SUPPLEMENTAL MATERIAL

Chronic consumption of a bergamot-based beverage does not affect glucose, lipid and inflammatory biomarkers of cardiometabolic risk in healthy subjects: a randomised controlled intervention study.

Supplementary Materials and Methods

Urine sample analysis for the determination of phenolic metabolites

Urinary extraction of phenolic metabolites was performed using microelution solid phase extraction (μSPE) through a 96 well μ-SPE HLB plate (Oasis® HLB μElution Plate 30 μm, Waters) according to validated protocols, with some modifications (Feliciano, R. P., Boeres, A., Massacessi, L., Istas, G., Ventura, M. R., Nunes dos Santos, C., Heiss, C., & Rodriguez-Mateos, A. Identification and quantification of novel cranberry-derived plasma and urinary (poly)phenols. Archives of Biochemistry and Biophysics, 2016, **599**, 31–41. Feliciano, R. P., Mecha, E., Bronze, M. R., & Rodriguez-Mateos, A. Development and validation of a high-throughput micro solid-phase extraction method coupled with ultra-high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry for rapid identification and quantification of phenolic metabolites in human plasma and urine. Journal of Chromatography A, 2016, **1464**, 21–31.). Briefly, urine samples (100 μL) were diluted with acidified water (0.1% formic acid) (1:5, v/v). Another sample dilution (350 μL) was performed with water containing 4% (v/v) of *orto*-phosphoric acid to reduce phenolic-protein interactions. Each sample (600 μL) was loaded on the 96-well plate, washed with water (200 μL) and water acidified with 0.2% (v/v) acetic acid (200 μL), and finally eluted in a clean plate, containing 40 μL of water in each well, in three steps, using acidified methanol (0.1% v/v formic acid) containing 10 nmol/L ammonium formate solution (30 μL for each elution step). Samples were analyzed by ultra-high performance liquid chromatography coupled with tandem mass spectrometry (uHPLC-ESI-MS/MS) to identify and quantify phenolic metabolites. An UHPLC DIONEX Ultimate 3000 fitted with a TSQ Vantage Triple Quadrupole Mass Spectrometer (QqQ-MS) (Thermo Fisher

Scientific Inc., San Jose, CA, USA), equipped with a heated-electrospray ionization source (H-ESI-II; Thermo Fisher Scientific Inc.) was used. Separation was carried out by means of Kinetex Evo C18 column (100 x 2.1 mm; 2.6 µm particle size; Pheneomenex, CA, USA) installed with a precolumn cartridge (Phenomenex, CA, USA). Urine separation was carried out by means of Kinetex Evo C18 column (100 x 2.1 mm; 2.6 µm particle size; Pheneomenex, CA, USA) installed with a precolumn cartridge (Phenomenex, CA, USA). For uHPLC, mobile phase A was water containing 0.01% formic acid and mobile phase B was acetonitrile containing 0.01% formic acid. The gradient started with 5% B, keeping isocratic conditions for 0.5 min, reaching 95% B at 7 min, followed by 1 min at 95% B and then 4 min at the start conditions to re-equilibrate the column. The flow rate was set at 0.4 mL/min, the injection volume was 5 µL, and the column was thermostatted at 40 °C. The MS worked in negative ionization mode with a capillary temperature of 270 °C, while the source was set at 300 °C. The sheath gas flow was 60 units, while auxiliary gas pressure was set to 10 units. The source voltage was 3 kV. Ultra-high purity argon gas was used for collision-induced dissociation (CID).

Up to 120 compounds were simultaneously monitored in selective reaction monitoring (SRM) mode (ESI Table S2). Chromatograms, mass spectral data and data processing were performed using Xcalibur software 2.1 (Thermo Fisher Scientific Inc.). Metabolite identification was carried out by comparison of the retention time with standards, when available, and/or MS/MS fragmentation patterns. Quantification was performed with calibration curves of standards, when available; when not available, compounds were quantified with the most structurally similar compound.

Urinary excretions of phenolic metabolites were reported for each identified compound. Moreover, total amounts of urinary metabolites belonging to the same (poly)phenolic class were calculated, defining 10 groups, as well as total amounts of metabolites belonging to the same aglycone moiety, defining 22 groups (23 if hippuric acid derivatives were considered). Standards used for urinary (poly)phenol metabolites and catabolites identification and quantification are the following: 4-Hydroxybenzaldehyde, 3,4-dihydroxybenzaldehyde, 4-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid, 3-(4′-hydroxyphenyl)propanoic acid, 3-(3′-hydroxyphenyl)propanoic acid, 3-(3′,4′-

dihydroxyphenyl)propanoic acid, 3-(4′-hydroxy-3′-methoxyphenyl)propanoic acid, 4′-hydroxy-3′-methoxycinnamic acid, 4′-hydroxyphenylacetic acid, 3′-hydroxyphenylacetic acid, 3′,4′-dihydroxyphenylacetic acid and hippuric acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). 3′-Methoxycinnamic acid-4′-sulfate, 3′-methoxycinnamic acid-4′-glucuronide, 4′-methoxycinnamic acid-3′-sulfate, 4′-methoxycinnamic acid-3′-glucuronide, 3-(3′-methoxyphenyl)propanoic acid-4′-sulfate, 3-(4′-methoxyphenyl)propanoic acid-3′-glucuronide, 3-(4′-hydroxyphenyl)propanoic acid-3′-sulfate, 3-(4′-hydroxyphenyl)propanoic acid-3′-glucuronide, 3′-hydroxycinnamic acid-4′-glucuronide, 4′-hydroxycinnamic acid-3′-glucuronide, naringenin-4′-glucuronide, naringenin-7-glucuronide and hesperetin-3′-glucuronide were purchased from Toronto Research Chemicals (Toronto, ON, Canada). Hesperetin-7-glucuronide was purchased from Santa Cruz Biotechnology (Santa Cruz, California, USA). 4-Hydroxy-3-methoxybenzoic acid was purchased from Alfa Aesar (Thermo Fisher (Kandel) GmbH, Postfach, Karlsruhe, Germany), and 4′-hydroxy-3′-phenylacetic acid were purchased from Extrasynthese (Genay Cedex, France). 4′-Hydroxyhippuric acid was purchased from Bachem Ltd. (St Helens, UK). Prof. Alan Crozier (University of Glasgow) kindly supplied feruloylglycine. 3-Methoxybenzoic acid-4-glucuronide, 3-methoxybenzoic acid-4-sulfate, 4-methoxybenzoic acid-3-glucuronide, 4-hydroxybenzoic acid-3-sulfate, 4-hydroxybenzoic acid-3-glucuronide, benzoic acid-4-sulfate, benzoic acid-4-glucuronide were provided by Dr. Colin Kay (N.C. State University′s Plants for Human Health Institute). Ultrapure water from MilliQ system (Millipore, Bedford, MA, USA) was used throughout the experiment.

Statistical analyses

Principal Component Analysis (PCA) (ESI Figure S1A) was used to assess the differences in the phenolic profiles among treatments (control or bergamot) and times (T1 and T2). Data were pre-treated with mean-centering and UV-scaling, and two principal components (PCs) were set (PC1 and PC2). Centering converted all the data to variations around zero instead of around the mean of the data; UV scaling used standard deviation as scaling factor to give all variables equal weight. The

parameters used to assess the quality of each model and subsequent data interpretability were R2X and Q2, namely the model fit (or explained variation) and the predictive ability, respectively.

