Phenolic and microbial-targeted metabolomics to discovering and evaluating wine intake biomarkers in human urine and plasma

Mireia Urpi-Sarda [1,2, †,*], María Boto-Ordóñez [1,†], María Isabel Queipo-Ortuño[3,4], Sara Tulipani [1,2,3], Dolores Corella [4,5], Ramon Estruch [4,6], Francisco J. Tinahones [4,7], Cristina Andres-Lacueva [1,2]

[1] Biomarkers and Nutritional & Food Metabolomics Research Group, Nutrition and Food Science Department, XaRTA, INSA, Pharmacy Faculty, University of Barcelona, Avenida Joan XXIII s/n, 08028 Barcelona, Spain.

[2] Ingenio-CONSOLIDER programme, FUN-C-FOOD, CSD2007-063, Barcelona, Spain.

[3]Research Laboratory, IMABIS Foundation, Virgen de la Victoria Clinical Hospital, Málaga, Spain.

[4] CIBER Fisiopatología de la Obesidad y la Nutrición, Instituto de Salud Carlos III, Madrid, Spain.

[5] Department of Preventive Medicine, University of Valencia, Valencia, Spain.

[6] Department of Internal Medicine, Hospital Clinic, Institut d'Investigació Biomèdica August Pi i Sunyer (IDIBAPS), University of Barcelona, Villarroel 170, 08036 Barcelona, Spain.

[7] Servicio Endocrinología y Nutrición del Hospital Universitario Virgen de la Victoria, Málaga, Spain.

[†] These authors contributed equally to this paper.

*__Correspondence:__ Dr Mireia Urpi-Sarda, Department of Nutrition and Food Science, XaRTA, INSA, Campus Torribera, Pharmacy School, University of Barcelona, Barcelona, Spain

E-mail: murpi@ub.edu

Fax: +34-934035931

Abbrevations used: AUC, area under the curve; DHPV, dihydroxyphenyl-γ-valerolactone; DRW, dealcoholized red wine; MRM, multiple reaction monitoring; PCA, principal component analysis; PM, prediction model; ROC, receiver operating characteristic; RW, red wine; Tmax, time to maximum; $t_{1/2,}$ half-life.

Keywords: exposure biomarkers / nutrimetabolomics / logistic regression models / targeted metabolomics / wine

Total Words: 4978 words (including references as well as table legends)

ABSTRACT

The discovery of biomarkers of intake in nutritional epidemiological studies is essential in establishing an association between dietary intake (considering their bioavailability) and diet-related risk factors for diseases. The aim is to study urine and plasma phenolic and microbial profile by targeted metabolomics approach in a wine intervention clinical trial for discovering and evaluating food intake biomarkers.

High-risk male volunteers (n=36) were included in a randomized, crossover intervention clinical trial. After a washout period, subjects received red wine or gin, or dealcoholized red wine over 4 weeks. Fasting plasma and 24-h urine were collected at baseline and after each intervention period. A targeted metabolomic analysis of 70 host and microbial phenolic metabolites was performed using UPLC-MS/MS. Metabolites were subjected to stepwise logistic regression to establish prediction models and received operation curves were performed to evaluate biomarkers.

Prediction models based mainly on gallic acid metabolites, obtained sensitivity, specificity and area under the curve (AUC) for the training and validation sets of between 91% and 98% for urine and between 74% and 91% for plasma. Resveratrol, ethylgallate and gallic acid metabolite groups in urine samples also resulted in being good predictors of wine intake (AUC>87%). However, lower values for metabolites were obtained in plasma samples. The highest correlations between fasting plasma and urine were obtained for the prediction model score (r=0.6, P<0.001), followed by gallic acid metabolites (r=0.5-0.6, P<0.001). This study provides new insights into the discovery of food biomarkers in different biological samples.

1 Introduction

Biomarkers in epidemiological and clinical trials have to be indicators of exposure and must have several characteristics, including being robust, sensitive to changes, specific to the dietary source and biologically and physiologically understandable [1]. In the food research field, this means that biomarkers have to be an objective measure of intake and an evaluated indicator of food intervention [2]. There has been much in-depth discussion concerning their ability to solve classical problems regarding estimating an index of quantitative exposure to individual food [1-3], and recently, identifying dietary patterns that may be related to major health benefits. Hence, there is an increased interest in biomarker research for the development of new functional foods, as well as for the validation of existing biomarkers [4]. Therefore, global metabolic approaches need to be carried out in order to evaluate the role of individual or groups of metabolites in the discrimination of selected food consumption.

