10.1017/S0007114510000462

Haug, K., Salek, R. M., Conesa, P., Hastings, J., de Matos, P., Rijnbeek, M., … Steinbeck, C. (2013). MetaboLights—an open-access general-purpose repository for metabolomics studies and associated meta-data. *Nucleic Acids Research*, *41*(D1), D781–D786. https://doi.org/10.1093/nar/gks1004

Henning, S. M., Wang, P., Abgaryan, N., Vicinanza, R., Oliveira, D. M. De, Zhang, Y., … William, J. (2013). Phenolic acid

concentrations in plasma and urine from men consuming green or black tea and potential chemopreventive properties for colon cancer, 483–492. https://doi.org/10.1002/mnfr.201200646

Herrick, K. A., Rossen, L. M., Nielsen, S. J., Branum, A. M., & Ogden, C. L. (2015). Fruit Consumption by Youth in the United States. *Pediatrics*, *136*(4), 664–71. https://doi.org/10.1542/peds.2015-1709

Hoek-van den Hil, E. F., Keijer, J., Bunschoten, A., Vervoort, J. J. M., Stankova, B., Bekkenkamp, M., … van Schothorst, E. M. (2013). Quercetin Induces Hepatic Lipid Omega-Oxidation and Lowers Serum Lipid Levels in Mice. *PLoS ONE*, *8*(1), e51588. https://doi.org/10.1371/journal.pone.0051588

Hooft, J. J. J. Van Der, Mihaleva, V., Vos, R. C. H. De, Bino, R. J., & Vervoort, J. (2012). A strategy for fast structural elucidation of metabolites in small volume plant extracts using automated MS-guided LC-MS-SPE-NMR †, (August 2011). https://doi.org/10.1002/mrc.2833

Hooft, J. J. J. Van Der, Vos, R. C. H. De, Mihaleva, V., & Bino, R. J. (n.d.). Structural elucidation and quantification of phenolic conjugates present in human urine after tea intake.

Hooft, J. J. J. Van Der, Vos, R. C. H. De, Mihaleva, V., Bino, R. J., Ridder, L., Roo, N. De, … Vervoort, J. (2012). Structural Elucidation and Quanti fi cation of Phenolic Conjugates Present in Human Urine after Tea Intake, (i).

Horai, H., Arita, M., Kanaya, S., Nihei, Y., Ikeda, T., Suwa, K., … Nishioka, T. (2010). Special Feature : Tutorial MassBank : a public repository for sharing mass spectral data for life sciences Naoshige Akimoto , h Takashi Maoka , i Hiroki Takahashi , d Takeshi Ara , j, (June), 703–714. https://doi.org/10.1002/jms.1777

Hyson, A. D. (2011). A comprehensive review of apples and apple components and their relationship to human health. *Advances in Nutrition: An International Review Journal*, *2*(5), 408–420. https://doi.org/10.3945/an.111.000513.408

Ito, H., Gonthier, M., Manach, C., Morand, C., & Mennen, L. (2005). Polyphenol levels in human urine after intake of six different polyphenol-rich beverages, 500–509. https://doi.org/10.1079/BJN20051522

Jaganath, I. B., Mullen, W., Lean, M. E. J., Edwards, C. A., & Crozier, A. (2009). Free Radical Biology & Medicine In vitro catabolism of rutin by human fecal bacteria and the antioxidant capacity of its catabolites. *Free Radical Biology and Medicine*, *47*(8), 1180–1189. https://doi.org/10.1016/j.freeradbiomed.2009.07.031

Kahle, K., Huemmer, W., Kempf, M., Scheppach, W., Erk, T., & Richling, E. (2007). Polyphenols are intensively metabolized in the human gastrointestinal tract after apple juice consumption. *Journal of Agricultural and Food Chemistry*, *55*(26), 10605–10614. https://doi.org/10.1021/jf071942r

Kahle, K., Kempf, M., Schreier, P., Scheppach, W., Schrenk, D., Kautenburger, T., … Richling, E. (2011). Intestinal transit and systemic metabolism of apple polyphenols. *European Journal of Nutrition*, *50*(7), 507–522. https://doi.org/10.1007/s00394-010-0157-0

Kemperman, R. A., Gross, G., Mondot, S., Possemiers, S., Marzorati, M., Van de Wiele, T., … Vaughan, E. E. (2013). Impact of polyphenols from black tea and red wine/grape juice on a gut model microbiome. *Food Research International*, *53*(2), 659–669. https://doi.org/10.1016/j.foodres.2013.01.034

Knekt, P., Kumpulainen, J., Järvinen, R., Rissanen, H., Heliövaara, M., Reunanen, A., & Hakulinen, T. (2002). Flavonoid intake and risk of chronic diseases 1,2, 560–568.

Konopacka, D., Jesionkowska, K., Kruczyńska, D., Stehr, R., Schoorl, F., Buehler, A., … Bonany, J. (2010). Apple and peach consumption habits across European countries. *Appetite*, *55*(3), 478–483. https://doi.org/10.1016/j.appet.2010.08.011

Lee, J., Ebeler, S. E., Zweigenbaum, J. A., & Mitchell, A. E. (2012). UHPLC-(ESI)QTOF MS/MS profiling of quercetin metabolites in human plasma postconsumption of applesauce enriched with apple peel and onion. *Journal of Agricultural and Food Chemistry*, *60*(34), 8510–8520. https://doi.org/10.1021/jf302637t

Liu, H., Garrett, T. J., Su, Z., Khoo, C., & Gu, L. (2017). UHPLC-Q-Orbitrap-HRMS-based global metabolomics reveal metabolome modifications in plasma of young women after cranberry juice consumption. *Journal of Nutritional Biochemistry*, *45*, 67–76. https://doi.org/10.1016/j.jnutbio.2017.03.007

Llorach, R., Garrido, I., Monagas, M., Urpi-Sarda, M., Tulipani, S., Bartolome, B., & Andres-Lacueva, C. (2010). Metabolomics Study of Human Urinary Metabolome Modifications After Intake of Almond (Prunus dulcis (Mill.) D.A. Webb) Skin Polyphenols. *Journal of Proteome Research*, *9*(11), 5859–5867. https://doi.org/10.1021/pr100639v

Llorach, R., Urpi-Sarda, M., Jauregui, O., Monagas, M., & Andres-Lacueva, C. (2009). An LC-MS-based metabolomics approach for exploring urinary metabolome modifications after cocoa consumption. *Journal of Proteome Research*, *8*(11), 5060–5068. https://doi.org/10.1021/pr900470a

Manach, C., Scalbert, A., Morand, C., Rémésy, C., & Jime, L. (2004). Polyphenols: food sources and bioavailability 1,2.

Manach, C., Williamson, G., Morand, C., & Scalbert, A. (2005). Bioavailability and bioefficacy of polyphenols in humans . I . Review of 97 bioavailability studies 1 – 3, *81*, 230–242.

Marks, S. C., Mullen, W., Borges, G., & Crozier, A. (2009). Absorption, Metabolism, and Excretion of Cider Dihydrochalcones in Healthy Humans and Subjects with an Ileostomy. *Journal of Agricultural and Food Chemistry*, *57*(5), 2009–2015. https://doi.org/10.1021/jf802757x

Masumoto, S., Akimoto, Y., Oike, H., & Kobori, M. (2009). Dietary Phloridzin Reduces Blood Glucose Levels and Reverses Sglt1 Expression in the Small Intestine in Streptozotocin-Induced Diabetic Mice. *Journal of Agricultural and Food Chemistry*, *57*(11), 4651–4656. https://doi.org/10.1021/jf9008197

McMurdie, P. J., & Holmes, S. (2013). Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS ONE*, *8*(4). https://doi.org/10.1371/journal.pone.0061217

Meselhy, Me., Nakamura, N., & Hattori, M. (1966). Biotransformation of epicatechin 3-O-Gallate by human intestinal bacteria. *Chemical and Pharmaceutical Bulletin*, *14*, 369–375. https://doi.org/10.1248/cpb.37.3229

Muraki, I., Imamura, F., Manson, J. E., Hu, F. B., Willett, W. C., van Dam, R. M., & Sun, Q. (2013). Fruit consumption and risk of type 2 diabetes: results from three prospective longitudinal cohort studies. *BMJ*, *347*(aug28 1), f5001–f5001. https://doi.org/10.1136/bmj.f5001

Najafian, M., Jahromi, M. Z., Nowroznejhad, M. J., Khajeaian, P., Kargar, M. M., Sadeghi, M., & Arasteh, A. (2012). Phloridzin reduces blood glucose levels and improves lipids metabolism in streptozotocin-induced diabetic rats. *Molecular Biology Reports*, *39*(5), 5299–5306. https://doi.org/10.1007/s11033-011-1328-7

Natella, F., Nardini, M., Belelli, F., & Scaccini, C. (2007). Coffee drinking induces incorporation of phenolic acids into LDL and increases the resistance of LDL to ex vivo oxidation in humans. *The American Journal of Clinical Nutrition*, *86*(3), 604–609. https://doi.org/10.1093/ajcn/86.3.604

Nieman, D. C., Gillitt, N. D., Knab, A. M., Shanely, R. A., Pappan, K. L., Jin, F., & Lila, M. A. (2013). Influence of a Polyphenol-Enriched Protein Powder on Exercise-Induced Inflammation and Oxidative Stress in Athletes : A Randomized Trial Using a Metabolomics Approach, *8*(8). https://doi.org/10.1371/journal.pone.0072215

Nørskov, N. P., Hedemann, M. S., Lærke, H. N., Erik, K., & Knudsen, B. (2013). Multicompartmental Nontargeted LC − MS Metabolomics : Explorative Study on the Metabolic Responses of Rye Fiber versus Re fi ned Wheat Fiber Intake in Plasma and Urine of Hypercholesterolemic Pigs.

O'Mahony, S. M., Clarke, G., Borre, Y. E., Dinan, T. G., & Cryan, J. F. (2015). Serotonin, tryptophan metabolism and the brain-gut-microbiome axis. *Behavioural Brain Research*, *277*, 32–48. https://doi.org/10.1016/j.bbr.2014.07.027

Olthof, M. R., Hollman, P. C. H., Buijsman, M. N. C. P., Van Amelsvoort, J. M. M., & Katan, M. B. (2003). Human Nutrition and Metabolism Chlorogenic Acid, Quercetin-3-Rutinoside and Black Tea Phenols Are Extensively Metabolized in Humans 1. *J. Nutr*, *133*(February), 1806–1814.

Papamandjaris, A. A., Macdougall, D. E., & Jones, P. J. H. (1998). Medium chain fatty acid metabolism and energy expenditure: Obesity treatment implications. *Life Sciences*, *62*(14), 1203–1215. https://doi.org/10.1016/S0024-3205(97)01143-0

Pimpão, R. C., Dew, T., Figueira, M. E., Mcdougall, G. J., Stewart, D., Ferreira, R. B., … Williamson, G. (2014). Urinary metabolite profiling identifies novel colonic metabolites and conjugates of phenolics in healthy volunteers. *Molecular Nutrition and Food Research*, *58*(7), 1414–1425. https://doi.org/10.1002/mnfr.201300822

Pimpão, R. C., Ventura, M. R., Ferreira, R. B., Williamson, G., & Santos, C. N. (2015). Phenolic sulfates as new and highly abundant metabolites in human plasma after ingestion of a mixed berry fruit purée. *British Journal of Nutrition*, *113*(3), 454–463. https://doi.org/10.1017/S0007114514003511

Queipo-Ortuño, M. I. (2012). Influence of red wine polyphenols on the gut microbiota ecology. *The American Journal of Cliniclal Nutrition*, *95*(2), 1323–1334. https://doi.org/10.3945/ajcn.111.027847.INTRODUCTION

R Core Team. (2013). *R: A Language and Environment for Statistical Computing*. *R Foundation for Statistical Computing* (Vol. 739). https://doi.org/10.1007/978-3-540-74686-7

Rago, D., Gurdeniz, G., Gitte, R.-H., & Dragsted, L. (2014). An explorative study of the effect of apple and apple products on the human plasma metabolome investigated by LC-MS profiling. *Metabolomics*, *11*(1), 27–39. https://doi.org/10.1007/s11306-014-0668-4

Rechner, A. R., Pannala, A. S., & Rice-Evans, C. A. (2001). Caffeic acid derivatives in artichoke extract are metabolised to phenolic acids in vivo. *Free Radical Research*, *35*(2), 195–202. https://doi.org/10.1080/10715760100300741

Rechner, A. R., Spencer, J. P. ., Kuhnle, G., Hahn, U., & Rice-Evans, C. A. (2001). Novel biomarkers of the metabolism of caffeic acid derivatives in vivo. *Free Radical Biology and Medicine*, *30*(11), 1213–1222. https://doi.org/10.1016/S0891-5849(01)00506-8

Rios, L. Y., Gonthier, M., Remesy, C., Mila, I., Lapierre, C., Lazarus, S. A., … Scalbert, A. (2003). Chocolate intake incrases urinary excretion of polyphenot-derived phenolic acids in healthy patients. *Am J Clin Nutr*, *77*(912), 918.