Partial least squares discriminant analysis (PLS-DA) (ESI Figure S1B) was applied to improve the stratification of subjects in the treatment groups and predict which metabolites characterized those groups. Two clusters (BBB group and control group) were defined according to the treatment type, and observations were assigned to clusters based on the treatment type. (Poly)phenolic classes were considered as variables and were autoscaled applying centering plus UV scaling. Model validity was assessed by R2X (variation in the data), R2Y (variation in the cluster), Q2 (goodness of fit of the validation), the random permutation test, and CV-ANOVA. The most relevant metabolites for clustering were identified using the Variable Importance in the Projection (VIP) scores (ESI Figure S2). Variables with VIP greater than 1.25 were considered relevant for the model. The ROC curve was built to assess the classification performance of the model (ESI Figure S3). Both PCA and PLS-DA were performed using SIMCA 16.0.1 software (Sartorius Stedim Data Analytics, Umea, Sweden).

ESI Table S1. Chromatographic and spectrometric properties of the monitored flavanone and flavone metabolites. Nomenclature of metabolites is reported as proposed by Kay et al (30). Synonyms are reported under brackets, as well as abbreviations. RT means retention time; CE means collision energy; / means not detected; STD means standard use for the quantification.

Compound	RT (min)	Parent ion $[M-H]^-$ *(m/z)*	S-Lens	Quantifier		Qualifier(s)		STD
				Product ion (*m/z*)	CE (V)	Product ion (*m/z*)	CE (V)	
HMG-flavanone metabolites								
Naringenin-7-*O*-glucoside-6”-*O*-HMG	-	577	100	271	25	463, 445, 433, 475	20, 20, 20, 20	
Naringenin-7-*O*-glucoside-6”-*O*-HMG-glucuronide	-	753	115	271	25	577	25	
Naringenin-7-*O*-glucoside-6”-*O*-HMG-sulfate	-	657	115	271	25	577, 113	25, 25	
Hesperetin-7-*O*-glucoside-6”-*O*-HMG	-	607	100	301	25	493, 475, 463, 505	20, 20, 20, 20	
Hesperetin-7-*O*-glucoside-6”-*O*-HMG-glucuronide	-	783	115	301	25	607	25	
Hesperetin-7-*O*-glucoside-6”-*O*-HMG-sulfate	-	687	115	301	25	607, 113	25, 25	
Structurally-related flavanone metabolites								
Naringenin (Nar)	-	271	84	177	16	118, 93	24, 24	
Naringenin-5-glucuronide (Nar-5-Glc)	-	447	100	271	25	151, 175, 113	34, 27, 27	
Naringenin-7-glucuronide (Nar-7-Glc)	5.25	447	100	271	25	151, 175, 113	34, 27, 27	Naringenin-7-glucuronide
Naringenin-4’-glucuronide (Nar-4’-Glc)	5.35	447	100	271	25	151, 113, 175	34, 27, 27	Naringenin-4’-glucuronide

Naringenin-sulfate (Nar-S)	7.80	351	100	271	26	151, 231	34, 25	Quercetin-3’-sulfate
Naringenin-diglucuronide (Nar-diGlc)	5.00	623	100	447	25	271, 175, 113	26, 27, 27	Naringenin-7-glucuronide
Naringenin-disulfate (Nar-diS)	-	431	100	271	26	151, 231	34, 25	
Naringenin-sulfate-glucuronide (Nar-S-Glc)	5.45	527	100	351	26	447, 271	26, 26	Naringenin-7-glucuronide
Isosakuranetin (Isok)	6.55	285	80	270	18			(−)-Epicatechin
Isosakuranetin-glucuronide (Isok-Glc)	-	461	90	285	35	113, 175, 151	18, 27, 39	
Isosakuranetin-sulfate (Isok-S)	-	365	90	285	20	270	20	
Isosakuranetin-sulfate-glucuronide (Isok-S-Glc)	-	541	115	461	26	365, 285	25, 25	
Eriodyctiol (Erio)	-	287	80	151	39			
Eriodyctiol-glucuronide (Erio-Glc)	-	463	90	287	25	151, 113	39, 27	
Eriodyctiol-sulfate (Erio-S)	-	367	90	287	20	151	39	
Eriodyctiol-sulfate-glucuronide (Erio-S-Glc)	-	543	115	463	26	367, 287	25, 25	
Hesperetin (Hes)	6.05	301	80	151	39	286	25	(−)-Epicatechin
Hesperetin-5-glucuronide (Hes-5-Glc)	-	477	100	301	27	151, 113	46, 27	
Hesperetin-7-glucuronide (Hes-7-Glc)	5.40	477	100	301	27	113, 286, 175, 151	27, 35, 27, 46	Hesperetin-7-glucuronide
Hesperetin-3’-glucuronide (Hes-3’-Glc)	5.50	477	100	301	27	113, 286, 175, 151	27, 35, 27, 46	Hesperetin-3’-glucuronide
Hesperetin-sulfate (Hes-S)	7.90	381	100	301	26	151, 286	39, 35	Quercetin-3’-sulfate
Hesperetin-diglucuronide (Hes-diGlc)	5.30	653	100	477	26	301, 113, 175, 151,	26, 27, 27,	Hesperetin-7-glucuronide

						286	39, 30	
Hesperetin-disulfate (Hes-diS)	-	461	100	381	26	301, 286, 151	26, 35, 39	
Hesperetin-sulfate-glucuronide (Hes-S-Glc)	5.80	557	100	381	26	113, 175, 477, 301	27, 27, 26, 26	Hesperetin-7-glucuronide
Structurally-related flavone metabolites								
Apigenin (Api)	-	269	98	151	30	149, 117	30, 26	
Apigenin-glucuronide (Api-Glc)	5.35	445	90	269	20	113, 225	30, 30	Naringenin-7-glucuronide
Apigenin-sulfate (Api-S)	-	349	100	269	20	225	30	
Luteolin (Lut)	-	285	88	151	20	133	20	
Luteolin-glucuronide (Lut-Glc)	-	461	90	285	27	113, 151	30, 39	
Luteolin-sulfate (Lut-S)	-	365	90	285	20	257	30	
Diosmetin (Dios)	6.00	299	88	284	25	256	25	(−)-Epicatechin
Diosmetin-glucuronide (Dios-Glc)	5.45	475	93	299	27	113	30	Hesperetin-7-glucuronide
Diosmetin-sulfate (Dios-Glc)	-	379	93	299	20	284	35	
Cinnamic acids (HCAs)								
3’,4’-Dihydroxycinnamic acid (Caffeic acid) (3’,4’-diHCA)	3.80	179	62	135	19			3’,4’-Dihydroxycinnamic acid
3’-Hydroxycinnamic acid-4’-glucuronide (Caffeic acid-4-glucuronide) (3’-HCA-4’-Glc)	3.10	355	98	179	21	113	18	3’-Hydroxycinnamic acid-4’-glucuronide
4’-Hydroxycinnamic acid-3’-glucuronide (Caffeic	4.10	355	98	179	21	113	18	4’-Hydroxycinnamic acid-