After consumption of polyphenols, beneficial health effects in the prevention of diseases have been widely analysed in *in vivo* and *in vitro* studies [5-7]. In particular, the consumption of grape-derived products such as red wine (RW) and dealcoholized RW (DRW) has been associated with a protective effect against cardiovascular diseases, possibly through their anti-inflammatory and antihypertensive activities [5, 8]. These associations were first linked to phytochemicals found in foods, which could exert their biological activity. However, in recent years there has been increasing attention paid to the metabolites formed in the organism, especially those formed by microbiota, due to their role in the prevention of some diseases such as obesity and diabetes [9, 10]. This supposes an increase in the variety of metabolites found in biofluids after consumption, and therefore an increased number of possible food biomarkers [11]. Moreover, new targeted and untargeted approaches have also increased the range of metabolites found

in biofluids, allowing the use of metabolomic tools for a new approach in biomarker research. In the case of RW, resveratrol metabolites have been described as being good biomarkers of wine intake [12, 13], and gallic acid has also been suggested as a marker, due to its increased excretion after wine consumption [14]. Both compounds were determined in 24-h urine. This sample has been suggested as being better for biomarker determination than others but since it is difficult to obtain in large epidemiological studies [1, 12-14], other samples such as fasting plasma need to be assessed for their potential to identify biomarkers.

Here, we study the phenolic and microbial profile by a targeted metabolomics approach in a wine intervention clinical trial for the discovery and evaluation of biomarkers of wine intake considering both fasting plasma and 24-h urine samples.

2 Materials and Methods

2.1 Subjects and study design

Thirty-six volunteers were included for the study of the targeted phenolic metabolite profile. The study was an open, randomized, crossover and controlled clinical intervention trial comprising three 4-week periods [5]. Baseline characteristics of participants and inclusion and exclusion criteria are given in detail in the Supporting Information and Supporting Information Table S1. After following a 15-day run-in period free of grape-derived products and alcoholic beverages, subjects were requested to consume 272 mL of RW (30 g ethanol/day), 272 mL of DRW and 100 mL of gin (30 g ethanol/day) every day for 4 weeks, following the same background diet. Fasting blood samples (n=33) and 24-h urine samples (n=36) were collected after each intervention period and immediately stored at −80 °C until analysis. The Institutional Review Board of the hospital approved the study protocol. All participants gave written consent before participation in the study. This trial was registered in the Current Controlled Trials at the International Standard Randomized Controlled Trial Number Register, at controlled-trials.com, as ISRCTN88720134.

2.2 Chemicals and reagents

Chemical reagents and solvents used in this study are detailed in Supporting Information.

2.3 Red wine, dealcoholized red wine and gin

The RW and DRW used in this study were made with the Merlot grape variety, from the Penedès appellation (Catalonia, Spain). No differences in phenolic composition were

found between wines (Supporting Information Table S2) [15]. Xoriguer gin was used to ensure the same alcoholic consumption as the RW period.

2.4 Sample extraction

The targeted analyses of microbial-derived and conjugated metabolites were performed using solid-phase extraction. Oasis® MCX and HLB 96-well plates (Waters, Milford, Massachusetts) were used in hydrolyzed and non-hydrolyzed samples, respectively, as previously described [16-18]. Briefly, urine and plasma samples (1 mL) were loaded onto the conditioned cartridge plate. Then the cartridges were washed and analytes were eluted with methanol or acidified methanol (0.1% formic acid), respectively. Eluates from both extraction methods were evaporated to dryness under a gentle stream of nitrogen gas [17]. Residues were reconstituted with 100 µL of taxifolin (1.64 µmol/L) dissolved in mobile phase [16, 18].

2.5 UPLC-MS/MS analysis

The analysis of metabolites in urine and plasma was performed by UPLC-MS/MS equipped with a binary solvent manager and a refrigerated autosampler plate (Waters Acquity UPLC system, Milford, MA, USA), coupled to an AB Sciex API 3000 triple quadrupole mass spectrometer equipped with a turbo ion spray, in a negative electrospray ionization mode (PE Sciex). An Acquity UPLC BEH C18 (Milford, MA, USA) (1.7 µm, 2.1 mm × 5 mm), using a pre-filter, working at 40 °C with 0.5 mL/min with an injection volume of 5 µL, was used as described before [16]. Mobile phase A (0.1% formic acid) and B (0.1% formic acid in acetonitrile) were used at a flow rate of 500 µL/min with the following proportions (v/v) of phase A [t(min),%A]: (0,92);

(2.5,50); (2.6,0); (3,0); (3.1,92); (3.5,92). The MS/MS parameters used were as previously described [16, 17].

2.6 Quantitative analysis

For quantification purposes, data were collected using the multiple reaction monitoring (MRM) mode (Table 1 and Table 2) with a dwell time of 10 ms. When commercial standards were not available, concentrations were quantified using the most similar compound standard curve. Results were expressed as their equivalents [16]. The mean recovery of analytes ranged from 87% to 109%, and accuracy and precision of analytes at different concentrations were <15% [16, 17].