Romani, L., Zelante, T., De Luca, A., Iannitti, R. G., Moretti, S., Bartoli, A., … Puccetti, P. (2014). Microbiota control of a tryptophan-AhR

pathway in disease tolerance to fungi. *European Journal of Immunology*, *44*(11), 3192–3200. https://doi.org/10.1002/eji.201344406

Rossi, M., Lugo, A., Lagiou, P., Zucchetto, A., Polesel, J., Serraino, D., … La vecchia, C. (2012). Proanthocyanidins and other flavonoids in relation to pancreatic cancer: A case-control study in Italy. *Annals of Oncology*, *23*(6), 1488–1493. https://doi.org/10.1093/annonc/mdr475

Salek, R. M., Haug, K., Conesa, P., Hastings, J., Williams, M., Mahendraker, T., … Steinbeck, C. (2013). The MetaboLights repository: curation challenges in metabolomics. *Database : The Journal of Biological Databases and Curation*, *2013*, 1–8. https://doi.org/10.1093/database/bat029

Scalbert, A., Brennan, L., Manach, C., Andres-Lacueva, C., Dragsted, L. O., Draper, J., … Wishart, D. S. (2014). The food metabolome: A window over dietary exposure. *American Journal of Clinical Nutrition*, *99*(6), 1286–1308. https://doi.org/10.3945/ajcn.113.076133

Scannell, J. W., & Bosley, J. (2016). When Quality Beats Quantity: Decision Theory, Drug Discovery, and the Reproducibility Crisis. *PLOS ONE*, *11*(2), e0147215. https://doi.org/10.1371/journal.pone.0147215

Scheline, R. R. (1967). 4-Methylcatechol, a Metabolite of Homoprotocatechuic acid, *23*(6), 493–494. https://doi.org/doi.org/10.1007/BF02142196

Scientific Opinion on the substantiation of a health claim related to cocoa flavanols and maintenance of normal endothelium-dependent vasodilation pursuant to Article 13(5) of Regulation (EC) No 1924/2006. (2012). *EFSA Journal*, *10*(7). https://doi.org/10.2903/j.efsa.2012.2809

Selma, M. V., Espín, J. C., & Tomás-Barberán, F. A. (2009). Interaction between Phenolics and Gut Microbiota: Role in Human Health. *Journal of Agricultural and Food Chemistry*, *57*(15), 6485–6501. https://doi.org/10.1021/jf902107d

Sesso, H. D., Gaziano, J. M., Liu, S., & Buring, J. E. (2003). Flavonoid intake and the risk of cardiovascular disease in women. *The American Journal of Clinical Nutrition*, *77*(6), 1400–8. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12791616

Smith, A., O 'maille, G., Want, E. J., Qin, C., Trauger, S. A., Brandon, T. R., … Siuzdak, G. (2005). METLIN A Metabolite Mass Spectral Database. *Proceedings of the 9Th International Congress of Therapeutic Drug Monitoring & Clinical Toxicology*, *27*(6), 747–751. https://doi.org/10.1097/01.ftd.0000179845.53213.39

Soriano-Maldonado, A., Hidalgo, M., Arteaga, P., de Pascual-Teresa, S., & Nova, E. (2014). Effects of regular consumption of vitamin C-rich or polyphenol-rich apple juice on cardiometabolic markers in healthy adults: a randomized crossover trial. *European Journal of Nutrition*, 1645–1657. https://doi.org/10.1007/s00394-014-0670-7

Stalmach, A., Edwards, C. A., Wightman, J. D., & Crozier, A. (2011). Identification of (Poly)phenolic compounds in concord grape juice and their metabolites in human plasma and urine after juice consumption. *Journal of Agricultural and Food Chemistry*, *59*(17), 9512–9522. https://doi.org/10.1021/jf2015039

Stalmach, A., Edwards, C. A., Wightman, J. D., & Crozier, A. (2013). Colonic catabolism of dietary phenolic and polyphenolic compounds from Concord grape juice. *Food & Function*, *4*(1), 52–62. https://doi.org/10.1039/C2FO30151B

Stalmach, A., Mullen, W., Barron, D., Uchida, K., Yokota, T., Cavin, C., … Crozier, A. (2009). Metabolite profiling of hydroxycinnamate derivatives in plasma and urine after the ingestion of coffee by humans: Identification of biomarkers of coffee consumption. *Drug Metabolism and Disposition*, *37*(8), 1749–1758. https://doi.org/10.1124/dmd.109.028019

Stalmach, A., Mullen, W., Steiling, H., Williamson, G., Lean, M. E. J., & Crozier, A. (2010). Absorption, metabolism, and excretion of green tea flavan-3-ols in humans with an ileostomy. *Molecular Nutrition and Food Research*, *54*(3), 323–334. https://doi.org/10.1002/mnfr.200900194

Stalmach, A., Steiling, H., Williamson, G., & Crozier, A. (2010). Bioavailability of chlorogenic acids following acute ingestion of coffee by humans with an ileostomy. *Archives of Biochemistry and Biophysics*, *501*(1), 98–105. https://doi.org/10.1016/j.abb.2010.03.005

Stoupi, S., Williamson, G., Drynan, J. W., Barron, D., & Clifford, M. N. (2010a). A comparison of the in vitro biotransformation of (-)-epicatechin and procyanidin B2 by human faecal microbiota. *Molecular Nutrition and Food Research*, *54*(6), 747–759. https://doi.org/10.1002/mnfr.200900123

Stoupi, S., Williamson, G., Drynan, J. W., Barron, D., & Clifford, M. N. (2010b). Procyanidin B2 catabolism by human fecal microflora: Partial characterization of "dimeric" intermediates. *Archives of Biochemistry and Biophysics*, *501*(1), 73–78. https://doi.org/10.1016/j.abb.2010.02.009

Strand, L. P., & Scheline, R. R. (1975). The metabolism of vanillin and isovanillin in the rat. *Xenobiotica; the Fate of Foreign Compounds in Biological Systems*, *5*(1), 49–63. https://doi.org/10.3109/00498257509056093

Sud, M., Fahy, E., Cotter, D., Brown, A., Dennis, E. A., Glass, C. K., … Subramaniam, S. (2007). LMSD: LIPID MAPS structure database. *Nucleic Acids Research*, *35*(SUPPL. 1), 527–532. https://doi.org/10.1093/nar/gkl838

TABAK, C., ARTS, I. C. W., SMIT, H. A., HEEDERIK, D., & KROMHOUT, D. (2001). Chronic Obstructive Pulmonary Disease and Intake of Catechins, Flavonols, and Flavones. *American Journal of Respiratory and Critical Care Medicine*, *164*(1), 61–64. https://doi.org/10.1164/ajrccm.164.1.2010025

Tamura, M., Hori, S., Nakagawa, H., Katada, K., Kamada, K., Uchiyama, K., … Yoshikawa, T. (2015). Relationships among fecal daidzein metabolites, dietary habit and BMI in healthy volunteers: a preliminary study. *Bioscience of Microbiota, Food and Health*, *34*(3), 59–65. https://doi.org/10.12938/bmfh.2014-019

Theodoratou, E., Kyle, J., Cetnarskyj, R., Farrington, S. M., Tenesa, A., Barnetson, R., … Campbell, H. (2007). Dietary Flavonoids and the Risk of Colorectal Cancer. *Cancer Epidemiology Biomarkers & Prevention*, *16*(4), 684–693. https://doi.org/10.1158/1055-9965.EPI-06-0785

Theuwissen, E., & Mensink, R. P. (2008). Water-soluble dietary fibers and cardiovascular disease. *Physiology and Behavior*, *94*(2), 285–292. https://doi.org/10.1016/j.physbeh.2008.01.001

Tuohy, K. M., Conterno, L., Gasperotti, M., & Viola, R. (2012). Up-regulating the Human Intestinal Microbiome Using Whole Plant Foods, Polyphenols, and/or Fiber.

Ulaszewska, M. M., Trost, K., Stanstrup, J., Tuohy, K. M., Franceschi, P., Chong, M. F. F., … Mattivi, F. (2016). Urinary metabolomic profiling to identify biomarkers of a flavonoid-rich and flavonoid-poor fruits and vegetables diet in adults: the FLAVURS trial. *Metabolomics*, *12*(2), 1–22. https://doi.org/10.1007/s11306-015-0935-z

Unno, T., Tamemoto, K., Yayabe, F., & Kakuda, T. (2003). Urinary Excretion of 5-(3',4'-Dihydroxyphenyl)-γ-valerolactone, a Ring-Fission Metabolite of (-)-Epicatechin, in Rats and Its in Vitro Antioxidant Activity. *Journal of Agricultural and Food Chemistry*, *51*, 6893–6898. https://doi.org/10.1021/jf034578e

Urpi-Sarda, M., Garrido, I., Monagas, M., Gómez-Cordovés, C., Medina-Remón, A., Andres-Lacuevaílnd, C., & Bartolomé, B. (2009). Profile of plasma and urine metabolites after the intake of almond [Prunus dulcis (Mill.) D.A. Webb] polyphenols in humans. *Journal of Agricultural and Food Chemistry*, *57*(21), 10134–10142. https://doi.org/10.1021/jf901450z

Urpi-Sarda, M., Monagas, M., Khan, N., Llorach, R., Lamuela-Raventós, R. M., Jáuregui, O., … Andrés-Lacueva, C. (2009). Targeted metabolic profiling of phenolics in urine and plasma after regular consumption of cocoa by liquid chromatography-tandem mass spectrometry. *Journal of Chromatography A*, *1216*(43), 7258–7267. https://doi.org/10.1016/j.chroma.2009.07.058

van Dorsten, F. A., Grün, C. H., van Velzen, E. J. J., Jacobs, D. M., Draijer, R., & van Duynhoven, J. P. M. (2009). The metabolic fate of red wine and grape juice polyphenols in humans assessed by metabolomics. *Molecular Nutrition & Food Research*, *54*(7), 897–908. https://doi.org/10.1002/mnfr.200900212

van Duynhoven, J., van der Hooft, J. J. J., van Dorsten, F. A., Peters, S., Foltz, M., Gomez-Roldan, V., … Jacobs, D. M. (2014). Rapid and Sustained Systemic Circulation of Conjugated Gut Microbial Catabolites after Single-Dose Black Tea Extract Consumption. *Journal of Proteome Research*, *13*(5), 2668–2678. https://doi.org/10.1021/pr5001253

van Duynhoven, J., van Velzen, E., & Jacobs, D. M. (2017). Nutrikinetic assessment of polyphenol exposure. *Current Opinion in Food Science*, *16*, 88–95. https://doi.org/10.1016/j.cofs.2017.09.004

Van Velzen, E. J. J., Westerhuis, J. A., Van Duynhoven, J. P. M., Van Dorsten, F. A., Grün, C. H., Jacobs, D. M., … Smilde, A. K. (2009). Phenotyping tea consumers by nutrikinetic analysis of polyphenolic end-metabolites. *Journal of Proteome Research*, *8*(7), 3317–3330. https://doi.org/10.1021/pr801071p

Vanholder, R., Glorieux, G., De Smet, R., & Lameire, N. (2003). New insights in uremic toxins. *Kidney International*, *63*, S6–S10. https://doi.org/10.1046/j.1523-1755.63.s84.43.x

Vanzo, A., Scholz, M., Gasperotti, M., Tramer, F., Passamonti, S., Vrhovsek, U., & Mattivi, F. (2013). Metabonomic investigation of rat tissues following intravenous administration of cyanidin 3-glucoside at a physiologically relevant dose. *Metabolomics*, *9*(1), 88–100. https://doi.org/10.1007/s11306-012-0430-8

Vrhovsek, U., Rigo, A., Tonon, D., & Mattivi, F. (2004). Quantitation of polyphenols in different apple varieties. *Journal of Agricultural and Food Chemistry*, *52*(21), 6532–6538. https://doi.org/10.1021/jf049317z

Westfall, P., & Young, S. (1993). *Resampling-Based Multiple Testing: Examples and Methods for p-Value Adjustment*. Wiley-Interscience. Retrieved from https://www.wiley.com/en-it/Resampling+Based+Multiple+Testing:+Examples+and+Methods+for+p+Value+Adjustment-p-9780471557616

Wiese, S., Esatbeyoglu, T., Winterhalter, P., Kruse, H.-P., Winkler, S., Bub, A., & Kulling, S. E. (2015). Comparative biokinetics and metabolism of pure monomeric, dimeric, and polymeric flavan-3-ols: A randomized cross-over study in humans. *Molecular Nutrition & Food Research*, *59*(4), 610–621. https://doi.org/10.1002/mnfr.201400422

Williamson, G., Dionisi, F., & Renouf, M. (2011). Flavanols from green tea and phenolic acids from coffee: Critical quantitative evaluation of the pharmacokinetic data in humans after consumption of single doses of beverages. *Molecular Nutrition and Food*