acid-3-glucuronide) (4’-HCA-3’-Glc)								3’-glucuronide
3’-Hydroxycinnamic acid-4’-sulfate (Caffeic acid-3-sulfate) (3’-HCA-4’-S)	5.00	259	62	179	20	135	25	3-(4’-Hydroxyphenyl)propanoic acid-3’-sulfate
4’-Hydroxycinnamic acid-3’-sulfate (Caffeic acid-4-sulfate) (4’-HCA-3’-S)	5.50	259	62	179	20	135	25	3-(4’-Hydroxyphenyl)propanoic acid-3’-sulfate
4’-Hydroxy-3’-methoxycinnamic acid (Ferulic acid) (3’-MeO-4’-HCA)	4.74	193	71	134	19	178	18	4’-Hydroxy-3’-methoxycinnamic acid
3’-Hydroxy-4’-methoxycinnamic acid (Isoferulic acid) (4’-MeO-3’-HCA)	-	193	71	134	19	178	18	
3’-Methoxycinnamic acid-4’-glucuronide (Ferulic acid-4-glucuronide) (3’-MeO-CA-4’-Glc)	3.90	369	93	193	20	113, 134, 178	18,39,32	3’-Methoxycinnamic acid-4’-glucuronide
4’-Methoxycinnamic acid-3’-glucuronide (Isoferulic acid-3-glucuronide) (4’-MeO-CA-3’-Glc)	4.44	369	93	113	18	178, 193, 134	32,20,39	4’-Methoxycinnamic acid-3’-glucuronide
3’-Methoxycinnamic acid-4’-sulfate (Ferulic acid-4-sulfate) (3’-MeO-CA-4’-S)	5.40	273	92	193	18	178, 134, 149	28,33,30	3’-Methoxycinnamic acid-4’-sulfate
4’-Methoxycinnamic acid-3’-sulfate (Isoferulic acid-3-sulfate) (4’-MeO-CA-3’-S)	5.64	273	92	193	18	178, 134	28,33	4’-Methoxycinnamic acid-3’-sulfate
Feruloylglycine (Fer-Gly)	4.38	250	79	206	14	134, 163	22,19	Feruloylglycine
2’-Hydroxycinnamic acid (*o*-Coumaric acid) (2’-HCA)	5.00	163	62	119	19	93	38	2’-Hydroxycinnamic acid
3’-Hydroxycinnamic acid (*m*-Coumaric acid) (3’-HCA)	4.77	163	62	119	19	93	38	3’-Hydroxycinnamic acid
4’-Hydroxycinnamic acid (*p*-Coumaric acid) (4’-	4.54	163	62	119	19	93	38	3’-Hydroxycinnamic acid

HCA)								
Cinnamic acid-4’-glucuronide (Coumaric acid-4-glucuronide) (CA-4’-Glc)	4.48	339	90	163	20	113	16	3-(3’-Hydroxyphenyl)propanoic acid-4’-glucuronide
Cinnamic acid-3’-glucuronide (Coumaric acid-4-glucuronide) (CA-3’-Glc)	4.64	339	90	163	20	113	16	3-(4’-Hydroxyphenyl)propanoic acid-3’-glucuronide
Cinnamic acid-4’-sulfate (Coumaric acid-4-sulfate) (CA-4’-S)	5.35	243	62	163	20	119	35	3-(4’-Hydroxyphenyl)propanoic acid-3’-sulfate
Cinnamic acid-3’-sulfate (Coumaric acid-3-sulfate) (CA-3’-S)	5.74	243	62	163	20	119	35	3-(4’-Hydroxyphenyl)propanoic acid-3’-sulfate
Phenylpropanoic acids (PPAs)								
3-(3’,4’-Dihydroxyphenyl)propanoic acid (Dihydrocaffeic acid) (3’,4’-diHPPA)	3.10	181	64	137	14	119, 121	13,10	3-(3’,4’-Dihydroxyphenyl)propanoic acid
3-(3’-Hydroxyphenyl)propanoic acid-4’-glucuronide (Dihydrocaffeic acid-4-glucuronide) (3’-HPPA-4’-Glc)	3.11	357	63	181	21	113	22	3-(4’-Hydroxyphenyl)propanoic acid-3’-glucuronide
3-(4’-Hydroxyphenyl)propanoic acid-3’-glucuronide (Dihydrocaffeic acid-3-glucuronide) (4’-HPPA-3’-Glc)	3.96	357	63	181	21	113	22	3-(4’-Hydroxyphenyl)propanoic acid-3’-glucuronide
3-(3’-Hydroxyphenyl)propanoic acid-4’-sulfate (Dihydrocaffeic acid-4-sulfate) (3’-HPPA-4’-S)	4.80	261	96	181	20	137	25	3-(4’-Hydroxyphenyl)propanoic acid-3’-sulfate

3-(4’-Hydroxyphenyl)propanoic acid-3’-sulfate (Dihydrocaffeic acid-3-sulfate) (4’-HPPA-3’-S)	5.22	261	96	181	20	137	25	3-(4’-Hydroxyphenyl)propanoic acid-3’-sulfate
3-(4’-Hydroxy-3’-methoxyphenyl)propanoic acid (Dihydroferulic acid) (3’-MeO-4’-HPPA)	4.56	195	73	136	21	151	22	3-(4’-Hydroxy-3’-methoxyphenyl)propanoic acid
3-(3’-Hydroxy-4’-methoxyphenyl)propanoic acid (Dihydroisoferulic acid) (4’-MeO-3’-HPPA)	4.99	195	73	136	21	151	22	3-(4’-Hydroxy-3’-methoxyphenyl)propanoic acid
3-(3’-Methoxy-phenyl)propanoic acid-4’-glucuronide (Dihydroferulic acid-4-glucuronide) (3’-MeO-PPA-4’-Glc)	4.08	371	90	195	26	113	21	3-(4’-Methoxy-phenyl)propanoic acid-3’-glucuronide
3-(4’-Methoxy-phenyl)propanoic acid-3’-glucuronide (Dihydroisoferulic acid-3-glucuronide) (4’-MeO-PPA-3’-Glc)	4.48	371	90	195	26	113	21	3-(4’-Methoxy-phenyl)propanoic acid-3’-glucuronide
3-(3’-Methoxy-phenyl)propanoic acid-4’-sulfate (Dihydroferulic acid-4-sulfate) (3’-MeO-PPA-4’-S)	5.25	275	75	195	31	136	17	3-(3’-Methoxy-phenyl)propanoic acid-4’-sulfate
3-(4’-Methoxy-phenyl)propanoic acid-3’-sulfate (Dihydroisoferulic acid-3-sulfate) (4’-MeO-PPA-3’-S)	5.55	275	75	195	31	136	17	3-(3’-Methoxy-phenyl)propanoic acid-4’-sulfate
3-(3’-Hydroxyphenyl)propanoic acid (3’-HPPA)	4.54	165	48	121	15	119	20	3-(3’-Hydroxyphenyl)propanoic acid
3-(Phenyl)propanoic acid-4’-glucuronide (PPA-4’-Glc)	4.13	341	90	165	20	113	18	3-(4’-Hydroxyphenyl)propanoic acid-3’-glucuronide

3-(Phenyl)propanoic acid-3'-glucuronide (PPA-3'-Glc)	4.44	341	90	165	20	113	18	3-(4'-Hydroxyphenyl)propanoic acid-3'-glucuronide
3-(Phenyl)propanoic acid-sulfate (PPA-S)	5.46	245	90	165	20	121	15	3-(4'-Hydroxyphenyl)propanoic acid-3'-sulfate
Phenylhydracrylic acids (PHAs)								
3-Hydroxy-3-(phenyl)propanoic acid (Phenylhydracrylic acid) (3-OH-PPA)	4.45	165	48	119	20	121, 103	15, 14	3-(3'-Hydroxyphenyl)propanoic acid
3-Methoxy-3-phenylpropanoic acid-glucuronide (3-Methylphenylhydracrylic acid-glucuronide) (3-MeO-PPA-GlcUA)	4.70	371	90	195	26	113	21	
3-Methoxy-3-phenylpropanoic acid-sulfate (3-Methylphenylhydracrylic acid-sulfate) (3-MeO-PPA-S)	6.00	275	86	195	31	136	17	
Phenylacetic acids (PAAs)								
3',4'-Dihydroxyphenylacetic acid (Homoprotocatechuic acid) (3',4'-DiHPAA)	-	167	60	123	17			
4'-Hydroxy-3'-methoxy-phenylacetic acid (Homovanillic acid) (3'-MeO-4'-HPAA)	3.93	181	64	137	14	121, 122, 119, 166	10,30,13,30	4'-Hydroxy-3'-methoxy-phenylacetic acid
3'-Hydroxy-4'-methoxy-phenylacetic acid (Isohomovanillic acid) (4'-MeO-3'-HPAA)	4.10	181	64	137	14	121, 122, 119, 166	10,30,13,30	4'-Hydroxy-3'-methoxy-phenylacetic acid
3'-Methoxy-phenylacetic acid-4'-glucuronide (Homovanillic acid-glucuronide) (3'-Met-PAA-4'-Glc)	4.30	261	96	137	25	181	20	3-Methoxybenzoic acid-4-glucuronide