2.7 Statistical analysis

Two statistical programs for data analysis were used: the MetaboAnalyst Web-based platform [19] and IBM SPSS Statistics software program for Windows version 20 (Chicago, IL). The overall approach is described with the following steps: i) Data normalization of quantified phenolic metabolites was performed by a cube root transformation and a range scaling of the data; ii) This data retrieved an unsupervised segregation by principal component analysis (PCA) and hierarchical clustering analysis; iii) ANOVA for repeated measures was used to compare changes in phenolic metabolites in plasma and urine after intervention treatments (Bonferroni post hoc test); iv) Among the metabolites that displayed significantly different levels between wine interventions and baseline or gin period, a binary stepwise logistic regression analysis was performed to assess which metabolite combination predicted the wine intervention. For this purpose, 80% of random samples of wine interventions and baseline or gin periods were used as a training set, in which the logistic regression model was

calculated, and the remaining samples of each group (20%) were used as a validation set; v) The sensitivity, specificity and area under the curve (AUC) of the model were compared with parameters of phenolic metabolite groups in the whole population through a receiver operating characteristic (ROC) curve. The phenolic metabolite groups in urine and plasma are described in Supporting Information Table S3. In addition to the metabolites analysed in this study, resveratrol data from previous analysis [15] were included to be evaluated and compared, since resveratrol has already been described as a wine intake biomarker [12, 13].

The optimal cut-off for the ROC curves was determined through the identification of the shortest distance to the optimal point (0,1) for which specificity and sensitivity was calculated.

To estimate the association between fasting plasma and 24-h urine in the prediction models and within the phenolic metabolite groups, the Spearman correlation coefficients were calculated. Statistical significance was defined as $P \leq 0.05$.

3 Results

3.1 Urine and plasma analysis of targeted polyphenol metabolomic pattern

Nineteen individual metabolites and conjugates of (epi)catechin, methyl(epi)catechin and dihydroxyphenyl-γ-valerolactone (DHPV), and 10 phenolic acids including methylgallic sulfate and the group of total resveratrol metabolites significantly increased in urine after RW or DRW interventions compared to the baseline or gin periods (Table 1). Only ethylgallate metabolites showed a statistically significant difference between both wine periods of intake. The plasma metabolites that increased after the wine interventions in relation to the baseline or gin periods included 10 phenolic acids, such as gallic acid and DHPV and their conjugates (Table 2).

The PCA differentiated easily between urinary samples from RW and DRW interventions and samples from those in the baseline or gin period (Supporting Information Fig. S1A). PC1 explained 41.9% of the total variance while PC2 explained 7.6% of the total variance, where the loading plot showed that gallic acid, ethylgallate and resveratrol metabolites were mainly responsible for this difference (data not shown). The clustering analysis executed by the heat map compared the metabolites of the participants in the four intervention periods. This was used as a first approach to assess the possible use of phenolic groups as biomarkers of wine consumption. A progression in the strongest discriminatory signals was observed in the heat map (Supporting Information Fig. S1B). The strongest discriminatory signals were observed for resveratrol, gallic acid and ethylgallate metabolites, followed by (epi)catechin and valerolactone metabolites, and the least discriminatory signals were those of phenolic acids.

3.2 Evaluation of food intake biomarkers

209 The applicability of a logistic regression model involving multiple metabolites was
210 examined to find the best markers of wine consumption in hydrolyzed and non-
211 hydrolyzed fasting plasma and 24-h urine samples from a clinical study in the training
212 set. Metabolites that displayed significantly different levels between wine interventions
213 and the baseline or gin period were subjected to a stepwise variable selection method.
214 The results of the model for each type of sample are shown in Table 3. Metabolites
215 included in the models did not display multicollinearity (data not shown). Both groups
216 of resveratrol (resveratrol biomarker and microbial resveratrol metabolites) showed
217 AUC over 96% and were analyzed only in non-hydrolyzed urine samples. Therefore,
218 they were excluded from the logistic regression to be able to compare models with the
219 same metabolites between different samples. The validity of the model was confirmed
220 with the validation set and then applied to the whole population. The results of
221 sensitivity, specificity and AUC for the model were higher than 92% and 74% for urine
222 and fasting plasma samples, respectively, among training and validation sets, and for the
223 whole population (Table 4). The global performance of the model for each kind of
224 sample considering the whole population was depicted in the ROC curves (Supporting
225 Information Fig. S2) and compared with the results obtained for the different phenolic
226 groups (Table 4 and Supporting Information Fig. S2). In hydrolyzed urine, the best
227 sensitivity, specificity and AUC were obtained for the model, followed by ethylgallate.
228 In non-hydrolyzed urine samples, the groups of ethylgallate, methylgallic and
229 resveratrol metabolites (AUC: 93–99%) resulted in being better discriminators of wine
230 intake than (epi)catechin and DHPV metabolites (AUC: 76–86%). The best sensitivity
231 and specificity were obtained for the model and for microbial resveratrol metabolites
232 (cut-off value: 1424.19 µmol/24-h), and closely followed by the resveratrol biomarker.
233 Plasma metabolites were weaker indicators of wine intake. Only the model in both

hydrolyzed and non-hydrolyzed samples and methylgallic sulfate in non-hydrolyzed samples had an AUC over 80%, which matched the results obtained in the prediction model.