Research, *55*(6), 864–873. https://doi.org/10.1002/mnfr.201000631

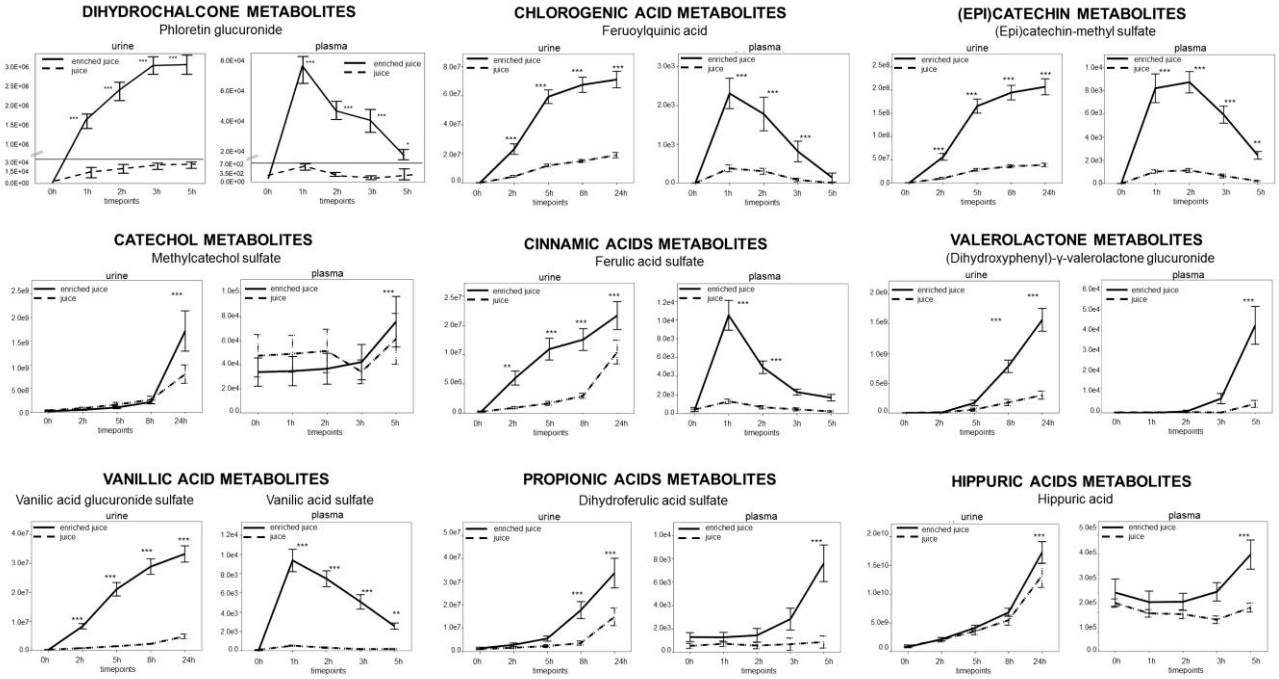
Wishart, D. S., Jewison, T., Guo, A. C., Wilson, M., Knox, C., Liu, Y., … Scalbert, A. (2013). HMDB 3.0-The Human Metabolome Database in 2013. *Nucleic Acids Research*, *41*(D1), 801–807. https://doi.org/10.1093/nar/gks1065

Wu, G. D., Compher, C., Chen, E. Z., Smith, S. A., Shah, R. D., Bittinger, K., … Lewis, J. D. (2016). Comparative metabolomics in vegans and omnivores reveal constraints on diet-dependent gut microbiota metabolite production. *Gut*, *65*(1), 63–72. https://doi.org/10.1136/gutjnl-2014-308209

Wu, J., Gao, H., Zhao, L., Liao, X., Chen, F., Wang, Z., & Hu, X. (2007). Chemical compositional characterization of some apple cultivars. *Food Chemistry*, *103*(1), 88–93. https://doi.org/10.1016/j.foodchem.2006.07.030

Zhang, T., Creek, D. J., Barrett, M. P., Blackburn, G., & Watson, D. G. (2012). Evaluation of coupling reversed phase, aqueous normal phase, and hydrophilic interaction liquid chromatography with orbitrap mass spectrometry for metabolomic studies of human urine. *Analytical Chemistry*, *84*(4), 1994–2001. https://doi.org/10.1021/ac2030738

Figure 1



*** p value <0.001; ** p value 0.01-0.001; * p value 0.05-0.01
Plasma graphs: X axis: timepoints ; Y axis: peak intensity (MS response). Urine graphs: X axis timepoints, Y axis: cumulative intensities of peak (MS response)

Figure 2

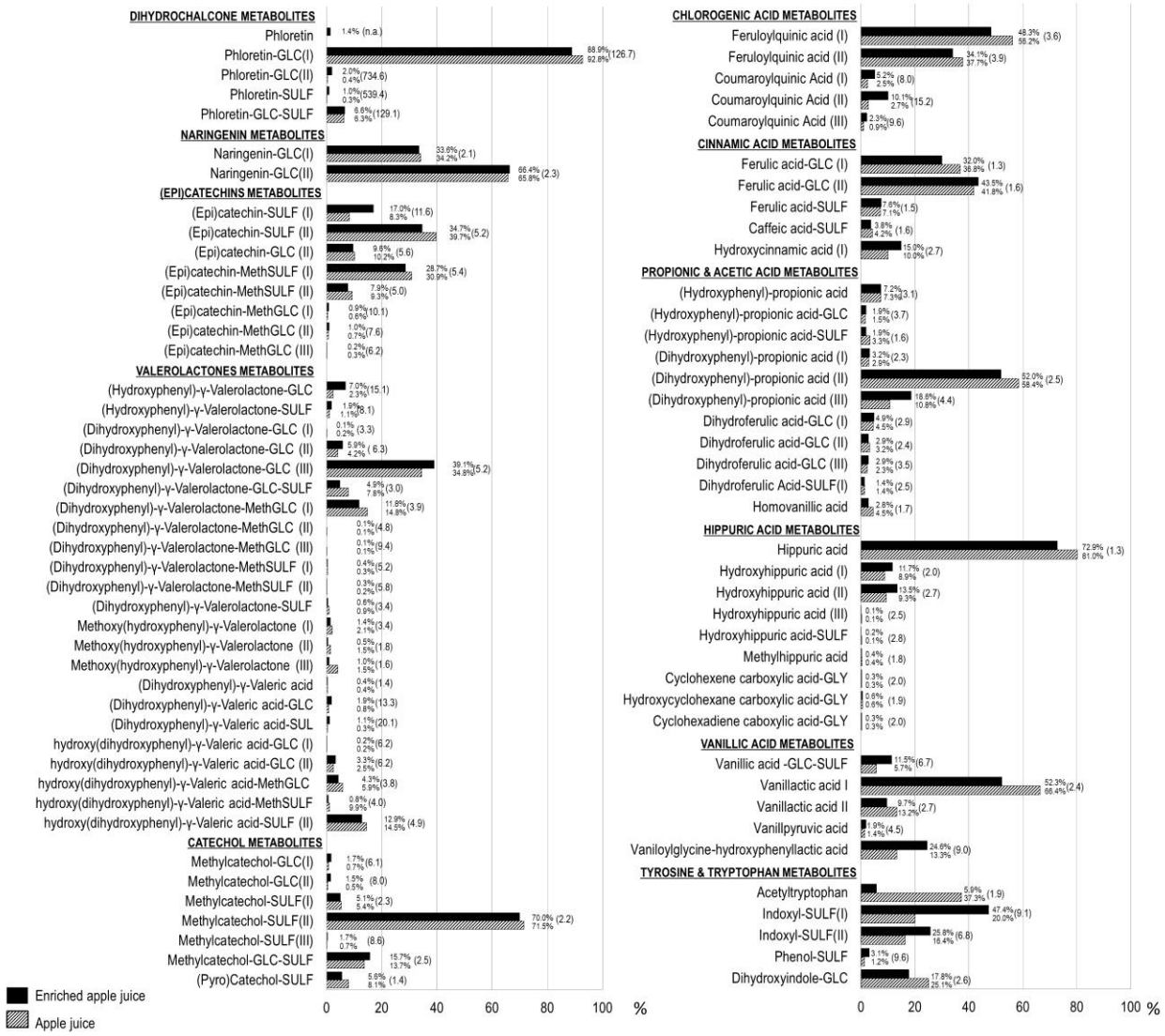

DIHYDROCHALCONE METABOLITES
Phloretin
Phloretin-GLC(I)
Phloretin-GLC(II)
Phloretin-SULF
Phloretin-GLC-SULF
NARINGENIN METABOLITES
Naringenin-GLC(I)
Naringenin-GLC(II)
(EPI)CATECHINS METABOLITES
(Epi)catechin-SULF (I)
(Epi)catechin-SULF (II)
(Epi)catechin-GLC (II)
(Epi)catechin-MethSULF (I)
(Epi)catechin-MethSULF (II)
(Epi)catechin-MethGLC (I)
(Epi)catechin-MethGLC (II)
(Epi)catechin-MethGLC (III)
VALEROLACTONES METABOLITES
(Hydroxyphenyl)-γ-Valerolactone-GLC
(Hydroxyphenyl)-γ-Valerolactone-SULF
(Dihydroxyphenyl)-γ-Valerolactone-GLC (I)
(Dihydroxyphenyl)-γ-Valerolactone-GLC (II)
(Dihydroxyphenyl)-γ-Valerolactone-GLC (III)
(Dihydroxyphenyl)-γ-Valerolactone-GLC-SULF
(Dihydroxyphenyl)-γ-Valerolactone-MethGLC (I)
(Dihydroxyphenyl)-γ-Valerolactone-MethGLC (II)
(Dihydroxyphenyl)-γ-Valerolactone-MethGLC (III)
(Dihydroxyphenyl)-γ-Valerolactone-MethSULF (I)
(Dihydroxyphenyl)-γ-Valerolactone-MethSULF (II)
(Dihydroxyphenyl)-γ-Valerolactone-SULF
Methoxy(hydroxyphenyl)-γ-Valerolactone (I)
Methoxy(hydroxyphenyl)-γ-Valerolactone (II)
Methoxy(hydroxyphenyl)-γ-Valerolactone (III)
(Dihydroxyphenyl)-γ-Valeric acid
(Dihydroxyphenyl)-γ-Valeric acid-GLC
(Dihydroxyphenyl)-γ-Valeric acid-SUL
hydroxy(dihydroxyphenyl)-γ-Valeric acid-GLC (I)
hydroxy(dihydroxyphenyl)-γ-Valeric acid-GLC (II)
hydroxy(dihydroxyphenyl)-γ-Valeric acid-MethGLC
hydroxy(dihydroxyphenyl)-γ-Valeric acid-MethSULF
hydroxy(dihydroxyphenyl)-γ-Valeric acid-SULF (II)
CATECHOL METABOLITES
Methylcatechol-GLC(I)
Methylcatechol-GLC(II)
Methylcatechol-SULF(I)
Methylcatechol-SULF(II)
Methylcatechol-SULF(III)
Methylcatechol-GLC-SULF
(Pyro)Catechol-SULF
Enriched apple juice
Apple juice
0 20 40 60 80 100 %
CHLOROGENIC ACID METABOLITES
Feruloylquinic acid (I)
Feruloylquinic acid (II)
Coumaroylquinic Acid (I)
Coumaroylquinic Acid (II)
Coumaroylquinic Acid (III)
CINNAMIC ACID METABOLITES
Ferulic acid-GLC (I)
Ferulic acid-GLC (II)
Ferulic acid-SULF
Caffeic acid-SULF
Hydroxycinnamic acid (I)
PROPIONIC & ACETIC ACID METABOLITES
(Hydroxyphenyl)-propionic acid
(Hydroxyphenyl)-propionic acid-GLC
(Hydroxyphenyl)-propionic acid-SULF
(Dihydroxyphenyl)-propionic acid (I)
(Dihydroxyphenyl)-propionic acid (II)
(Dihydroxyphenyl)-propionic acid (III)
Dihydroferulic acid-GLC (I)
Dihydroferulic acid-GLC (II)
Dihydroferulic acid-GLC (III)
Dihydroferulic Acid-SULF(I)
Homovanillic acid
HIPPURIC ACID METABOLITES
Hippuric acid
Hydroxyhippuric acid (I)
Hydroxyhippuric acid (II)
Hydroxyhippuric acid (III)
Hydroxyhippuric acid-SULF
Methylhippuric acid
Cyclohexene carboxylic acid-GLY
Hydroxycyclohexane carboxylic acid-GLY
Cyclohexadiene caboxylic acid-GLY
VANILLIC ACID METABOLITES
Vanillic acid -GLC-SULF
Vanillactic acid I
Vanillactic acid II
Vanillpyruvic acid
Vaniloylglycine-hydroxyphenyllactic acid
TYROSINE & TRYPTOPHAN METABOLITES
Acetyltryptophan
Indoxyl-SULF(I)
Indoxyl-SULF(II)
Phenol-SULF
Dihydroxyindole-GLC
0 20 40 60 80 100 %