4’-Methoxy-phenylacetic acid-3’-glucuronide (Isohomovanillic acid-glucuronide) (4’-Met-PAA-3’-Glc)	4.60	261	96	137	25	181	20	4-Methoxybenzoic acid-3-glucuronide
3’-Methoxy-phenylacetic acid-4’-sulfate (Homovanillic acid-sulfate) (3’-Met-PAA-4’-S)	4.80	261	96	137	25	181	20	3-Methoxybenzoic acid-4-sulfate
4’-Methoxy-phenylacetic acid-3’-sulfate (Isohomovanillic acid-sulfate) (4’-Met-PAA-3’-S)	5.20	261	96	137	25	181	20	4-Methoxybenzoic acid-3-sulfate
3’-Hydroxyphenylacetic acid (3’-HPAA)	3.60	151	40	107	10			3’-Hydroxyphenylacetic acid
4’-Hydroxyphenylacetic acid (4’-HPAA)	2.90	151	40	107	10			4’-Hydroxyphenylacetic acid
Benzoic acids (BAs)								
3,4-Dihydroxybenzoic acid (Protocatechuic acid) (3,4-DiHBA)	1.53	153	64	109	20	108	27	3,4-Dihydroxybenzoic acid
3-Hydroxybenzoic acid-4-glucuronide (Protocatechuic acid-4-glucuronide) (3-HBA-4-Glc)	-	329	81	153	21	113	18	
4-Hydroxybenzoic acid-3-glucuronide (Protocatechuic acid-3-glucuronide) (4-HBA-3-Glc)	-	329	81	153	21	113	18	
Hydroxybenzoic acid-sulfate (Protocatechuic acid-sulfate) (HBA-S)	4.45	233	85	153	20	109	33	3-Hydroxybenzoic acid-4-sulfate
4-Hydroxy-3-methoxybenzoic acid (Vanillic acid) (3-MeO-4-HBA)	3.65	167	60	152	18	123, 108, 121	17,21,18	4’-Hydroxy-3’-methoxyphenylacetic acid
3-Methoxybenzoic acid-4-glucuronide (Vanillic acid-4-glucuronide) (3-MeO-BA-4-Glc)	1.79	343	92	167	23	152, 113	40,17	3-Methoxybenzoic acid-4-glucuronide
4-Methoxybenzoic acid-3-glucuronide (Isovanillic	-	343	92	113	17	167, 152	23,40	

acid-3-glucuronide) (4-MeO-BA-3-Glc)								
3-Methoxybenzoic acid-4-sulfate (Vanillic acid-4-sulfate) (3-MeO-BA-4-S)	4.67	247	72	167	17	152, 123	27,17	3-Methoxybenzoic acid-4-sulfate
4-Methoxybenzoic acid-3-sulfate (Isovanillic acid-3-sulfate) (4-MeO-BA-3-S)	4.90	247	72	167	17	152	27	4-Methoxybenzoic acid-3-sulfate
3-Hydroxybenzoic acid (3-HBA)	3.50	137	70	93	16	92	26	4-Hydroxybenzoic acid
4-Hydroxybenzoic acid (4-HBA)	2.60	137	70	93	16	92	26	4-Hydroxybenzoic acid
Benzoic acid-4-glucuronide (BA-4-Glc)	1.26	313	78	113	15	137	20	Benzoic acid-4-glucuronide
Benzoic acid-3-glucuronide (BA-3-Glc)	1.82	313	78	113	15	137	20	Benzoic acid-4-glucuronide
Benzoic acid-4-sulfate (BA-4-S)	4.50	217	70	137	20	93	16	Benzoic acid-4-sulfate
Benzoic acid-3-sulfate (BA-3-S)	4.95	217	70	137	20	93	16	Benzoic acid-4-sulfate
Hippuric acids (HAs)								
4-Hydroxyhippuric acid (4-HHA)	1.65	194	72	100	11	150	15	4-Hydroxyhippuric acid
Hippuric acid (HA)	3.50	178	61	134	15	77.2	22	Hippuric acid
Benzaldehydes (BALs)								
4-Hydroxybenzaldehyde (4-HBAL)	3.55	121	89	92	27	91	22	4-Hydroxybenzaldehyde
Benzene derivatives (BZs)								
3,5-Dihydroxybenzene-1-glucuronide (Phloroglucinol-glucuronide) (Phlglu-Glc)	-	301	87	125	27	113	15	
3,5-Dihydroxybenzene-1-sulfate (Phloroglucinol-sulfate) (Phlglu-S)	-	205	83	125	20	97	15	
5-Hydroxy-3-methoxybenzene-1-sulfate	5.53	219	68	139	20	124	24	3-Methoxybenzoic acid-4-

(Methoxyphloroglucinol-sulfate) (MeO-Phlglu-S)								sulfate
2-Hydroxy-benzene-1-glucuronide (Catechol-glucuronide) (Cat-Glc)	2.84	285	88	109	27	113	15	Benzoic acid-4-glucuronide
2-Hydroxy-benzene-1-sulfate (Catechol-sulfate) (Cat-S)	4.93	189	70	109	20	81	20	Benzoic acid-4-sulfate
2-Hydroxy-4/5-methylbenzene-1-sulfate isomer 2 (4-Methylcatechol-sulfate isomer 1) (4-Me-Cat-S_iso1)	5.40	203	70	108	26	123	20	3-Methoxybenzoic acid-4-sulfate
2-Hydroxy-4/5-methylbenzene-1-sulfate isomer 2 (4-Methylcatechol-sulfate isomer 2) (4-Me-Cat-S_iso2)	5.96	203	70	108	26	123	20	3-Methoxybenzoic acid-4-sulfate

Supplementary Results

ESI Table S2: Mean daily intake of energy and nutrients for total population and by intervention groups.

	Total n=44		Intervention n=24		Control n=20		P Group	P Time	P Interaction
	T1	*T2*	*T1*	*T2*	*T1*	*T2*			
Energy (kJ/day)	7560.06 ± 1563.87	7127.97 ± 1871.53	7261.14 ± 1206.40	6689.00 ± 1643.01	7918.75 ± 1877.48	7654.74 ± 2030.69	0.081	0.085	0.519
Energy (kcal/day)	1799.48 ± 372.06	1696.14 ± 446.07	1729.13 ± 287.72	1591.35 ± 391.28	1883.89 ± 446.45	1821.89 ± 484.23	0.083	0.084	0.505
Protein (g/day)	68.94 ± 15.24	66.14 ± 16.45	64.14 ± 13.85	60.83 ± 15.00	74.69 ± 15.15	72.52 ± 16.19	**0.007**	0.252	0.809
(%E)	15	16	15	15	16	16			
Lipids (g/day)	68.82 ± 17.44	62.51 ± 19.96	69.18 ± 19.06	56.76 ± 17.51	68.38 ± 15.75	69.42 ± 20.94	0.230	**0.041**	**0.017**
(%E)	34	33	36	32	33	34			
Saturated fatty acids (g/day)	22.09 ± 5.85	21.22 ± 8.49	21.48 ± 5.83	18.83 ± 6.24	22.83 ± 5.93	24.09 ± 10.00	0.078	0.543	0.091
(%E)	11	11	11	11	11	12			
Monounsaturated fatty acids (g/day)	31.23 ± 9.42	27.22 ± 8.47	32.15 ± 11.10	24.79 ± 8.09	30.12 ± 7.02	30.14 ± 8.16	0.470	**0.012**	**0.011**
(%E)	16	14	17	14	15	15			
Polyunsaturated fatty acids (g/day)	9.81 ± 2.64	8.78 ± 3.37	9.65 ± 2.34	8.09 ± 3.54	10.01 ± 3.01	9.61 ± 3.02	0.254	**0.024**	0.171
(%E)	5	5	5	5	5	5			
Cholesterol (mg/day)	244.62 ± 80.43	224.92 ± 87.23	226.59 ± 72.19	198.84 ± 66.93	266.25 ± 86.19	256.22 ± 99.54	**0.021**	0.179	0.525
Carbohydrates (g/day)	210.46 ± 57.97	202.67 ± 56.47	197.66 ± 43.82	196.33 ± 54.08	225.81 ± 69.48	210.27 ± 59.72	0.163	0.339	0.419
(%E)	47	48	46	50	48	46			
Sugars (g/day)	67.62 ± 23.39	72.44 ± 25.29	65.99 ± 24.98	76.70 ± 28.24	69.58 ± 21.80	67.34 ± 20.78	0.653	0.263	0.090
(%E)	15	17	15	20	15	15			