3.3 Correlations between fasting plasma and 24-h urine

Correlations of individual metabolites, phenolic metabolite groups and the prediction model between 24-h urine and fasting plasma samples were performed (Table 5). The highest correlations were obtained for the prediction model score in both hydrolyzed and non-hydrolyzed samples (r=0.565 and 0.599, P<0.001, respectively) (Table 5 and Supporting Information Fig. S3), followed by the gallic acid metabolite group (r=0.451 and 0.587, P<0.001, respectively). The group of flavan-3-ols and DHPV metabolites had lower but significant correlation values (r=0.4, P<0.001) in non-hydrolyzed samples.

4 Discussion

This is the first study in which phenolic metabolites from wine intake have been systematically evaluated and trialled in quantitative approach for the discovery and discrimination of food intake biomarkers.

In this work, up to 70 and 30 phenolic metabolites have been identified and quantified in 24-h urine and fasting plasma samples, respectively, at baseline and after RW, DRW and gin interventions using a UPLC-MS/MS targeted analysis. Only 19 metabolites of (epi)catechin, methyl(epi)catechin and DHPV, 10 phenolic acids and resveratrol metabolites resulted in being higher in urine after RW and DRW compared with baseline or gin periods (Table 1). No differences were observed between RW and DRW metabolites in plasma and urine except for urinary concentrations of ethylgallate and its metabolites, whose concentration increased after the RW period ($P<0.001$). Ethylgallate is a wine compound derived from ethanol and gallic acid esterification [20], with similar concentration values in both wines (Supporting Information Table S1). The increment observed after RW intake may be due to the fact that ethylgallate could also be formed in the organism influenced by ethanol and gallate consumption through ethyl esterification by human esterases or by microbial metabolism [21, 22]. Up to this point, the results have shown individual statistical differences for metabolites between groups or the baseline time period. Previous targeted studies on the metabolism of polyphenols have also used these kind of approaches to evaluate statistical differences between groups in searching for polyphenol biomarkers [14, 17, 23] and sometimes they only focused on a few metabolites that could not represent the global fingerprint [14].

In this study, the metabolites that displayed significant differences between both wine interventions and the baseline or gin period were selected as metabolite biomarker candidates to be evaluated in the stepwise logistic regression analysis. This approach,

traditionally used in clinical diagnosis [24], allows the identification of combinations of metabolites from several origins that increased their discriminate power regarding single metabolites. To our knowledge, this approach has been applied for the first time in targeted studies of polyphenol food research. Advantages over previous works were the high number of metabolites quantified that were added to this new step, which allowed the discrimination of those metabolites as better predictors of wine intake.

All of the metabolites included in the model (Table 3) could come from the microbial degradation of several wine phenolics [11] and some of them are also present in wine composition, such as gallic acid, ethylgallate and 2,4-dihydroxybenzoic acid [16, 25, 26]. Gallic acid could also be released from several compounds present in wine, such as gallates and anthocyanins [14, 27]. 2,4-Dihydroxybenzoic acid has also been described as coming from the degradation of anthocyanins [28] and 3-hydroxyphenylacetic and *p*-coumaric acids, derived from procyanidins and anthocyanins, respectively [18, 29], which can be found in high content in wine [25]. Other analysed phenolic acids were not considered in the model since they were less discriminant as most arise from several food compounds. Thus, these metabolites could be misleading if they were considered as biomarkers, as has previously been suggested after the intake of berries [11, 30].

Once the model for each kind of sample was obtained, the AUC, sensitivity and specificity and ROC curves evaluated their capacity to discriminate wine consumers. In addition, these values were compared with those corresponding to phenolic metabolite groups (Table 4 and Supporting Information Fig. S2). The resveratrol biomarker and microbial resveratrol metabolites had similar values to the model, with AUC values of 96.5 and 98.8%, respectively. Until now, phase II metabolites of resveratrol have been proposed as good biomarkers of wine intake [12, 13], but microbial-derived metabolites have not been evaluated before. As was discussed above, one of the characteristics of a