Figure 3

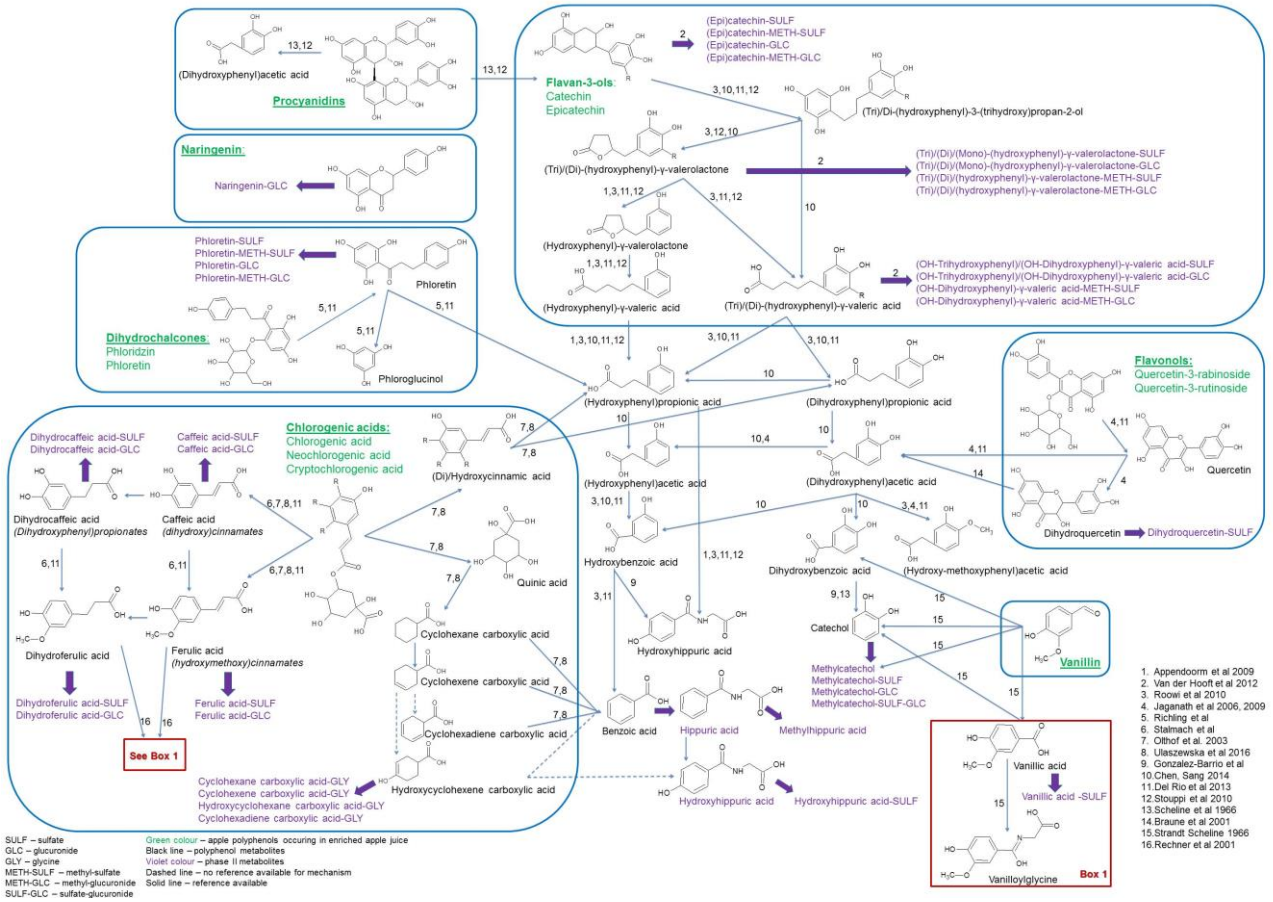

Procyanidins
(Dihydroxyphenyl)acetic acid
Naringenin:
Naringenin-GLC
Dihydrochalcones:
Phloridzin
Phloretin
Phloretin-SULF
Phloretin-METH-SULF
Phloretin-GLC
Phloretin-METH-GLC
Phloroglucinol
Flavan-3-ols:
Catechin
Epicatechin
(Epi)catechin-SULF
(Epi)catechin-METH-SULF
(Epi)catechin-GLC
(Epi)catechin-METH-GLC
(Tri)/Di-(hydroxyphenyl)-3-(trihydroxy)propan-2-ol
(Tri)/(Di)-(hydroxyphenyl)-γ-valerolactone
(Tri)/(Di)/(Mono)-(hydroxyphenyl)-γ-valerolactone-SULF
(Tri)/(Di)/(Mono)-(hydroxyphenyl)-γ-valerolactone-GLC
(Tri)/(Di)/(hydroxyphenyl)-γ-valerolactone-METH-SULF
(Tri)/(Di)/(hydroxyphenyl)-γ-valerolactone-METH-GLC
(Hydroxyphenyl)-γ-valerolactone
(Hydroxyphenyl)-γ-valeric acid
(Tri)/(Di)-(hydroxyphenyl)-γ-valeric acid
(OH-Trihydroxyphenyl)/(OH-Dihydroxyphenyl)-γ-valeric acid-SULF
(OH-Trihydroxyphenyl)/(OH-Dihydroxyphenyl)-γ-valeric acid-GLC
(OH-Dihydroxyphenyl)-γ-valeric acid-METH-SULF
(OH-Dihydroxyphenyl)-γ-valeric acid-METH-GLC
Flavonols:
Quercetin-3-rabinoside
Quercetin-3-rutinoside
Quercetin
Dihydroquercetin
Dihydroquercetin-SULF
Chlorogenic acids:
Chlorogenic acid
Neochlorogenic acid
Cryptochlorogenic acid
(Di)/Hydroxycinnamic acid
Dihydrocaffeic acid-SULF
Dihydrocaffeic acid-GLC
Caffeic acid-SULF
Caffeic acid-GLC
Dihydrocaffeic acid
(Dihydroxyphenyl)propionates
Caffeic acid
(dihydroxy)cinnamates
Quinic acid
Dihydroferulic acid
Ferulic acid
(hydroxymethoxy)cinnamates
Dihydroferulic acid-SULF
Dihydroferulic acid-GLC
Ferulic acid-SULF
Ferulic acid-GLC
See Box 1
Cyclohexane carboxylic acid
Cyclohexene carboxylic acid
Cyclohexadiene carboxylic acid
Hydroxycyclohexene carboxylic acid
Cyclohexane carboxylic acid-GLY
Cyclohexene carboxylic acid-GLY
Hydroxycyclohexane carboxylic acid-GLY
Cyclohexadiene carboxylic acid-GLY
(Hydroxyphenyl)propionic acid
(Dihydroxyphenyl)propionic acid
(Hydroxyphenyl)acetic acid
(Dihydroxyphenyl)acetic acid
Hydroxybenzoic acid
Dihydroxybenzoic acid
(Hydroxy-methoxyphenyl)acetic acid
Hydroxyhippuric acid
Catechol
Methylcatechol
Methylcatechol-SULF
Methylcatechol-GLC
Methylcatechol-SULF-GLC
Vanillin
Benzoic acid
Hippuric acid
Methylhippuric acid
Hydroxyhippuric acid
Hydroxyhippuric acid-SULF
Vanillic acid
Vanillic acid -SULF
Vanilloylglycine
Box 1
SULF – sulfate
GLC – glucuronide
GLY – glycine
METH-SULF – methyl-sulfate
METH-GLC – methyl-glucuronide
SULF-GLC – sulfate-glucuronide
Green colour – apple polyphenols occuring in enriched apple juice
Black line – polyphenol metabolites
Violet colour – phase II metabolites
Dashed line – no reference available for mechanism
Solid line – reference available
1. Appendoorm et al 2009
2. Van der Hooft et al 2012
3. Roowi et al 2010
4. Jaganath et al 2006, 2009
5. Richling et al
6. Stalmach et al
7. Olthof et al. 2003
8. Ulaszewska et al 2016
9. Gonzalez-Barrio et al
10. Chen, Sang 2014
11. Del Rio et al 2013
12. Stouppi et al 2010
13. Scheline et al 1966
14. Braune et al 2001
15. Strandt Scheline 1966
16. Rechner et al 2001

Figure 4

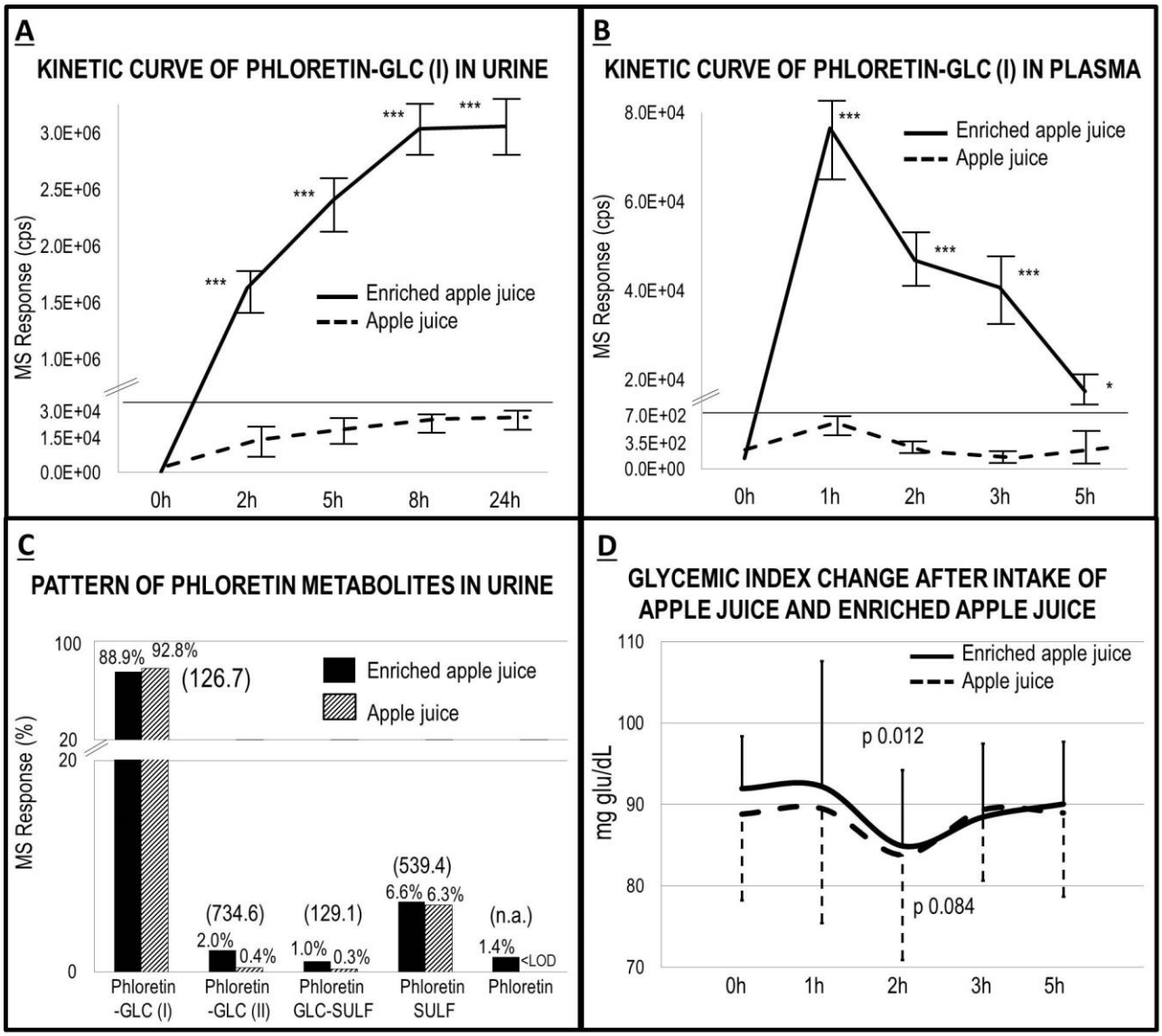

A
KINETIC CURVE OF PHLORETIN-GLC (I) IN URINE
MS Response (cps)
3.0E+06
2.5E+06
2.0E+06
1.5E+06
1.0E+06
3.0E+04
1.5E+04
0.0E+00
0h
2h
5h
8h
24h
Enriched apple juice
Apple juice
B
KINETIC CURVE OF PHLORETIN-GLC (I) IN PLASMA
MS Response (cps)
8.0E+04
6.0E+04
4.0E+04
2.0E+04
7.0E+02
3.5E+02
0.0E+00
0h
1h
2h
3h
5h
Enriched apple juice
Apple juice
C
PATTERN OF PHLORETIN METABOLITES IN URINE
MS Response (%)
100
20
0
88.9%
92.8%
(126.7)
(734.6)
2.0%
0.4%
(129.1)
1.0%
0.3%
(539.4)
6.6%
6.3%
(n.a.)
1.4%
<LOD
Phloretin -GLC (I)
Phloretin -GLC (II)
Phloretin GLC-SULF
Phloretin SULF
Phloretin
Enriched apple juice
Apple juice
D
GLYCEMIC INDEX CHANGE AFTER INTAKE OF APPLE JUICE AND ENRICHED APPLE JUICE
mg glu/dL
110
100
90
80
70
p 0.012
p 0.084
0h
1h
2h
3h
5h
Enriched apple juice
Apple juice