Dietary fibre (g/day)	19.07 ± 5.60	16.84 ± 5.72	17.64 ± 4.09	15.39 ± 5.25	20.79 ± 6.71	18.58 ± 5.90	**0.044**	**0.002**	0.978
Alcohol (g/day)	11.10 ± 14.41	10.78 ± 13.65	10.60 ± 10.23	9.98 ± 11.14	11.71 ± 18.51	11.75 ± 16.43	0.733	0.759	0.730
(%E)	4	4	4	4	4	4			

Data are reported as mean ± standard deviation and percentage of the main nutrients at baseline (T1) and at 12-weeks (T2) and p values from generalized linear model (GLM) for repeated measures.

ESI Table S3. Total urinary excretion (μmol) of phenolic metabolites in control and bergamot groups during the 24 h after the first intervention day (T1) and the 24 h after the last intervention day (T2). Data are indicated as mean ± SEM. Nomenclature of metabolites is reported as proposed by Kay et al (30)

Urinary metabolites	Control group (μmol)		Intervention group (μmol)	
	T1	T2	T1	T2
Structurally-related flavanone metabolites (Flavanones)				
Naringenin-7-glucuronide (Nar-7-Glc)	0.45±0.14	0.25±0.09	1.23±0.23	1.44±0.43
Naringenin-4'-glucuronide (Nar-4'-Glc)	2.05±0.64	1.17±0.34	7.69±1.92	8.22±2.54
Naringenin-sulfate (Nar-S)	0.13±0.07	0.21±0.09	0.25±0.08	0.17±0.04
Naringenin-diglucuronide (Nar-diGlc)	0.03±0.01	0.03±0.02	0.10±0.02	0.14±0.04
Naringenin-sulfate-glucuronide (Nar-S-Glc)	0.04±0.03	0.07±0.04	0.54±0.14	1.68±0.33
Isosakuranetin (Isok)	-	0.001±0.00	0.04±0.00	0.04±0.01
Hesperetin (Hes)	-	-	0.01±0.00	0.01±0.00
Hesperetin-7-glucuronide (Hes-7-Glc)	0.14±0.07	0.03±0.01	0.75±0.16	1.10±0.44
Hesperetin-3'-glucuronide (Hes-3'-Glc)	2.91±1.45	1.21±0.54	6.95±1.57	10.41±3.82
Hesperetin-sulfate (Hes-S)	1.30±0.61	0.49±0.26	4.92±1.04	7.82±2.48
Hesperetin-diglucuronide (Hes-diGlc)	0.09±0.04	0.05±0.03	0.50±0.15	0.69±0.21
Hesperetin-sulfate-glucuronide (Hes-S-Glc)	0.07±0.02	0.08±0.02	0.04±0.01	0.04±0.01
Structurally-related flavone metabolites (Flavones)				
Apigenin-glucuronide (Api-Glc)	0.13±0.02	0.21±0.06	0.35±0.13	0.28±0.08
Diosmetin (Dios)	-	0.02±0.01	0.07±0.02	0.09±0.03
Diosmetin-glucuronide (Dios-Glc)	0.23±0.08	0.22±0.07	0.52±0.10	0.53±14
Cinnamic acids (CAs)				
3',4'-Dihydroxycinnamic acid (Caffeic acid) (3',4'-diHCA)	0.06±0.01	0.07±0.03	0.06±0.02	0.06±0.02
3'-Hydroxycinnamic acid-4'-glucuronide (Caffeic acid-4-glucuronide) (3'-HCA-4'-Glc)	0.03±0.02	0.02±0.01	0.05±0.02	0.04±0.02
4'-Hydroxycinnamic acid-3'-glucuronide (Caffeic acid-3-glucuronide) (4'-HCA-3'-Glc)	0.07±0.02	0.06±0.03	0.07±0.01	0.05±0.01
3'-Hydroxycinnamic acid-4'-sulfate (Caffeic acid-3-sulfate) (3'-HCA-4'-S)	0.20±0.05	0.30±0.07	0.30±0.06	0.24±0.06
4'-Hydroxycinnamic acid-3'-sulfate (Caffeic acid-4-sulfate) (4'-HCA-3'-S)	1.39±0.26	1.40±0.23	1.35±0.18	1.16±0.18

4’-Hydroxy-3’-methoxycinnamic acid (Ferulic acid) (3’-MeO-4’-HCA)	0.06±0.01	0.20±0.09	0.06±0.01	0.13±0.05
3’-Methoxycinnamic acid-4’-glucuronide (Ferulic acid-4-glucuronide) (3’-MeO-CA-4’-Glc)	3.90±0.95	2.92±0.42	3.83±0.65	3.19±0.59
4’-Methoxycinnamic acid-3’-glucuronide (Isoferulic acid-3-glucuronide) (4’-MeO-CA-3’-Glc)	0.98±0.18	0.84±0.15	1.07±0.17	1.00±0.16
3’-Methoxycinnamic acid-4’-sulfate (Ferulic acid-4-sulfate) (3’-MeO-CA-4’-S)	2.64±0.51	3.00±0.44	2.34±0.29	2.06±0.24
4’-Methoxycinnamic acid-3’-sulfate (Isoferulic acid-3-sulfate) (4’-MeO-CA-3’-S)	0.10±0.02	0.11±0.02	0.10±0.02	0.14±0.04
Feruloylglycine (Fer-Gly)	2.69±0.49	2.50±0.39	2.81±0.68	2.47±0.37
2’-Hydroxycinnamic acid (*o*-Coumaric acid) (2’-HCA)	0.01±0.00	-	0.01±0.00	0.01±0.00
3’-Hydroxycinnamic acid (*m*-Coumaric acid) (3’-HCA)	0.06±0.03	0.08±0.02	0.07±0.02	0.09±0.02
4’-Hydroxycinnamic acid (*p*-Coumaric acid) (4’-HCA)	0.17±0.08	0.20±0.04	0.25±0.05	0.15±0.01
Cinnamic acid-4’-glucuronide (Coumaric acid-4-glucuronide) (CA-4’-Glc)	0.09±0.03	0.05±0.02	0.06±0.02	0.03±0.01
Cinnamic acid-3’-glucuronide (Coumaric acid-3-glucuronide) (CA-3’-Glc)	0.04±0.01	0.04±0.02	0.06±0.01	0.07±0.02
Cinnamic acid-4’-sulfate (Coumaric acid-4-sulfate) (CA-4’-S)	0.31±0.06	0.29±0.04	0.27±0.04	0.24±0.04
Cinnamic acid-3’-sulfate (Coumaric acid-3-sulfate) (CA-3’-S)	0.42±0.16	0.37±0.06	0.36±0.04	0.52±0.09
Phenylpropanoic acids (PPAs)				
3-(3’,4’-Dihydroxyphenyl)propanoic acid (Dihydrocaffeic acid) (3’,4’-diHPPA)	0.56±0.14	0.50±0.14	2.44±0.39	3.13±0.52
3-(3’-Hydroxyphenyl)propanoic acid-4’-glucuronide (Dihydrocaffeic acid-4-glucuronide) (3’-HPPA-4’-Glc)	0.06±0.04	0.02±0.02	0.03±0.03	0.06±0.05
3-(4’-Hydroxyphenyl)propanoic acid-3’-glucuronide (Dihydrocaffeic acid-3-glucuronide) (4’-HPPA-3’-Glc)	0.13±0.06	0.01±0.01	0.12±0.06	0.04±0.02
3-(3’-Hydroxyphenyl)propanoic acid-4’-sulfate (Dihydrocaffeic acid-4-sulfate) (3’-HPPA-4’-S)	0.30±0.05	0.35±0.03	0.24±0.03	0.25±0.04
3-(4’-Hydroxyphenyl)propanoic acid-3’-sulfate (Dihydrocaffeic acid-3-sulfate) (4’-HPPA-3’-S)	2.55±0.69	1.99±0.35	2.33±0.75	2.21±0.44
3-(4’-Hydroxy-3’-methoxyphenyl)propanoic acid (Dihydroferulic acid) (3’-MeO-4’-HPPA)	0.79±0.27	0.90±0.25	0.77±0.17	1.01±0.35
3-(3’-Hydroxy-4’-methoxyphenyl)propanoic acid (Dihydroisoferulic acid) (4’-MeO-3’-	0.01±0.01	0.29±0.23	1.16±0.22	1.45±0.31