298 good biomarker is being specific to food intake, thus resveratrol is well known for being
299 almost exclusively distributed in grape products [31]. The fact to validate biomarkers is
300 of great importance since there is the need for objective measures of food exposure that
301 allow accurate measures taking into account their bioavailability [32]. Other phenolic
302 groups with good but lower AUC values than the model were ethylgallate and gallic
303 acid metabolites (Table 4). Thus, they could also be considered as biomarkers of wine
304 intake. Previously, some authors positively associated gallic acid and methylgallic acid
305 with the consumption of wine [33], but, to our knowledge, no associations have been
306 published for ethylgallate. Gallic acid has even been described as the main metabolite of
307 ethylgallate, with longer Tmax and $t_{1/2}$ than its parent compound [34]. Both ethylgallate
308 and gallic acid have been described in other foods, such as grape products, wine and
309 vinegar, and tea, nuts and berries [25]. Other metabolites such as (epi)catechin and
310 valerolactones were less discriminant than those described metabolites and the model.
311 Although the concentrations of flavan-3-ols are high in wine, they are not exclusively of
312 wine since metabolites have been described after cocoa, tea or nut consumption [18, 35,
313 36]. As far as we know, previous studies have evaluated a single or groups of
314 metabolites as biomarkers of specific food consumption. Therefore, as commented
315 above, a same biomarker could be associated to different foods. Here, the application of
316 this targeted metabolomic approach allows to define a specific biomarker imprinting of
317 wine intake.
318 The type of sample matrix in which biomarkers are measured also influences biomarker
319 evaluation [37]. Twenty-four hour urine has been described as the gold standard sample
320 for biomarker evaluation [38] and it provides a better measure of total polyphenol
321 metabolites than fasting plasma as it provides a better index of intake [1]. However, for
322 practical reasons, 24-h urine is not an easy sample to obtain in large-scale

323 epidemiological studies [1]. Consequently, we have assessed that fasting plasma should
324 be considered for biomarker determination. In this study, individual and phenolic
325 metabolite groups along with the score obtained from the prediction model (Supporting
326 Information Fig. S3) were correlated between fasting plasma and 24-h urine (Table 5).
327 The best correlations were observed among model scores from hydrolyzed and non-
328 hydrolyzed samples, indicating that those volunteers that were better classified as wine
329 consumers were done so through both urine and plasma samples (r=0.565 and r=0.599,
330 respectively P<0.001). Valerolactones and gallic acid microbial metabolites that also
331 had significant correlations were selected for their important role as biomarkers in urine,
332 and possible presence in fasting plasma due to their longer half-life [34, 36].
333 Ethylgallate could not be evaluated due to the low concentrations obtained in plasma
334 since the Tmax and half-lives of ethylgallate were expected to be lower than its main
335 metabolite gallic acid [34]. Although the coefficients of correlation were significant, the
336 r values were clinically moderate (r < 0.750) [37], which was similar to previous studies
337 that correlated urinary and plasma alkylresorcinol metabolites [37]. Correlations
338 between 24-h urine and fasting plasma have been previously described for total
339 flavonols in a crossover trial with a low flavonoid diet or with the same diet
340 supplemented with flavonols (r=0.624) [39], as well as for isoflavones, using spot
341 plasma (r=0.99) [40]. These correlations could open the possibility of finding those
342 metabolites in plasma and establishing them as biomarkers of consumption and effect,
343 but larger studies in a free-living population are needed to confirm and generalize this
344 statement. In addition, a problem with the fasting plasma, as suggested previously [41],
345 could be the substantial number of concentrations that are lower than the limit of
346 quantification due to the short half-lives of polyphenol metabolites.

This study proposes the use of a quantitative targeted metabolomics approach that combines phenolic and microbial analysis, logistic regression joining with ROC curves in interventional studies to identify, evaluate and compare single, groups of biomarkers and the biomarker imprinting of wine intake. Correlations between fasting plasma and urine provide the opportunity to discriminate metabolites that could be good urinary biomarkers of consumption, both in urine and plasma. This approach is a promising tool that has great potential for identifying possible food biomarkers to evaluate compliance in clinical studies, identify eating patterns and make associations between polyphenol consumption and health benefits.

Acknowledgments

The authors are grateful to the Spanish National Grants from the Ministry of Economy and Competitiveness (MINECO) and cofounded by FEDER (Fondo Europeo de Desarrollo Regional): AGL2006-14228-C03-02/ALI, AGL2009-13906-C02-01, AGL2010-10084-E, the CONSOLIDER INGENIO 2010 Programme, FUN-C-FOOD (CSD2007-063), CIberOBN, as well as PI13/01172 Project, (Plan N de I+D+i 2013-2016) by ISCII-Subdirección General de Evaluación y Fomento de la Investigación. We also thank the award of 2014SGR1566 from the Generalitat de Catalunya's Agency AGAUR and INSA-UB FRI 2013 Grant. M.U.-S. would like to thank the "Ramon y Cajal" (RYC-2011-09677) from the MINECO and Fondo Social Europeo. M.B.-O. and S.-T. would like to thank the FPU fellowship and "Juan de la Cierva" programmes, respectively, from the MINECO. We thank Torres SA for providing the red wine and dealcoholized red wine used in the study, and Xoriguer Gin for providing the gin used in the study.