Figure 5

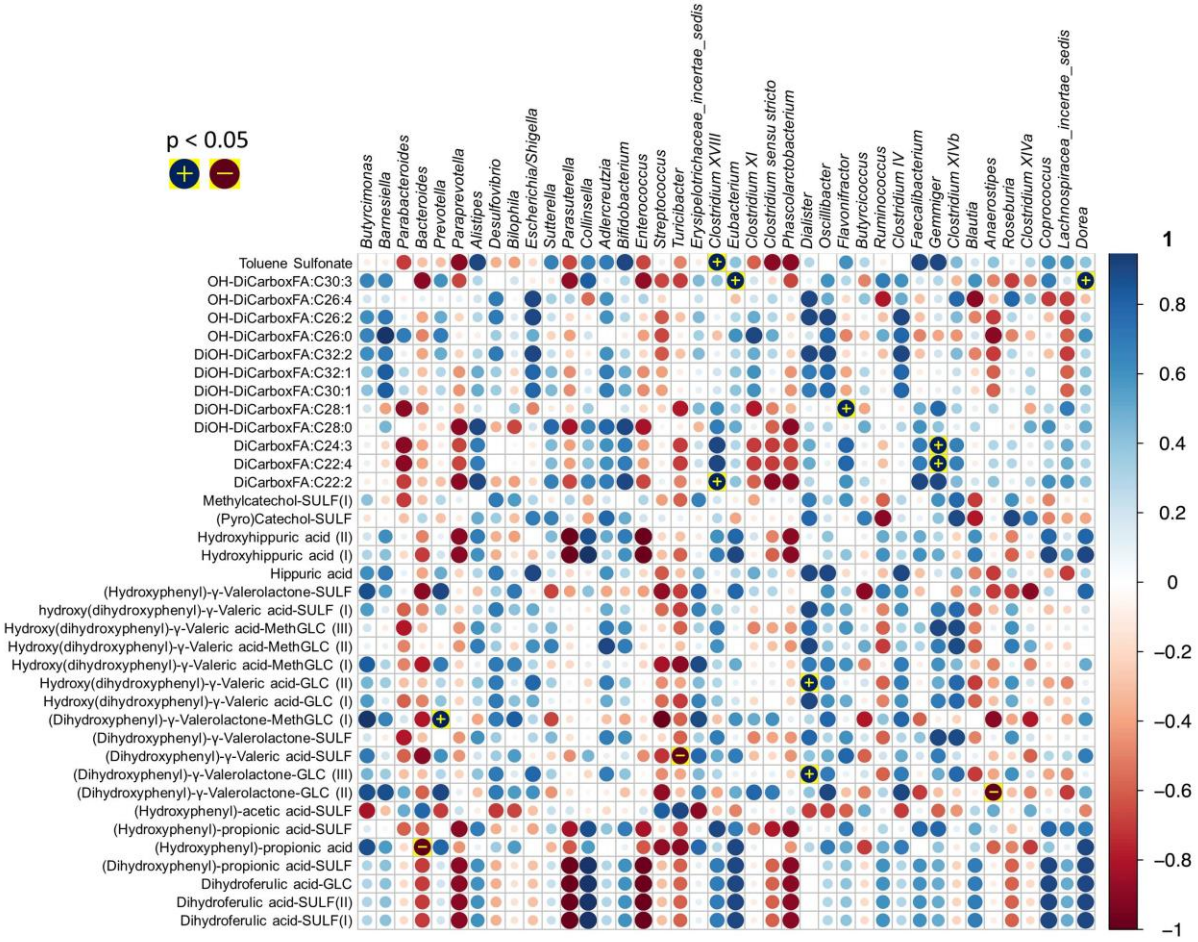
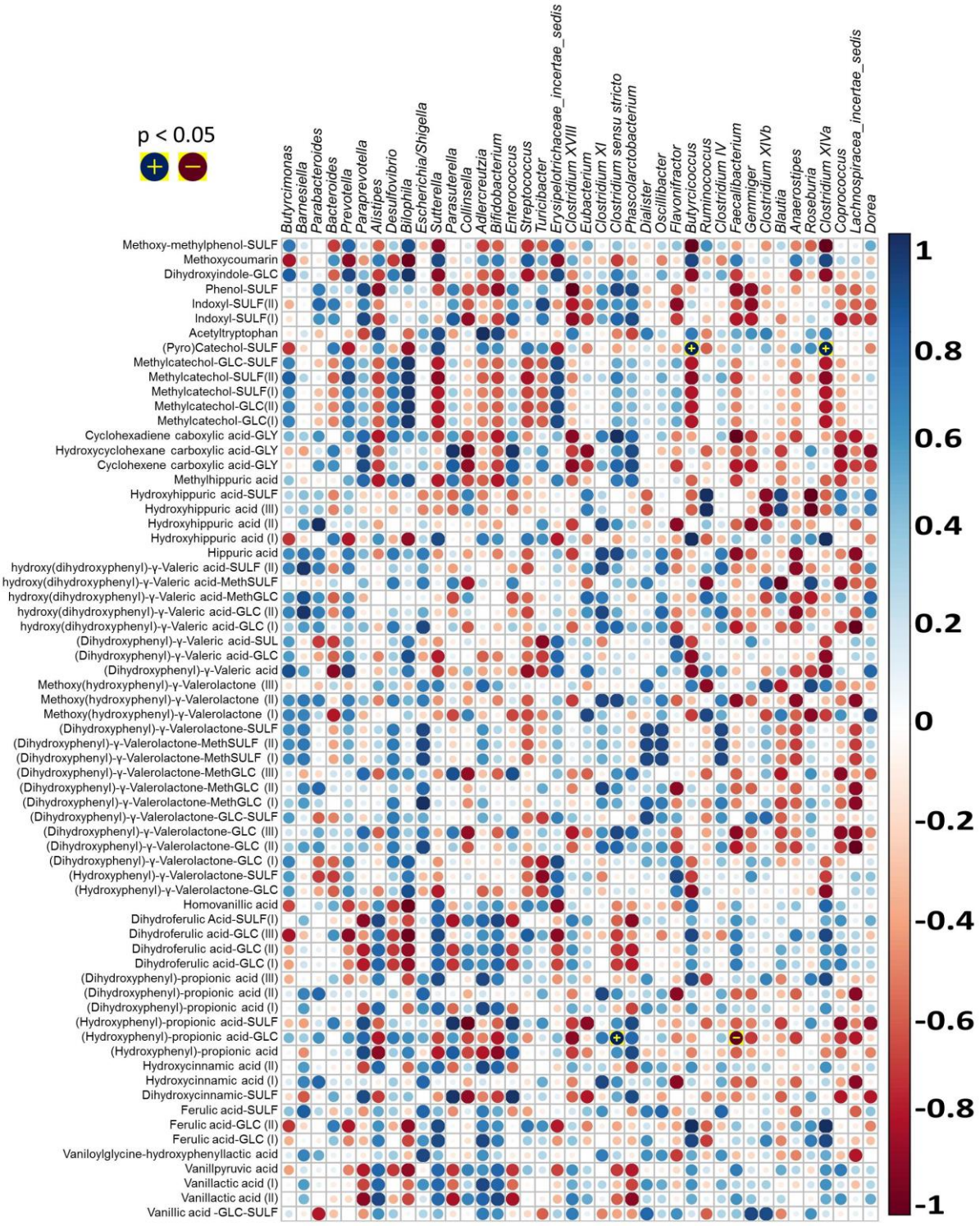

p < 0.05
Butyricimonas
Barnesiella
Parabacteroides
Bacteroides
Prevotella
Paraprevotella
Alistipes
Desulfovibrio
Bilophila
Escherichia/Shigella
Sutterella
Parasutterella
Collinsella
Adlercreutzia
Bifidobacterium
Enterococcus
Streptococcus
Turicibacter
Erysipelotrichaceae_incertae_sedis
Clostridium XVIII
Eubacterium
Clostridium XI
Clostridium sensu stricto
Phascolarctobacterium
Dialister
Oscillibacter
Flavonifractor
Butyricicoccus
Ruminococcus
Clostridium IV
Faecalibacterium
Gemmiger
Clostridium XIVb
Blautia
Anaerostipes
Roseburia
Clostridium XIVa
Coprococcus
Lachnospiracea_incertae_sedis
Dorea
Toluene Sulfonate
OH-DiCarboxFA:C30:3
OH-DiCarboxFA:C26:4
OH-DiCarboxFA:C26:2
OH-DiCarboxFA:C26:0
DiOH-DiCarboxFA:C32:2
DiOH-DiCarboxFA:C32:1
DiOH-DiCarboxFA:C30:1
DiOH-DiCarboxFA:C28:1
DiOH-DiCarboxFA:C28:0
DiCarboxFA:C24:3
DiCarboxFA:C22:4
DiCarboxFA:C22:2
Methylcatechol-SULF(I)
(Pyro)Catechol-SULF
Hydroxyhippuric acid (II)
Hydroxyhippuric acid (I)
Hippuric acid
(Hydroxyphenyl)-γ-Valerolactone-SULF
hydroxy(dihydroxyphenyl)-γ-Valeric acid-SULF (I)
Hydroxy(dihydroxyphenyl)-γ-Valeric acid-MethGLC (III)
Hydroxy(dihydroxyphenyl)-γ-Valeric acid-MethGLC (II)
Hydroxy(dihydroxyphenyl)-γ-Valeric acid-MethGLC (I)
Hydroxy(dihydroxyphenyl)-γ-Valeric acid-GLC (II)
Hydroxy(dihydroxyphenyl)-γ-Valeric acid-GLC (I)
(Dihydroxyphenyl)-γ-Valerolactone-MethGLC (I)
(Dihydroxyphenyl)-γ-Valerolactone-SULF
(Dihydroxyphenyl)-γ-Valeric acid-SULF
(Dihydroxyphenyl)-γ-Valerolactone-GLC (III)
(Dihydroxyphenyl)-γ-Valerolactone-GLC (II)
(Hydroxyphenyl)-acetic acid-SULF
(Hydroxyphenyl)-propionic acid-SULF
(Hydroxyphenyl)-propionic acid
(Dihydroxyphenyl)-propionic acid-SULF
Dihydroferulic acid-GLC
Dihydroferulic acid-SULF(II)
Dihydroferulic acid-SULF(I)
1
0.8
0.6
0.4
0.2
0
−0.2
−0.4
−0.6
−0.8
−1

Figure 6


p < 0.05
Butyricimonas
Barnesiella
Parabacteroides
Bacteroides
Prevotella
Paraprevotella
Alistipes
Desulfovibrio
Bilophila
Escherichia/Shigella
Sutterella
Parasuterella
Collinsella
Adlercreutzia
Bifidobacterium
Enterococcus
Streptococcus
Turicibacter
Erysipelotrichaceae_incertae_sedis
Clostridium XVIII
Eubacterium
Clostridium XI
Clostridium sensu stricto
Phascolarctobacterium
Dialister
Oscillibacter
Flavonifractor
Butyricicoccus
Ruminococcus
Clostridium IV
Faecalibacterium
Gemmiger
Clostridium XIVb
Blautia
Anaerostipes
Roseburia
Clostridium XIVa
Coprococcus
Lachnospiracea_incertae_sedis
Dorea
Methoxy-methylphenol-SULF
Methoxycoumarin
Dihydroxyindole-GLC
Phenol-SULF
Indoxyl-SULF(II)
Indoxyl-SULF(I)
Acetyltryptophan
(Pyro)Catechol-SULF
Methylcatechol-GLC-SULF
Methylcatechol-SULF(II)
Methylcatechol-SULF(I)
Methylcatechol-GLC(II)
Methylcatechol-GLC(I)
Cyclohexadiene caboxylic acid-GLY
Hydroxycyclohexane carboxylic acid-GLY
Cyclohexene carboxylic acid-GLY
Methylhippuric acid
Hydroxyhippuric acid-SULF
Hydroxyhippuric acid (III)
Hydroxyhippuric acid (II)
Hydroxyhippuric acid (I)
Hippuric acid
hydroxy(dihydroxyphenyl)-γ-Valeric acid-SULF (II)
hydroxy(dihydroxyphenyl)-γ-Valeric acid-MethSULF
hydroxy(dihydroxyphenyl)-γ-Valeric acid-MethGLC
hydroxy(dihydroxyphenyl)-γ-Valeric acid-GLC (II)
hydroxy(dihydroxyphenyl)-γ-Valeric acid-GLC (I)
(Dihydroxyphenyl)-γ-Valeric acid-SUL
(Dihydroxyphenyl)-γ-Valeric acid-GLC
(Dihydroxyphenyl)-γ-Valeric acid
Methoxy(hydroxyphenyl)-γ-Valerolactone (III)
Methoxy(hydroxyphenyl)-γ-Valerolactone (II)
Methoxy(hydroxyphenyl)-γ-Valerolactone (I)
(Dihydroxyphenyl)-γ-Valerolactone-SULF
(Dihydroxyphenyl)-γ-Valerolactone-MethSULF (II)
(Dihydroxyphenyl)-γ-Valerolactone-MethSULF (I)
(Dihydroxyphenyl)-γ-Valerolactone-MethGLC (III)
(Dihydroxyphenyl)-γ-Valerolactone-MethGLC (II)
(Dihydroxyphenyl)-γ-Valerolactone-MethGLC (I)
(Dihydroxyphenyl)-γ-Valerolactone-GLC-SULF
(Dihydroxyphenyl)-γ-Valerolactone-GLC (III)
(Dihydroxyphenyl)-γ-Valerolactone-GLC (II)
(Dihydroxyphenyl)-γ-Valerolactone-GLC (I)
(Hydroxyphenyl)-γ-Valerolactone-SULF
(Hydroxyphenyl)-γ-Valerolactone-GLC
Homovanillic acid
Dihydroferulic Acid-SULF(I)
Dihydroferulic acid-GLC (III)
Dihydroferulic acid-GLC (II)
Dihydroferulic acid-GLC (I)
(Dihydroxyphenyl)-propionic acid (III)
(Dihydroxyphenyl)-propionic acid (II)
(Dihydroxyphenyl)-propionic acid (I)
(Hydroxyphenyl)-propionic acid-SULF
(Hydroxyphenyl)-propionic acid-GLC
(Hydroxyphenyl)-propionic acid
Hydroxycinnamic acid (II)
Hydroxycinnamic acid (I)
Dihydroxycinnamic-SULF
Ferulic acid-SULF
Ferulic acid-GLC (II)
Ferulic acid-GLC (I)
Vaniloylglycine-hydroxyphenyllactic acid
Vanillpyruvic acid
Vanillactic acid (I)
Vanillactic acid (II)
Vanillic acid -GLC-SULF
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1