HPPA)				
3-(3’-Methoxy-phenyl)propanoic acid-4’-glucuronide (Dihydroferulic acid-4-glucuronide) (3’-MeO-PPA-4’-Glc)	0.53±0.13	0.41±0.08	0.54±0.15	0.52±0.13
3-(4’-Methoxy-phenyl)propanoic acid-3’-glucuronide (Dihydroisoferulic acid-3-glucuronide) (4’-MeO-PPA-3’-Glc)	0.55±0.11	0.35±0.08	0.97±0.16	1.03±0.19
3-(3’-Methoxy-phenyl)propanoic acid-4’-sulfate (Dihydroferulic acid-4-sulfate) (3’-MeO-PPA-4’-S)	0.51±0.12	0.48±0.10	0.43±0.10	0.47±0.08
3-(4’-Methoxy-phenyl)propanoic acid-3’-sulfate (Dihydroisoferulic acid-3-sulfate) (4’-MeO-PPA-3’-S)	0.22±0.05	0.16±0.03	0.32±0.05	0.32±0.05
3-(3’-Hydroxyphenyl)propanoic acid (3’-HPPA)	0.39±0.15	0.54±0.16	0.58±0.14	0.84±0.25
3-(Phenyl)propanoic acid-4’-glucuronide (PPA-4’-Glc)	0.24±0.09	0.16±0.03	0.21±0.05	0.29±0.09
3-(Phenyl)propanoic acid-3’-glucuronide (PPA-3’-Glc)	0.11±0.04	0.08±0.01	0.08±0.01	0.15±0.05
3-(Phenyl)propanoic acid-sulfate (PPA-S)	1.21±0.35	1.50±0.22	1.28±0.19	1.78±0.35
Phenylhydracrylic acids (PHAs)				
3-Hydroxy-3-(phenyl)propanoic acid (Phenylhydracrylic acid) (3-OH-PPA)	0.05±0.01	0.06±0.01	0.05±0.01	0.05±0.01
3-Methoxy-3-phenylpropanoic acid-glucuronide (3-Methylphenylhydracrylic acid-glucuronide) (3-MeO-PPA-GlcUA)	0.09±0.03	0.15±0.05	1.17±0.14	1.29±0.21
3-Methoxy-3-phenylpropanoic acid-sulfate (3-Methylphenylhydracrylic acid-sulfate) (3-MeO-PPA-S)	0.02±0.02	0.05±0.02	0.27±0.03	0.36±0.06
Phenylacetic acids (PAAs)				
4’-Hydroxy-3’-methoxy-phenylacetic acid (Homovanillic acid) (3’-MeO-4’-HPAA)	4.72±0.65	5.60±0.75	3.99±0.49	3.76±0.38
3’-Hydroxy-4’-methoxy-phenylacetic acid (Isohomovanillic acid) (4’-MeO-3’-HPAA)	3.23±0.66	2.58±0.69	2.57±0.50	3.51±0.94
3’-Methoxy-phenylacetic acid-4’-glucuronide (Homovanillic acid-glucuronide) (3’-Met-PAA-4’-Glc)	0.65±0.09	0.96±0.21	0.73±0.10	0.89±0.14
4’-Methoxy-phenylacetic acid-3’-glucuronide (Isohomovanillic acid-glucuronide) (4’-Met-PAA-3’-Glc)	0.04±0.01	0.16±0.10	0.17±0.0.02	0.14±0.02
3’-Methoxy-phenylacetic acid-4’-sulfate (Homovanillic acid-sulfate) (3’-Met-PAA-4’-S)	0.70±0.13	0.76±0.09	0.55±0.06	0.54±0.07
4’-Methoxy-phenylacetic acid-3’-sulfate (Isohomovanillic acid-sulfate) (4’-Met-PAA-3’-	3.33±0.92	2.54±0.45	3.02±0.99	2.80±0.56

S)				
3’-Hydroxyphenylacetic acid (3’-HPAA)	3.02±0.50	3.66±0.54	4.64±0.77	4.16±0.63
4’-Hydroxyphenylacetic acid (4’-HPAA)	3.46±0.80	3.02±0.52	2.88±0.50	2.86±0.48
Benzoic acids (BAs)				
3,4-Dihydroxybenzoic acid (Protocatechuic acid) (3,4-DiHBA)	0.29±0.06	0.47±0.07	0.43±0.09	0.35±0.05
Hydroxybenzoic acid-sulfate (Protocatechuic acid-sulfate) (HBA-S)	0.58±0.20	0.46±0.06	0.56±0.10	0.55±0.10
4-Hydroxy-3-methoxybenzoic acid (Vanillic acid) (3-MeO-4-HBA)	2.66±1.22	6.88±2.74	2.94±0.87	2.51±0.89
3-Methoxybenzoic acid-4-glucuronide (Vanillic acid-4-glucuronide) (3-MeO-BA-4-Glc)	0.68±0.12	1.23±0.65	0.78±0.17	0.73±0.21
3-Methoxybenzoic acid-4-sulfate (Vanillic acid-4-sulfate) (3-MeO-BA-4-S)	2.45±0.58	4.44±1.89	1.98±0.35	1.98±0.45
4-Methoxybenzoic acid-3-sulfate (Isovanillic acid-3-sulfate) (4-MeO-BA-3-S)	0.11±0.01	0.11±0.02	0.16±0.03	0.14±0.02
3-Hydroxybenzoic acid (3-HBA)	2.32±1.96	0.51±0.39	0.48±0.32	0.12±0.07
4-Hydroxybenzoic acid (4-HBA)	3.94±1.80	2.76±0.55	3.41±1.03	2.47±0.07
Benzoic acid-4-glucuronide (BA-4-Glc)	0.02±0.01	0.01±0.00	0.02±0.01	0.02±0.01
Benzoic acid-3-glucuronide (BA-3-Glc)	0.08±0.02	0.06±0.02	0.12±0.04	0.08±0.02
Benzoic acid-4-sulfate (BA-4-S)	5.20±1.31	4.54±1.12	3.78±0.62	3.23±0.42
Benzoic acid-3-sulfate (BA-3-S)	0.42±0.08	0.85±0.25	0.72±0.12	0.66±0.11
Hippuric acids (HAs)				
4-Hydroxyhippuric acid	6.63±1.16	7.19±1.06	6.52±0.50	9.09±1.72
Hippuric acid	144.55±32.51	186.94±32.80	162.34±23.85	149.48±21.27
Benzaldehydes (BALs)				
4-Hydroxybenzaldehyde	0.14±0.04	0.14±0.04	0.15±0.03	0.13±0.02
Benzenes (BZs)				
5-Hydroxy-3-methoxybenzene-1-sulfate (Methoxyphloroglucinol-sulfate) (MeO-Phlglu-S)	0.84±0.14	1.14±0.22	0.80±0.11	0.74±0.16
2-Hydroxy-benzene-1-glucuronide (Catechol-glucuronide) (Cat-Glc)	0.35±0.10	0.27±0.06	0.31±0.08	0.27±0.08
2-Hydroxy-benzene-1-sulfate (Catechol-sulfate) (Cat-S)	18.06±3.54	22.91±3.53	15.64±2.37	15.27±2.17
2-Hydroxy-4/5-methylbenzene-1-sulfate isomer 2 (4-Methylcatechol-sulfate isomer 1)	1.26±0.23	1.67±0.32	0.95±0.16	0.94±0.17