371 The authors have declared no conflict of interest.

372

5 References

[1] Spencer, J. P., Abd El Mohsen, M. M., Minihane, A. M., Mathers, J. C., *Br. J. Nutr.* 2008, *99*, 12-22.
[2] Hedrick, V. E., Dietrich, A. M., Estabrooks, P. A., Savla, J., Serrano, E., Davy, B. M., *Nutr. J.* 2012, *11*, 109.
[3] Jenab, M., Slimani, N., Bictash, M., Ferrari, P., Bingham, S., *Hum. Genet.* 2009, *125*, 507-525.
[4] van Loveren, H., Sanz, Y., Salminen, S., *Annu. Rev. Food Sci. Technol.* 2012, *3*, 247-261.
[5] Chiva-Blanch, G., Urpi-Sarda, M., Llorach, R., Rotches-Ribalta, M., Guillen, M., Casas, R., Arranz, S., Valderas-Martinez, P., Portoles, O., Corella, D., Tinahones, F., Lamuela-Raventos, R. M., Andres-Lacueva, C., Estruch, R., *Am. J. Clin. Nutr.* 2012, *95*, 326-334.
[6] Verzelloni, E., Pellacani, C., Tagliazucchi, D., Tagliaferri, S., Calani, L., Costa, L. G., Brighenti, F., Borges, G., Crozier, A., Conte, A., Del Rio, D., *Mol. Nutr. Food Res.* 2011, *55 Suppl 1*, S35-43.
[7] Scalbert, A., Manach, C., Morand, C., Remesy, C., Jimenez, L., *Crit. Rev. Food Sci. Nutr.* 2005, *45*, 287-306.
[8] Chiva-Blanch, G., Urpi-Sarda, M., Ros, E., Arranz, S., Valderas-Martinez, P., Casas, R., Sacanella, E., Llorach, R., Lamuela-Raventos, R. M., Andres-Lacueva, C., Estruch, R., *Circ. Res.* 2012, *111*, 1065-1068.
[9] Devaraj, S., Hemarajata, P., Versalovic, J., *Clin. Chem.* 2013, *59*, 617-628.
[10] Manach, C., Williamson, G., Morand, C., Scalbert, A., Remesy, C., *Am. J. Clin. Nutr.* 2005, *81*, 230S-242S.
[11] Boto-Ordoñez, M., Rothwell, J. A., Andres-Lacueva, C., Manach, C., Scalbert, A., Urpi-Sarda, M., *Mol. Nutr. Food Res.* 2013.
[12] Zamora-Ros, R., Urpi-Sarda, M., Lamuela-Raventos, R. M., Estruch, R., Vazquez-Agell, M., Serrano-Martinez, M., Jaeger, W., Andres-Lacueva, C., *Clin. Chem.* 2006, *52*, 1373-1380.
[13] Zamora-Ros, R., Urpi-Sarda, M., Lamuela-Raventos, R. M., Estruch, R., Martinez-Gonzalez, M. A., Bullo, M., Aros, F., Cherubini, A., Andres-Lacueva, C., *Free Radic. Biol. Med.* 2009, *46*, 1562-1566.
[14] Ito, H., Gonthier, M. P., Manach, C., Morand, C., Mennen, L., Remesy, C., Scalbert, A., *Br. J. Nutr.* 2005, *94*, 500-509.
[15] Rotches-Ribalta, M., Urpi-Sarda, M., Llorach, R., Boto-Ordoñez, M., Jauregui, O., Chiva-Blanch, G., Perez-Garcia, L., Jaeger, W., Guillen, M., Corella, D., Tinahones, F. J., Estruch, R., Andres-Lacueva, C., *J. Chromatogr. A* 2012, *1265*, 105-113.
[16] Boto-Ordoñez, M., Urpi-Sarda, M., Queipo-Ortuño, M., Corella, D., Tinahones, F., Estruch, R., Andres-Lacueva, C., *J. Agric. Food. Chem.* 2013, *61*, 9166-9175.
[17] Urpi-Sarda, M., Monagas, M., Khan, N., Lamuela-Raventos, R. M., Santos-Buelga, C., Sacanella, E., Castell, M., Permanyer, J., Andres-Lacueva, C., *Anal. Bioanal. Chem.* 2009, *394*, 1545-1556.
[18] Urpi-Sarda, M., Monagas, M., Khan, N., Llorach, R., Lamuela-Raventos, R. M., Jauregui, O., Estruch, R., Izquierdo-Pulido, M., Andres-Lacueva, C., *J. Chromatogr. A* 2009, *1216*, 7258-7267.
[19] Xia, J., Wishart, D. S., *Nat. Protoc.* 2011, *6*, 743-760.