1181

Table 1

Substrate	No	Name (isomers)	Rt (min) plasma/ urine/ meth.extr	Formula; (MS Identification Level)	$T_{max(P)}$ $T_{max(U)}$, (In hours)	matrix; direction PAJ vs CAJ; adjusted p value (Adj. p)	Reference
			DIHYDROCHALCONES METABOLITES				
	M1	Phloretin	-/ 12.40/ -	$C_{15}H_{14}O_5$ (I)	$T_{max(U)}$ 8h	Urine ↑; Adj. p 7.3E-16	STD Lab
	M2	Phloretin glucuronide (I)	7.76/ 9.70 / -	$C_{21}H_{22}O_{11}$ (II)	$T_{max(P)}$ 1h $T_{max(U)}$ 8h	Plasma ↑; Adj.p 1.3E-15 ; Urine ↑; Adj.p 4.4E-49	Marks et al 2009; STD Lab
Phlorizin							
Phloretin	M3	Phloretin glucuronide (II)	-/ 9.88/ -	$C_{21}H_{22}O_{11}$ (II)	$T_{max(U)}$ 8h	Urine ↑; Adj. p 1.9E-56	Marks et al 2009,STD Lab
Naringenin	M4	Phloretin sulfate	-/ 9.05/ -	$C_{15}H_{14}O_8S$ (II)	$T_{max(U)}$ 8h	Urine ↑; Adj. p 4.9E-36	Marks et al 2009, STD Lab
	M5	Phloretin glucuronide sulfate	-/ 9.20/ -	$C_{21}H_{22}O_{14}S$ (II)	$T_{max(U)}$ 8h	Urine ↑; Adj.p 1.3E-18	Marks et al 2009, STD Lab
	M6	Naringenin glucuronide (I)	-/ 9.23/ -	$C_{21}H_{20}O_{11}$ (III)	$T_{max(U)}$ > 24h	Urine ↑; Adj. p 2.6E-17	
	M7	Naringenin glucuronide (II)	-/ 9.48/ -	$C_{21}H_{20}O_{11}$ (III)	$T_{max(U)}$ > 24h	Urine ↑; Adj. p 1.6E-14	
			QUERCETIN METABOLITES				
Quercetin-3-rabinoside Quercetin-3-rutinoside	M8	Dihydroquercetin sulfate	-/ 8.95 /-	$C_{15}H_{12}O_{10}S$ (III)	$T_{max(U)}$ 8h	Urine ↑; Adj. p 2.7E-53	Metlin, HMDB, In-house MS Library, STD Lab.
			(EPI)CATECHIN METABOLITES				
	M9	(Epi)catechin-glucuronide	-/ 6.24/-	$C_{21}H_{22}O_{12}$ (II)	$T_{max(U)}$ 8h	Urine ↑; Adj.p 3.0E-29	Blount et al 2012
Catechin	M10	(Epi)catechin-sulfate (I)	-/ 6.16/-	$C_{15}H_{13}O_9S$ (II)	$T_{max(U)}$ 8h	Urine ↑; Adj.p 7.1E-49	Van der Hooft et al 2012
Epicatechin	M11	(Epi)catechin-sulfate (II)	-/ 6.90/-	$C_{15}H_{13}O_9S$ (II)	$T_{max(U)}$ 8h	Urine ↑; Adj.p 3.5E-43	Van der Hooft et al 2012
Procyanidins	M12	(Epi)catechin-methyl glucuronide (I)	-/ 5.50/ -	$C_{22}H_{24}O_{12}$ (II)	$T_{max(U)}$ > 24h	Urine ↑; Adj.p 1.5E-20	Blount et al 2012; Liu et al 2015
	M13	(Epi)catechin-methyl glucuronide (II)	-/ 6.35/ -	$C_{22}H_{24}O_{12}$ (II)	$T_{max(U)}$ 8h	Urine ↑; Adj.p 3.3E-21	Blount et al 2012; Liu et al 2015
	M14	(Epi)catechin-methyl glucuronide (III)	-/ 6.65/ -	$C_{22}H_{24}O_{12}$ (II)	$T_{max(U)}$ 8h	Urine ↑; Adj.p 1.3E-18	Blount et al 2012; Liu et al 2015

	M15	(Epi)catechin-methyl sulfate (I)	5.90/ 7.35/ -	$C_{16}H_{16}O_9S$ (II)	$T_{max(P)}$ 3h $T_{max(U)}$ 8h	Plasma ↑; Adj p 1.1E-32; Urine ↑; Adj.p 7.7E-44	Van Der Hooft et al 2012
	M16	(Epi)catechin-methyl sulfate (II)	6.27/ 7.85/ -	$C_{16}H_{16}O_9S$ (II)	$T_{max(P)}$ 3h $T_{max(U)}$ 8h	Plasma ↑; Adj. p 1.1E-32; Urine ↑; Adj.p 3.2E-48	Van Der Hooft et al 2012
		VALEROLACTONE METABOLITES					
	M17	Hydroxyphenyl-γ-valerolactone glucuronide	-/ 6.80 /-	$C_{17}H_{20}O_9$ (II)	$T_{max(U)}$ > 24 h	Urine ↑; Adj. p 6.7E-10	Van der Hooft et al 2012 , Llorach et al 2010
	M18	Hydroxyphenyl-γ-valerolactone sulfate	-/ 7.25 / 6.12	$C_{11}H_{12}O_6S$ (II)	$T_{max(P)}$ >5 h $T_{max(U)}$ > 24 h	Meth.Ext.↑; Adj. p 1.9E-03; Urine ↑; Adj.p 1.2E-12	Van der Hooft et al 2012 ; Llorach et al 2010
	M19	Dihydroxyphenyl-γ-valerolactone sulfate	5.88/ 6.70 /-	$C_{11}H_{12}O_7S$ (II)	$T_{max(P)}$ >5 h $T_{max(U)}$ > 24 h	Plasma ↑;Adj.p 4.5E-05; Urine ↑; Adj.p 4.0E-23	Van der Hooft et al 2012; STD Lab
	M20	Dihydrohyphenyl-γ-valerolactone glucuronide (I)	-/ 5.20 /-	$C_{17}H_{20}O_{10}$ (II)	$T_{max(U)}$ > 24 h	Urine ↑; Adj.p 1.2E-12	Van der Hooft et al 2012; STD Lab
	M21	Dihydrohyphenyl-γ-valerolactone glucuronide (II)	-/ 6.05 / 5.35	$C_{17}H_{20}O_{10}$ (II)	$T_{max(P)}$ >5 h $T_{max(U)}$ > 24 h	Urine ↑; Adj. p 7.2E-22 ; Meth.Extr. ↑; Adj. p 1.1E-06	Van der Hooft et al 2012; STD Lab
Catechin							
Epicatechin	M22	Dihydrohyphenyl-γ-valerolactone glucuronide (III)	5.58/ 6.40 /-	$C_{17}H_{20}O_{10}$ (II)	$T_{max(P)}$ >5 h $T_{max(U)}$ > 24 h	Plasma ↑; Adj. p 1.3E-07; Urine ↑; Adj. p 6.5E-26	Van der Hooft et al 2012; STD Lab
Procyanidins							
	M23	Dihydroxyphenyl-γ-valerolactone methyl glucuronide (I)	-/ 6.55 / 5.75	$C_{18}H_{22}O_{10}$ (II)	$T_{max(P)}$ >5 h $T_{max(U)}$ > 24 h	Meth.Extr. ↑; Adj. p 1.3E-05; Urine ↑; Adj.p 7.1E-29	Van der Hooft et al 2012; STD Lab
	M24	Dihydroxyphenyl-γ-valerolactone methyl glucuronide (II)	-/ 7.20 /-	$C_{18}H_{22}O_{10}$ (II)	$T_{max(U)}$ > 24 h	Urine ↑; Adj.p2.8E-19	Van der Hooft et al 2012; STD Lab
	M25	Dihydroxyphenyl-γ-valerolactone methyl glucuronide (III)	-/ 7.95 /-	$C_{18}H_{22}O_{10}$ (II)	$T_{max(U)}$ > 24 h	Urine ↑; Adj.p 1.1E-06	Van der Hooft et al 2012; STD Lab
	M26	Dihydroxyphenyl-γ-valerolactone methyl sulfate (I)	-/ 7.00 /-	$C_{12}H_{14}O_7S$ (II)	$T_{max(U)}$ > 24 h	Urine ↑; Adj.p 1.2E-16	Van der Hooft et al 2012
	M27	Dihydroxyphenyl-γ-valerolactone methyl sulfate (II)	-/ 7.50 /-	$C_{12}H_{14}O_7S$ (II)	$T_{max(U)}$ > 24 h	Urine ↑; Adj.p 1.4E-20	Van der Hooft et al 2012
	M28	Dihydroxyphenyl-γ-valerolactone glucuronide sulfate	-/ 5.18 /-	$C_{17}H_{20}O_{13}S$ (II)	$T_{max(U)}$ > 24 h	Urine ↑; Adj.p 6.8E-20	Van der Hooft et al 2012
	M29	Methoxy-hydroxyphenyl-γ-valerolactone (I)	-/ 5.35 /-	$C_{12}H_{14}O_4$ (II)	$T_{max(U)}$ > 24 h	Urine ↑; Adj.p 1.8E-24	Urpi-Sarda et al 2009
	M30	Methoxy-hydroxyphenyl-γ-valerolactone (II)	-/ 7.20 / -	$C_{12}H_{14}O_4$ (II)	$T_{max(U)}$ > 24 h	Urine ↑; Adj.p 1.0E-11	Urpi-Sarda et al 2009