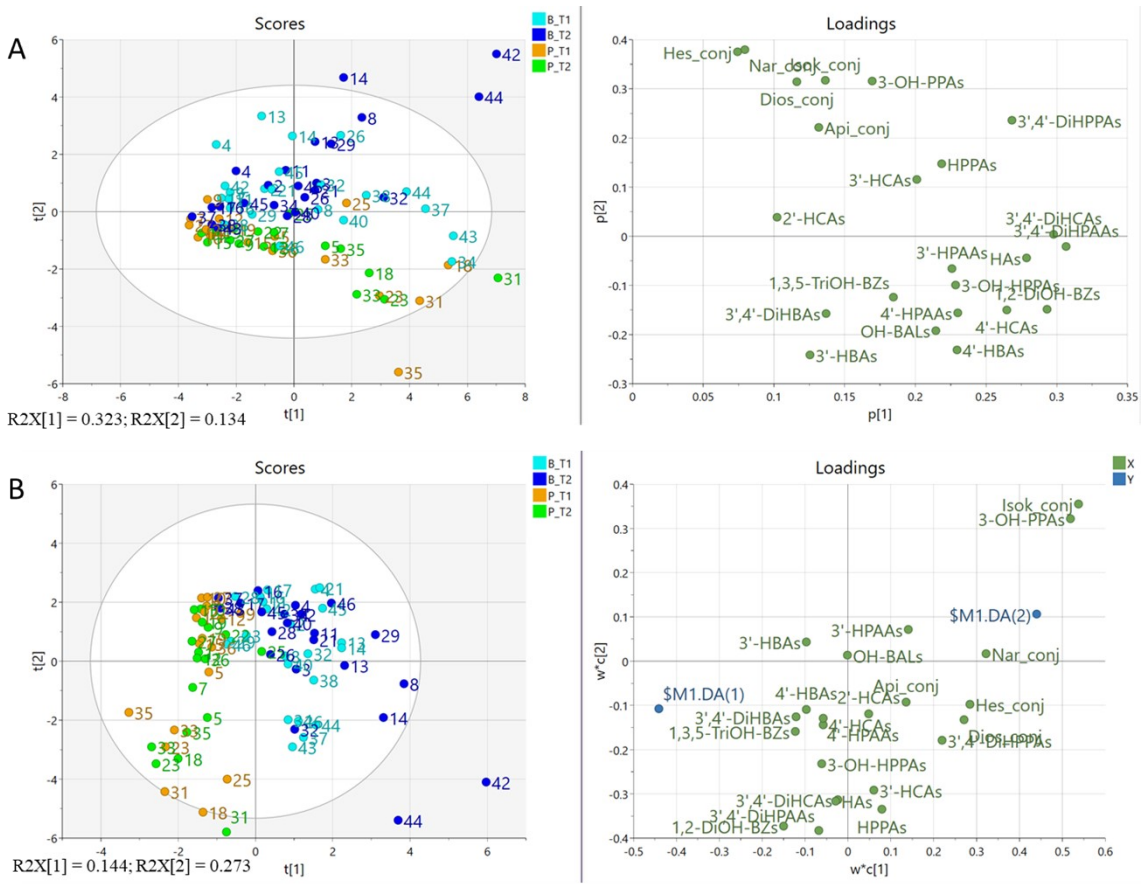
(4-Me-Cat-S_iso1)				
2-Hydroxy-4/5-methylbenzene-1-sulfate isomer 2 (4-Methylcatechol-sulfate isomer 2) (4-Me-Cat-S_iso2)	0.03±0.02	0.14±0.12	0.31±0.12	0.39±0.15

Principal Component Analysis (PCA) and Partial least squares discriminant analysis (PLS-DA)

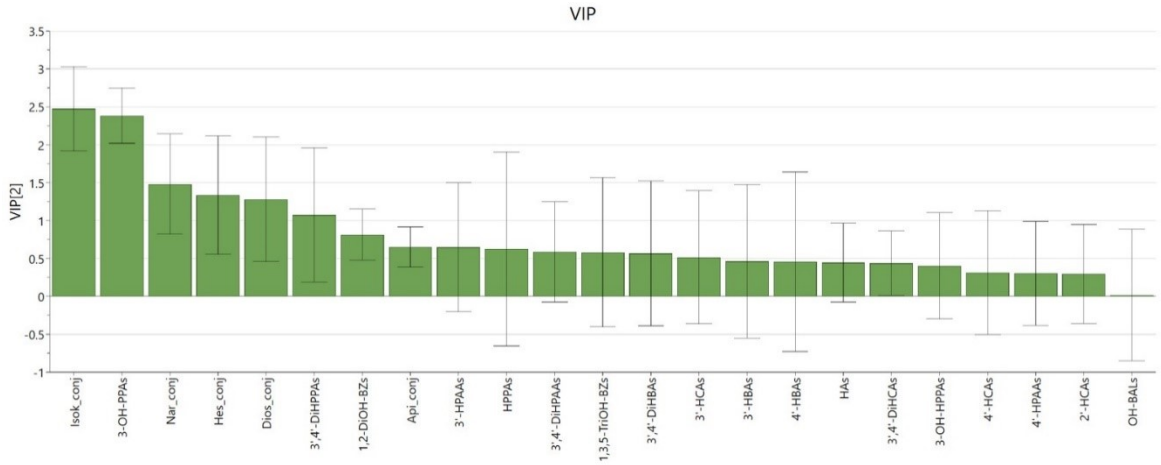
PCA was applied to further investigate the effect of BBB intervention and time on the urinary phenolic profile of the volunteers. Specifically, to analyze the effect of time, the acute effect of BBB consumption (detectable through T1 collection) was compared with the combined acute and chronic effect, (detectable through T2 collection). (Poly)phenolic classes grouping metabolites with the same aglycone moiety were considered as variables, to analyze possible differences arising from different catabolic pathways, and variables were autoscaled to give them equal weight, independently from the excretion level. Two PCs explained 45.7% of the total variability, with PC1 and PC2 explaining 32.3% and 13.4%, respectively (R2X parameter). The model predicted 26.5% of the total variability (Q2 parameter), with a major contribution from PC1 (23.5%). It was possible to identify a pattern of observation and variable distribution in the score and loading plots, respectively (ESI Figure 1A), which was in line with the intervention. Observations in the upper quadrants (positive values for PC2) belonged mainly to the BBB group and were characterized by a higher excretion of specific metabolites deriving from BBB (or other citrus fruits), namely structurally-related flavanones and flavones, and some phenylhydracrylic acids (hydroxy-3-(phenyl)propanoic acids). On the contrary, observations in the lower quadrants (negative values for PC2) belonged mainly to the control group and were characterized by a higher excretion of unspecific phenolic acids (mainly phenylacetic and benzoic acid derivatives) and benzene derivatives, which could derive from other sources of (poly)phenols present in the daily diet and from various endogenous and exogenous sources other than (poly)phenols (36). Observations were discriminated by PC1 according to the excreted amounts of metabolites, while PC2 discriminated them on a qualitative basis, resulting in the separation of metabolites specific of BBB from unspecific ones. No patterns depending on intervention time (T1 versus T2) were identified, but it was interesting to notice that all observations belonging to BBB group and characterized by the highest excretion of specific bergamot-derived metabolites were taken at T2 (observations 8, 14, 42 and 44). A PLS-DA (ESI Figure S1B) was applied to improve the