[20] Monagas, M., Gomez-Cordoves, C., Bartolome, B., Laureano, O., Ricardo da Silva, J. M., *J. Agric. Food. Chem.* 2003, *51*, 6475-6481.
[21] Lange, L. G., *Proc. Natl. Acad. Sci. U S A* 1982, *79*, 3954-3957.
[22] Couteau, D., McCartney, A. L., Gibson, G. R., Williamson, G., Faulds, C. B., *J. Appl. Microbiol.* 2001, *90*, 873-881.
[23] Gonthier, M. P., Cheynier, V., Donovan, J. L., Manach, C., Morand, C., Mila, I., Lapierre, C., Remesy, C., Scalbert, A., *J Nutr* 2003, *133*, 461-467.
[24] Moons, K. G., de Groot, J. A., Linnet, K., Reitsma, J. B., Bossuyt, P. M., *Clin. Chem.* 2012, *58*, 1408-1417.
[25] Neveu, V., Perez-Jimenez, J., Vos, F., Crespy, V., du Chaffaut, L., Mennen, L., Knox, C., Eisner, R., Cruz, J., Wishart, D., Scalbert, A., *Database (Oxford)* 2010, *2010*, bap024.
[26] Sanz, M., Fernandez de Simon, B., Esteruelas, E., Munoz, A. M., Cadahia, E., Hernandez, M. T., Estrella, I., Martinez, J., *Anal. Chim. Acta* 2012, *732*, 83-90.
[27] van't Slot, G., Humpf, H. U., *J. Agric. Food. Chem.* 2009, *57*, 8041-8048.
[28] Zhang, K., Zuo, Y., *J. Agric. Food. Chem.* 2004, *52*, 222-227.
[29] Ávila, M., Hidalgo, M., Sánchez-Moreno, C., Pelaez, C., Requena, T., Pascual-Teresa, S. d., *Food Res. Int.* 2009, *42*, 1453-1461.
[30] Williamson, G., Clifford, M. N., *Br. J. Nutr.* 2010, *104 Suppl 3*, S48-66.
[31] Chiva-Blanch, G., Urpi-Sarda, M., Rotches-Ribalta, M., Zamora-Ros, R., Llorach, R., Lamuela-Raventos, R. M., Estruch, R., Andres-Lacueva, C., *J. Chromatogr. A* 2011, *1218*, 698-705.
[32] Scalbert, A., Brennan, L., Manach, C., Andres-Lacueva, C., Dragsted, L. O., Draper, J., Rappaport, S. M., van der Hooft, J. J., Wishart, D. S., *Am. J. Clin. Nutr.* 2014, *99*, 1286-1308.
[33] Mennen, L. I., Sapinho, D., Ito, H., Bertrais, S., Galan, P., Hercberg, S., Scalbert, A., *Br. J. Nutr.* 2006, *96*, 191-198.
[34] Gao, S., Zhan, Q., Li, J., Yang, Q., Li, X., Chen, W., Sun, L., *Biomed. Chromatogr.* 2010, *24*, 472-478.
[35] Meng, X., Sang, S., Zhu, N., Lu, H., Sheng, S., Lee, M. J., Ho, C. T., Yang, C. S., *Chem. Res. Toxicol.* 2002, *15*, 1042-1050.
[36] Bartolome, B., Monagas, M., Garrido, I., Gomez-Cordoves, C., Martin-Alvarez, P. J., Lebron-Aguilar, R., Urpi-Sarda, M., Llorach, R., Andres-Lacueva, C., *Arch. Biochem. Biophys.* 2010, *501*, 124-133.
[37] Aubertin-Leheudre, M., Koskela, A., Marjamaa, A., Adlercreutz, H., *Cancer Epidemiol. Biomarkers Prev.* 2008, *17*, 2244-2248.
[38] Urpi-Sarda, M., Chiva-Blanch, G., Andres-Lacueva, C., Estruch, R., *Am. J. Clin. Nutr.* 2012, *95*, 1497-1498.
[39] Noroozi, M., Burns, J., Crozier, A., Kelly, I. E., Lean, M. E., *Eur. J. Clin. Nutr.* 2000, *54*, 143-149.
[40] Ritchie, M. R., Morton, M. S., Deighton, N., Blake, A., Cummings, J. H., *Br. J. Nutr.* 2004, *91*, 447-457.
[41] Stumpf, K., Adlercreutz, H., *Clin. Chem.* 2003, *49*, 178-181.

TABLE LEGENDS

Table 1. Urinary concentrations of metabolites in 36 subjects at baseline and after the three intervention periods.

Table 2. Fasting plasma concentrations of metabolites in 33 subjects at baseline and after the three intervention periods.

Table 3. Urine and plasma metabolites in hydrolyzed and non-hydrolyzed samples selected by the stepwise logistic regression model for discriminating wine consumers obtained from the training set.