	M31	Methoxy-hydroxyphenyl-γ-valerolactone (III)	-/ 8.95 / -	$C_{12}H_{14}O_4$ (II)	$T_{max(U)}$ > 24 h	Urine ↑; Adj.p 1.5E-08	Urpi-Sarda et al 2009
	M32	Dihydroxyphenyl-γ-valeric acid	-/ 7.00/ -	$C_{11}H_{14}O_4$ (I)	$T_{max(U)}$ > 24 h	Urine ↑; Adj.p 5.1E-08	Van der Hooft et al 2012, STD Lab
	M33	Dihydroxyphenyl-γ-valeric acid sulfate	5.28/ 5.93/-	$C_{11}H_{14}O_7S$ (II)	$T_{max(P)}$ > 5h $T_{max(U)}$ > 24 h	Plasma ↑; Adj.p 5.7E-03; Urine ↑; Adj. p 4.9E-10	Van der Hooft et al 2012, STD Lab
	M34	Dihydroxyphenyl-γ-valeric acid glucuronide(I)	5.60/- / 5.35	$C_{17}H_{22}O_{10}$ (II)	$T_{max(P)}$ > 5h $T_{max(ME)}$ > 5h	Plasma ↑; Adj.p 1.3E-07 ; Meth.Extr ↑; Adj.p 1.1E-06	STD Lab
	M35	Dihydroxyphenyl-γ-valeric acid glucuronide(II)	- / 5.61 / -	$C_{17}H_{22}O_{10}$ (II)	$T_{max(U)}$ > 24 h	Urine ↑; Adj. 1.4E-06	STD Lab
	M36	Hydroxy(dihydroxyPhenyl)-γ-valeric acid glucuronide (I)	4.38/ 4.77 / -	$C_{17}H_{22}O_{11}$ (II)	$T_{max(P)}$ >5h $T_{max(U)}$ > 24 h	Plasma ↑; Adj.p 6.0E-12; Urine ↑; Adj. p 1.7E-17	Van der Hooft et al 2012, Llorach et al 2010
	M37	Hydroxy(dihydroxyphenyl)-γ-valeric acid glucuronide (II)	4.68/ 5.13 /-	$C_{17}H_{22}O_{11}$ (II)	$T_{max(P)}$ >5h $T_{max(U)}$ > 24 h	Plasma ↑; Adj.p 6.0E-12; Urine ↑; Adj. p 8.6E-20	Van der Hooft et al 2012, Llorach et al 2010
	M38	Hydroxy(dihydroxy)phenyl-γ-valeric acid sulfate	4.95/ 5.41 /-	$C_{11}H_{14}O_8S$ (II)	$T_{max(P)}$ >5h $T_{max(U)}$ > 24 h	Plasma ↑; Adj. p 2.4E-08; Urine ↑; Adj.p 3.7E-28	Van der Hooft et al 2012, Llorach et al 2010
	M39	Hydroxy(dihydroxyphenyl)-γ-valeric acid methyl glucuronide (I)	4.82/ 5.35/ -	$C_{18}H_{24}O_{11}$ (II)	$T_{max(P)}$ >5h $T_{max(U)}$ > 24 h	Plasma ↑; Adj. p 4.6E-10; Urine ↑; Adj.p 1.2E-25	Van der Hooft et al 2012, Llorach et al., 2010
	M40	Hydroxy(dihydroxyphenyl)-γ-valeric acid methyl glucuronide (II)	5.55 /- /-	$C_{18}H_{24}O_{11}$ (II)	$T_{max(P)}$ >5h	Plasma ↑; Adj.p 4.5E-07	Van der Hooft et al 2012, Llorach et al., 2010
	M41	Hydroxy(dihydroxyphenyl)-γ-valeric acid methyl glucuronide (III)	5.85/ -/ -	$C_{18}H_{24}O_{11}$ (II)	$T_{max(P)}$ >5h	Plasma ↑; Adj. p 4.5E-07	Van der Hooft et al 2012, Llorach et al., 2010
	M42	Hydroxy(dihydroxy)phenyl-γ-valeric acid methyl sulfate	6.29 / 7.40/ -	$C_{12}H_{16}O_8S$ (II)	$T_{max(P)}$ >5h $T_{max(U)}$ > 24 h	Plasma ↑; Adj.p 4.5E-05; Urine ↑; Adj.p 6.0E-06	Van der Hooft et al 2012
				CATECHOL METABOLITE			
Vanillin	M43	(Pyro)Catechol sulfate	4.19/ 4.27/-	$C_6H_6O_5S$ (II)	$T_{max(P)}$ > 5h $T_{max(U)}$ > 24h	Plasma ↑; Adj.p 2.2E-02; Urine ↑; Adj.p 1.4E-03	Van der Hooft et al 2012,Stalmach et al 2013
	M44	Methylcatechol glucuronide (I)	-/ 6.86 /-	$C_{13}H_{16}O_8$ (II)	$T_{max(U)}$ > 24h	Urine ↑; Adj.p 4.2E-07	mzCloud; Pimpao et al 2014
	M45	Methylcatechol glucuronide (II)	-/ 7.00 /-	$C_{13}H_{16}O_8$ (II)	$T_{max(U)}$ > 24h	Urine ↑; Adj.p 7.6E-06	mzCloud; Pimpao et al 2014

	M46	Methylcatechol sulfate (I)	6.24/ 6.90/ -	$C_7H_8O_5S$ (II)	$T_{max(P)}$ > 5h $T_{max(U)}$ > 24h	Plasma ↓; Adj.p 4.4E-02; Urine ↑; Adj.p 1.6E-05	Pimpao et al 2015, mzCloud
	M47	Methylcatechol sulfate (II)	-/ 7.12 /-	$C_7H_8O_5S$ (II)	$T_{max(U)}$ > 24h	Urine ↑; Adj.p 3.8E-04	mzCloud; Pimpao et al 2015
	M48	Methylcatechol sulphate glucuronide	-/ 5.50/ -	$C_{13}H_{16}O_{11}S$ (III)	$T_{max(U)}$ > 24h	Urine ↑; Adj.p 1.6E-05	n.a
CHLOROGENIC ACID METABOLITES							
	M49	Feruloylquinic acid (I)	6.11/ 7.20 /-	$C_{17}H_{20}O_9$ (II)	$T_{max(P)}$ 1h $T_{max(U)}$ 8h	Pasma↑; Adj.p 1.7E-06; Urine ↑; Adj.p 7.0E-40	Quifer-Rada et al 2015
Caffeoylquinic acid Neochlorogenic acid	M50	Feruloylquinic acid (II)	6.36/ 7.60 / -	$C_{17}H_{20}O_9$ (II)	$T_{max(P)}$ 1h $T_{max(U)}$ 8h	Plasma ↑; Adj. p 1.7E-06; Urine ↑; Adj. p4.5E-24	Quifer-Rada et al 2015
Cryptochlorogenic acid	M51	Coumaroylquinic Acid (I)	-/ 5.50 /-	$C_{16}H_{18}O_8$ (II)	$T_{max(U)}$ 8h	Urine ↑; Adj.p 8.8E-36	Clifford et al 2006
	M52	Coumaroylquinic Acid (II)	-/ 6.73 /-	$C_{16}H_{18}O_8$ (II)	$T_{max(U)}$ 8h	Urine ↑; Adj.p 9.9E-44	Clifford et al 2006
	M53	Coumaroylquinic Acid (III)	-/ 6.92 /-	$C_{16}H_{18}O_8$ (II)	$T_{max(U)}$ 8h	Urine ↑; Adj.p 2.3E-42	Clifford et al 2006
CINNAMIC ACIDS METABOLITES							
	M54	Ferulic acid sulfate	5.65 /6.50 /-	$C_{10}H_{10}O_7S$ (II)	$T_{max(P)}$ 1h $T_{max(U)}$ > 24h	Plasma ↑;Adj. p 4.5E-14; Urine ↑;Adj.p 1.2E-16	Van der Hooft et al 2012; Pimpao et al 2014; STD Lab
Caffeoylquinic acid	M55	Ferulic acid glucuronide (I)	-/ 5.48 /-	$C_{16}H_{18}O_{10}$ (II)	$T_{max(U)}$ > 24h	Urine ↑; Adj. p 2.1E-10	Van der Hooft et al 2012; Pimpao et al 2014; STD Lab
Neochlorogenic acid	M56	Ferulic acid glucuronide (II)	-/ 6.50 /-	$C_{16}H_{18}O_{10}$ (II)	$T_{max(U)}$ > 24h	Urine ↑; Adj. p 2.0E-11	Van der Hooft et al 2012; Pimpao et al 2014; STD Lab
Cryptochlorogenic acid	M57	Caffeic acid Sulfate	-/ 6.35/ -	$C_9H_8O_7S$ (I)	$T_{max(U)}$ > 24h	Urine ↑; Adj. p 3.6E-07	Lab STD; Pimpao et al 2014
	M58	Hydroxycinnamic acid (I)	-/ 4.95 /-	$C_9H_8O_3$ (III)	$T_{max(U)}$ > 24h	Urine ↑; Adj.p 1.6E-16	mzCloud
	M59	Hydroxycinnamic acid (II)	-/ 3.50 /-	$C_9H_8O_3$ (III)	$T_{max(U)}$ > 24h	Urine ↑; Adj.p 2.5E-07	
PROPIONIC AND ACETIC ACIDS METABOLITES							
Phlorizin Phloretin	M60	Hydroxyphenylpropionic acid	- / 6.30/ 6.77	$C_9H_{10}O_3$ (II)	$T_{max(P)}$ > 5h $T_{max(U)}$ > 24h	Urine ↑; Adj. p 1.4E-21; Meth.Extr ↑; Adj. p 1.4E-06	mzCloud
Caffeoylquinic acid	M61	Hydroxyphenylpropionic acid glucuronide	-/ 6.10 /-	$C_{15}H_{18}O_9$ (II)	$T_{max(U)}$ > 24h	Urine ↑; Adj. p 3.5E-13	

Neochlorogenic acid Cryptochlorogenic acid	M62	Hydroxyphenylpropionic acid sulfate	5.55/6.40 /-	$C_9H_{10}O_6S$ (II)	$T_{max(P)}$ > 5h $T_{max(U)}$ > 24h	Plasma ↑; Adj. p 1.6E-08; Urine ↑; 5.7E-04	Robio et al 2012
Quercetin-3-arabinoside	M63	DiHydroxyphenyl propionic acid (I)	-/ 3.48 /-	$C_9H_{10}O_4$ (II)	$T_{max(U)}$ > 24h	Urine ↑; Adj.p 1.1E-09	mzCloud
Quercetin-3-rutinoside	M64	DiHydroxyphenyl propionic acid (II)	-/ 5.00 /-	$C_9H_{10}O_4$ (III)	$T_{max(U)}$ > 24h	Urine ↑; Adj.p 1.2E-15	mzCloud
Catechin	M65	DiHydroxyphenyl propionic acid (III)	-/ 5.78 /-	$C_9H_{10}O_4$ (III)	$T_{max(U)}$ > 24h	Urine ↑; Adj.p 1.8E-19	mzCloud
Epicatechin	M66	Dihydroxyphenylpropionic acid sulphate	5.19 /- /-	$C_9H_{10}O_7S$ (III)	$T_{max(P)}$ > 5h	Plasma ↑; Adj. p 5.4E-07	mzCloud
Procyanidins	M67	DihydroFerulic acid sulfate (I)	5.28 / -	$C_{10}H_{12}O_7S$ (II)	$T_{max(P)}$ > 5h	Plasma ↑; Adj. p.5.0E-07	Redeuil et al 2011; Pimpao et al 2014; STD Lab
	M68	DihydroFerulic acid sulfate (II)	5.73/ 6.06	$C_{10}H_{12}O_7S$ (II)	$T_{max(P)}$ > 5h $T_{max(U)}$ > 24h	Plasma ↑; Adj. p 5.0E-07; Urine ↑; Adj. p1.1E-08	Redeuil et al 2011; Pimpao et al 2014; STD Lab
	M69	DihydroFerulic acid glucuronide (I)	5.10/ 5.87/ -	$C_{16}H_{20}O_{10}$ (I)	$T_{max(P)}$ > 5h $T_{max(U)}$ > 24h	Plasma ↑; Adj. p 1.6E-08; Urine ↑; Adj. p 5.5E-14	Redeuil et al 2011; Pimpao et al 2014; STD Lab
	M70	DihydroFerulic acid glucuronide (II)	- / 6.37/ -	$C_{16}H_{20}O_{10}$ (I)	$T_{max(U)}$ > 24h	Urine ↑; Adj.p 1.2E-15	Redeuil et al 2011; Pimpao et al 2014; STD Lab
	M71	DihydroFerulic acid glucuronide (III)	- / 6.52/ -	$C_{16}H_{20}O_{10}$ (I)	$T_{max(U)}$ > 24h	Urine ↑; Adj.p 1.7E-25	Redeuil et al 2011; Pimpao et al 2014, STD Lab
	M72	Hydroxyphenyl acetic acid sulfate	6.55 /- /-	$C_8H_8O_6S$ (II)	$T_{max(P)}$ > 5h	Plasma ↑; Adj. p 6.3E-03	mzCloud
	M73	Homovanilic acid	-/ 6.45 /-	$C_9H_{10}O_4$ (I)	$T_{max(U)}$ > 24h	Urine ↑; Adj.p 2.8E-11	STD Lab
HIPPURIC ACID METABOLITES							
Phlorizin Phloretin	M74	Hippuric acid	5.32 / 5.84 /-	$C_9H_9NO_3$ (I)	$T_{max(P)}$ > 5h $T_{max(U)}$ > 24h	Plasma ↑; Adj.p 5.8E-07 ; Urine ↑; Adj.p 4.5E-03	STD Lab
Caffeoylquinic acid Neochlorogenic acid	M75	Hydroxyhippuric acid (I)	3.77 / 3.95 /-	$C_9H_9NO_4$ (I)	$T_{max(P)}$ > 5h $T_{max(U)}$ > 24h	Plasma ↑; Adj.p 2.5E-11; Urine ↑; Adj.p 1.4E-21	STD Lab
Cryptochlorogenic acid Quercetin-3-arabinoside	M76	Hydroxyhippuric acid (II)	4.27 / 4.52 /-	$C_9H_9NO_4$ (I)	$T_{max(P)}$ > 5h $T_{max(U)}$ > 24h	Plasma ↑; Adj.p 2.8E-08; Urine ↑; Adj.p 4.1E-20	STD Lab
Quercetin-3-rutinoside	M77	Hydroxyhippuric acid (III)	-/ 4.65 /-	$C_9H_9NO_4$ (I)	$T_{max(U)}$ > 24h	Urine ↑; Adj.p 7.7E-16	STD Lab
Catechin	M78	Hydroxyhippuric acid sulfate	-/ 3.45 /-	$C_9H_9NO_7S$ (II)	$T_{max(U)}$ > 24h	Urine ↑; Adj.p 1.8E-09	STD Lab; mzCloud