stratification of subjects into the two clusters (BBB group and control group), and to predict metabolites characterizing each group, using VIP scores (ESI Figure S2). The PLS-DA model passed cross-validation by CV-ANOVA ($p=8.9e^{-12}$) and by random permutation test. The explained variance (R2X) was 41.7%, with a greater contribution of the second PLS-DA component (27.3%). The predictive ability was 52.8%, with a major contribution from the first PLS-DA component (48.6%), which contributed greatly also to the variation explained by the clusters (R2Y=61.5%) (ESI Figure S1B). The ROC curves confirmed the good classification performance of the PLS-DA model (ESI Figure S3). The first component discriminated observations on a qualitative basis, with specific bergamot-derived metabolites in the right quadrants (positive value), stratifying subjects according to the intervention (BBB group in the right quadrants and control group in the left quadrants) (ESI Figure S1B). As suggested by PCA, the most relevant metabolites (VIP score > 1.25) for the clustering belonged to the classes of structurally-related flavanone metabolites (isasakuranetin, naringenin, and hesperetin derivatives), structurally-related flavone metabolites (diosmetin derivatives), and phenylhydracrylic acids (hydroxy-3-(phenyl)propanoic acids) (ESI Figure S2).

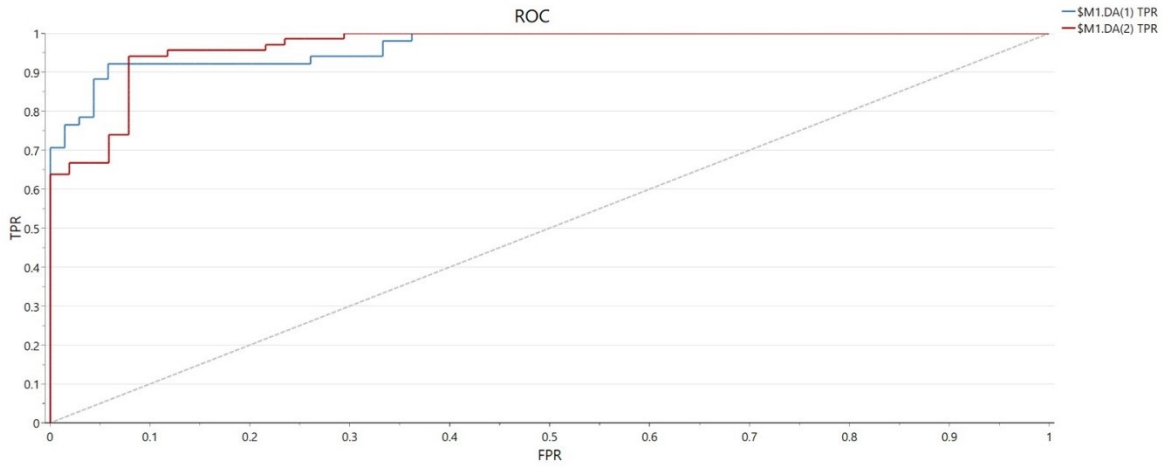
ESI Figure S1. (A) Score and loading plots resulting after applying PCA on autoscaled data for (poly)phenolic classes (including hippuric acids) and considering 2 PCs (PC1 and PC2). B_T1 indicates excretion values in the bergamot group during the 24 hours after the first intervention day, B_T2 indicates excretion values in the bergamot group during the 24 hours after the last intervention day, P_T1 indicates excretion values in the control group during the 24 hours after the first intervention day, and P_T2 indicates excretion values in the control group during the 24 hours after the last intervention day. (B) Score and loading plots resulting after applying PLS-DA with clusters based on the treatment type, namely bergamot-based beverage (BBB) group and control group. Data (excretion of (poly)phenolic classes) were autoscaled, and two components were considered. The abbreviations used for observations in the loading plot in panels A and B indicate the following classes: hesperetin conjugates (Hes_conj), naringenin conjugates (Nar_conj), isasakuranetin conjugates (Isok_conj), diosmetin conjugates (Dios_conj), apigenin conjugates (Api_conj), 3′,4′-hydroxycinnamic acids (3′,4′-DiHCAs), 2′-hydroxycinnamic acids (2′-HCAs), 3′-hydroxycinnamic acids (3′-HCAs), 4′-hydroxycinnamic acids (4′-HCAs), 3′,4′-dihydroxyphenylpropanoic acids (3′,4′-DiHPPAs), hydroxyphenylpropanoic acids (HPPAs), phenylhydracrylic acids (3-OH-PPAs), hydroxyphenylhydracrylic acids (3-OH-HPPAs), 3′,4′-hydroxyphenylacetic acids (3′,4′-DiHPAAs), 3′-hydroxyphenylacetic acids (3′-HPAAs), 4′-hydroxyphenylacetic acids (4′-HPAAs), 3,4-dihydroxybenzoic acids (3,4-DiHBAs), 3-hydroxybenzoic acids (3-HBAs), 4-hydroxybenzoic acids (4-HBAs), hippuric acids (HAs), hydroxybenzaldehydes (OH-BALs), 1,3,5-trihydroxybenzenes (1,3,5-TriOH-BZs), 1,2-dihydroxybenzenes (1,2-DiOH-BZs).



ESI Figure S2. Variable Importance in Projection (VIP) scores obtained from the PLS-DA model. The abbreviations used indicate the following classes: hesperetin conjugates (Hes_conj), naringenin conjugates (Nar_conj), isasakuranetin conjugates (Isok_conj), diosmetin conjugates (Dios_conj), apigenin conjugates (Api_conj), 3′,4′-hydroxycinnamic acids (3′,4′-DiHCAs), 2′-hydroxycinnamic acids (2′-HCAs), 3′-hydroxycinnamic acids (3′-HCAs), 4′-hydroxycinnamic acids (4′-HCAs), 3′,4′-dihydroxyphenylpropanoic acids (3′,4′-DiHPPAs), hydroxyphenylpropanoic acids (HPPAs), phenylhydracrylic acids (3-OH-PPAs), hydroxyphenylhydracrylic acids (3-OH-HPPAs), 3′,4′-hydroxyphenylacetic acids (3′,4′-DiHPAAs), 3′-hydroxyphenylacetic acids (3′-HPAAs), 4′-hydroxyphenylacetic acids (4′-HPAAs), 3,4-dihydroxybenzoic acids (3,4-DiHBAs), 3-hydroxybenzoic acids (3-HBAs), 4-hydroxybenzoic acids (4-HBAs), hippuric acids (HAs), hydroxybenzaldehydes (OH-BALs), 1,3,5-trihydroxybenzenes (1,3,5-TriOH-BZs), 1,2-dihydroxybenzenes (1,2-DiOH-BZs).



ESI Figure S3. ROC plot obtained from the PLS-DA model. TPR and FPR indicate true positive and false positive rates, respectively. Blue color is used for placebo group, red for BBB group.



ESI Figure S4. Distribution of phenolic classes of bergamot metabolites (without considering hippuric acid derivatives) excreted in urine (A) during the 24 hours after the first intervention day (T1) in the BBB group, (B) during the 24 hours after the first intervention day (T1) in the control group, (C) during the 24 hours after the last day of intervention (T2) in the BBB group, (D) during the 24 hours after the last day of intervention (T2) in the control group.