Epicatechin	M79	Hydroxycyclohexane carboxylic acid glycine	-/ 7.40 /-	$C_9H_{17}NO_4$ (III)	$T_{max(U)}$ > 24h	Urine ↑; Adj.p 2.1E-05	n.a
Procyanidins	M80	Cyclohexadiene carboxylic acid glycine	-/ 6.30/ -	$C_9H_{11}NO_3$ (II)	$T_{max(U)}$ > 24h	Urine ↑; Adj.p 4.0E-09	Ulaszewska et al 2015; Cuparencu et al 2015
	M81	Cyclohexene carboxylic acid glycine	-/ 7.10/ -	$C_9H_{13}NO_3$ (III)	$T_{max(U)}$ > 24h	Urine ↑; Adj.p 1.9E-07	Ulaszewska et al 2015; Cuparencu et al 2015
	M82	Methylhippuric acid	-/ 7.70 /-	$C_{10}H_{11}NO_3$ (I)	$T_{max(U)}$ > 24h	Urine ↑; Adj.p 5.8E-04	mzCloud; STD Lab
VANILLIC ACID METABOLITES							
	M83	Vanillic Acid Sulphate	6.26 /- /-	$C_8H_8O_7S$ (II)	$T_{max(P)}$ 1h	Plasma ↑; Adj. p 4.9E-20	Pekkinen et al 2012; Ulaszewska et al 2015; STD.Lab
Vanillin	M84	Vanillic acid glucuronide sulfate	-/ 5.28 /-	$C_{14}H_{16}O_{13}S$ (III)	$T_{max(U)}$ > 24h	Urine ↑; Adj.p 4.0E-44	Pekkinen et al 2012; Ulaszewska et al 2015; STD.Lab
Caffeoylquinic acid	M85	(Hydroxy-methoxyphenyl)lactic acid (I) (Vanillactic acid I)	-/ 4.42 /-	212.0684 (III)	$T_{max(U)}$ > 24h	Urine ↑; Adj.p 6.1E-13	Neveu et al 2010; mzCloud
Neochlorogenic acid	M86	(Hydroxy-methoxyphenyl)lactic acid (II) (Vanillactic acid II)	-/ 5.20 /-	212.0684 (III)	$T_{max(U)}$ > 24h	Urine ↑; Adj.p 1.6E-17	Neveu et al 2010; Mz Cloud
Cryptochlorogenic acid	M87	Vanilloylglycine hydroxyphenyllactic acid conjugate	-/ 4.90/ -	$C_{19}H_{21}NO_9$ (III)	$T_{max(U)}$ > 24h	Urine ↑; Adj.p 4.3E-04	Pekkinen et al 2012; mzCloud
	M88	Vanillpyruvic acid	-/ 6.60/ -	$C_{10}H_{10}O_5$ (III)	$T_{max(U)}$ 8h	Urine ↑; Adj.p 1.3E-14	mzCloud
TYROSINE/TRYPTOPHAN METABOLITES							
	M89	AcetylTryptophan	-/ 8.25/ -	$C_{13}H_{14}N_2O_3$ (III)	$T_{max(U)}$ 8h	Urine ↓; Adj.p 3.0E-10	mzCloud
	M90	Indoxyl sulfate (I)	-/ 6.00/ -	$C_8H_7NO_4S$ (I)	$T_{max(U)}$ > 24h	Urine ↑; Adj.p 5.1E-23	STD Lab; mzCloud
Clearance effect of all polyphenols	M91	Indoxyl sulfate (II)	-/ 6.35/ -	$C_8H_7NO_4S$ (I)	$T_{max(U)}$ > 24h	Urine ↑; Adj.p 1.6E-12	STD Lab; mzCoud
	M92	Phenol Sulphate	-/ 5.07 /-	$C_6H_6O_4S$ (III)	$T_{max(U)}$ > 24h	Urine ↑; Adj.p 3.0E-20	Van der Hooft et al 2012
	M93	Toluene sulfonate	4.32 /- /-	$C_7H_8O_3S$ (III)	$T_{max(P)}$ 3h	Plasma ↓; Adj.p 4.2E-02	n.a
	M94	Dihydroxyindole glucuronide	-/ 4.61 /-	$C_{14}H_{15}NO_8$ (III)	$T_{max(U)}$ > 24h	Urine↑; Adj.p 3.0E-12	n.a
FATTY ACIDS METABOLITES							
	M95	Dicarboxylic Fatty Acid C22:2	16.33 /- /-	$C_{22}H_{36}O_4$ (III)	-	Plasma ↓; Adj. p 4.0E-03	n.a
	M96	Dicarboxylic fatty acid C22:4	17.15 /- /-	$C_{22}H_{38}O_4$ (III)	-	Plasma ↑; Adj.p 4.3E-02	n.a
	M97	Dicarboxylic fatty acid C24:3	18.48 /- /-	$C_{24}H_{40}O_4$ (III)	-	Plasma ↑; Adj.p 1.7E-02	n.a
	M98	HydroxyDicarboxylic Fatty Acid C26:0	18.27 /- /-	$C_{26}H_{50}O_5$ (III)	-	Plasma ↑; Adj. p 6.6E-05	n.a
	M99	HydroxyDicarboxylic Fatty Acid C26:2	18.30 /- /-	$C_{26}H_{46}O_5$ (III)	-	Plasma ↑; Adj.p 3.2E-02	n.a

M100	HydroxyDicarboxylic Fatty Acid C26:4	11.45 /- /-	$C_{26}H_{42}O_5$ (III)	-	Plasma ↓; Adj.p 4.6E-03	n.a
M101	Hydroxy dicarboxylic Fatty Acid C30:3	21.15 /- /-	$C_{30}H_{52}O_5$ (III)	-	Plasma ↑; Adj. p 3.5E-02	n.a
M102	Dihydroxy dicarboxylic Fatty Acid C28:0	19.60 /- /-	$C_{28}H_{54}O_6$ (III)	-	Plasma ↓; Adj. p 3.6E-05	n.a
M103	Dihydroxy dicarboxylic Fatty Acid C28:1	17.83 /- /-	$C_{28}H_{52}O_6$ (III)	-	Plasma ↓; Adj.p 8.8E-04	n.a
M104	Dihydroxy dicarboxylic Fatty Acid C30:1	20.65 /- /-	$C_{30}H_{56}O_6$ (III)	-	Plasma ↑; Adj. p 2.3E-02	n.a
M105	Dihydroxy dicarboxylic Fatty Acid C32:1	20.38 /- /-	$C_{32}H_{60}O_6$ (III)	-	Plasma ↑;Adj. p 1.6E-04	n.a
M106	Dihydroxy dicarboxylic Fatty Acid C32:2	20.20 /- /-	$C_{32}H_{58}O_6$ (III)	-	Plasma ↑;Adj. p 3.6E-04	n.a
			OTHERS			
M107	Pyridoxic Acid	- / - / 5.0	$C_8H_9NO_4$ (II)	$T_{max(ME)}$ 3h	Meth.Extr. ↓; Adj. p 1.6E-03	n.a
M108	γ-Glutamyl-Leucine	- / - / 4.60	$C_{11}H_{20}N_2O_5$ (III)	$T_{max(ME)}$ 0h	Meth.Extr. ↓; Adj.p 2.3E-03	Wilkoff et al 2009
M109	Methoxycoumarin	6.75 /- /-	$C_{10}H_8O_3$ (III)	$T_{max(U)}$ > 24h	Urine ↑; Adj.p 2.5E-14	n.a
M110	Methoxy-methylphenol sulfate	7.70 /- /-	$C_8H_{10}O_5S$ (III)	$T_{max(U)}$ > 24h	Urine ↑; Adj.p 1.1E-05	n.a

1182

1183

Table 2

Parent polyphenol	Mechanism and/or effect	Metabolites statistically significant correlated in plasma (Metabolite Number)	Bacterial Genus, Correlations: (+) positive; (-) negative
Catechin Epicatechin procyanidins	dimer cleavage, deglycosylation , ring fission, C&A-rings cleavage (lactone formation), phase II metabolism	(dihydroxyphenyl) valerolactone glucuronide (M20) (dihydroxyphenyl) valerolactone methylglucuronide (M23) (dihydroxyphenyl) valerolactone glucuronide (M21)	*(+) Dialister* *(+) Prevotella* *(-) Anaerostipes*
	dimer cleavage, ring fission, c&a-rings cleavage (lactone formation), degradation, phase II metabolism	(dihydroxyphenyl) valeric acid sulfate (M19) hydroxy(dihydroxyphenyl) valeric acid glucuronide (M37)	*(-) Turicibacter* *(+)Dialister*
phlorizin phloretin naringenin quercetin-3-rabinoside quercetin-3-rutinoside Catechin Epicatechin procyanidins caffeoylquinic acid neochlorogenic acid cryptochlorogenic acid	dimer cleavage, deglycosylation, ring fission, C&A-rings cleavage (lactone formation), dehydroxylation, β-oxidation hydrolysis, reduction, dehydroxylation, decarboxylation	hydroxyphenyl propionic acid (M60)	*(-) Bacteroides*
-	clearance effect of polyphenol	toluene sulfonate (M93)	*(+) Clostridium XVIII*
-	ω-oxidation	hydroxydicarboxylic fatty acid C30:3 (M101) dihydroxydicarboxylic fatty acid C28:1 (M103) dicarboxylic fatty acid C22:4 (M96); dicarboxylic fatty acid C24:3 (M97) dicarboxylic fatty acid C22:2 (M95)	*(+) Eubacterium and Dorea* *(+) Flavonifractor* *(+) Gemmiger* *(+) Clostridium XVIII*
Parent polyphenol	**Mechanism and/or effect**	**Metabolites statistically significant correlated in urine:**	**Bacterial Genus, Correlations: (+) positive; (-) negative**
vanillin	dehydroxylation, demethylation, phase II metabolism	catechol sulfate (M43)	*(+)Butyrcicoccus, (+)Clostridium XIVa*
caffeoylquinic acid neochlorogenic acid cryptochlorogenic acid	hydrolysis, dehydroxylation, phase II metabolism	cyclohexadiene carboxylic acid-glycine (M80)	*(+)Clostrium sensu stricto; (-) Faecalibacterium*
phlorizin phloretin naringenin quercetin-3-rabinoside quercetin-3-rutinoside Catechin Epicatechin	dimer cleavage, deglycosylation, ring fission, C&A-rings cleavage (lactone formation), degradation, phase II metabolism hydrolysis, dehydroxylation, phase II metabolism	hydroxyhippuric acid (M77) hydroxyhippuric acid sulfate (M78) hydroxyhippuric acid (M76)	*(+) Ruminococcus, (-) Roseburia* *(+) Ruminococcus, (-) Roseburia* *(+) Bacteroides*

procyanidins caffeoylquinic acid neochlorogenic acid cryptochlorogenic acid		hydroxyhippuric acid (M75)	*(+) Butyricoccus, Clostridium XIVa*
Catechin Epicatechin procyanidins	dimer cleavage, phase II metabolism dimer cleavage, ring fission, C&A-rings cleavage (lactone formation), degradation, phase II metabolism dimer cleavage, ring fission, C&A-rings cleavage (lactone formation), phase II metabolism	epicatechin-sulfate (M10) epicatechin-methylglucuronide (M13-M14) hydroxy(dihydroxyphenyl)valeric acid methyl sulfate (M42) hydroxy(dihydroxyphenyl)valeric acid glucuronide (M39) (dihydroxyphenyl)valerolactone methylglucuronide (M23) (dihydroxyphenyl)valerolactone glucuronide (M21)	*(+)Escherichia/Shigella* *(-) Coprococcus* *(-) Blautia* *(-) Lachnospiracea* *(+)Escherichia/Shigella* *(-) Lachnospiracea*
caffeoylquinic acid neochlorogenic acid cryptochlorogenic acid	hydrolysis, reduction, dehydroxylation, decarboxylation	homovanillic acid (M73)	*(-) Dorea*
phlorizin phloretin naringenin cryptochlorogenic acid quercetin-3-rabinoside quercetin-3-rutinoside Catechin Epicatechin procyanidins caffeoylquinic acid neochlorogenic acid cryptochlorogenic acid	dimer cleavage, deglycosylation, ring fission, C&A-rings cleavage (lactone formation), degradation, β-oxidation hydrolysis, reduction, dehydroxylation, decarboxylation, phase II metabolism	hydroxyphenyl propionic acid glucuronide (M61)	*(-) Faecalibacterium, (+) Butyrcicoccus*
- -	clearance effect of polyphenol	methoxymethylphenol sulfate (M110) indoxyl sulfate (M90) Acetyltryptophan (M89)	*(-) Butyrcicoccus, (-) Clostridium XIVa* *(-) Clostridium XVIII* *(+) Adlercreutzia*

1184