Table 4. Threshold (cut-off), sensitivity, specificity, AUC and confidence interval of phenolic metabolite group biomarkers and the prediction model.

Table 5. Spearman's correlations between fasting plasma and 24-h urine samples for individual, phenolic metabolite groups and the prediction model.

Table 1. Urinary concentrations of phenolic metabolites in 36 subjects at baseline and after the three intervention periods.[a]

Metabolites	MRM	Urine samples (µmol, 24-h) [b]				
		BAS	RW	DRW	GIN	*P* [c]
Hydroxybenzoic acids						
4-Hydroxybenzoic acid	137/93	25.79±2.21[a]	29.84±3.52[a,b]	34.30±2.81[b]	27.07±2.21[a]	0.006
3-Hydroxybenzoic acid	137/93	3.77±1.27[a,b]	4.11±0.89[a,b]	5.67±1.57[b]	2.97±0.9[a]	0.001
2,4-Dihydroxybenzoic acid	153/109	1.57±0.17[a]	2.47±0.35[b]	2.67±0.37[b]	1.62±0.22[a]	<0.001
2,6-Dihydroxybenzoic acid	153/109	6.19±0.6[a]	8.35±0.91[b]	8.74±0.88[b]	6.08±0.59[a]	<0.001
2,5-Dihydroxybenzoic acid	153/109	16.23±1.65[a]	24.79±2.91[b]	27.29±2.9[b]	17.2±2.2[a]	<0.001
3,5-Dihydroxybenzoic acid	153/109	3.93±0.66[a,c]	6.41±1.01[a,b]	7.57±1.26[b]	3.97±0.72[c]	0.006
Protocatechuic acid	153/109	12.10±1.15	13.07±1.27	14.45±1.66	11.29±1.19	0.09
Syringic acid	197/121	0.73±0.15[a]	1.91±0.43[b]	2.03±0.32[b]	0.70±0.17[a]	<0.001
4-Hydroxyhippuric acid	194/100	54.05±5.42	58.83±4.47	72.13±9.02	53.63±5.69	0.09
3-Hydroxyhippuric acid	194/150	192.30±39.81	204.09±38.07	237.58±54.21	169.25±34.57	0.19
Gallic acid metabolites						
Gallic acid	169/125	0.85±0.18[a]	5.61±0.49[b]	4.76±0.53[b]	0.73±0.17[a]	<0.001
Methylgallic acid [e]	167/108	2.97±0.42[a]	4.37±0.62[b]	4.76±0.68[b]	3.03±0.41[a]	<0.001
Methylgallic sulfate[d,e]	263/183	2.97±0.74[a]	24.8±5.64[b]	19.94±3.08[b]	2.00±0.60[a]	<0.001
Ethylgallate metabolites						
Ethylgallate	197/169	1.06±0.37[a]	8.19±0.93[b]	4.97±0.73[c]	0.22±0.09[a]	<0.001
Ethylgallate sulfate [d,e]	277/197	2.16±0.76[a]	24.18±2.73[b]	15.81±1.64[c]	0.36±0.14[a]	<0.001
Ethylgallate glucuronide 1 [d,e]	373/197	36.73±6.01[a]	176.89±20.38[b]	114.52±10.77[b]	31.49±5.43[a]	<0.001
Ethylgallate glucuronide 2 [d,e]	373/197	101.74±22.4[a]	366.5±37.6[b]	240.9±24.23[c]	64.5±5.75[a]	<0.001
Hydroxyphenylacetic acids						
Phenylacetic acid	135/91	22.15±2.21[a,b]	25.49±2.40[a,b]	27.66±3.00[a]	21.31±2.17[b]	0.005
3-Hydroxyphenylacetic acid	151/107	24.72±3.50[a]	52.27±6.76[b]	56.57±6.9[b]	19.74±2.51[a]	<0.001
2-Hydroxyphenylacetic acid	151/107	5.89±0.40[a,b]	6.48±0.55[a,b]	7.41±0.54[b]	5.76±0.49[a]	0.008
3,4-Dihydroxyphenylacetic acid	167/123	1.61±0.17[a]	1.98±0.17[a,b]	2.37±0.24[b]	2.12±0.32[a,b]	0.026
Homovanillic acid	181/137	164.35±13.99	185.49±21.12	215.13±25.55	166.92±23.28	0.09
Hydroxycinnamic acids						
m-Coumaric acid	163/119	0.54±0.09[a,b]	0.86±0.20[a]	0.83±0.20[a,b]	0.40±0.06[b]	0.005
p-Coumaric acid	163/119	0.64±0.07[a]	1.75±0.35[b]	1.48±0.15[b]	0.55±0.08[a]	<0.001

o-Coumaric acid	163/119	0.07±0.02	0.11±0.05	0.10±0.03	0.13±0.03	0.19
Caffeic acid	179/135	5.42±0.34[a]	5.84±0.47[a,b]	7.05±0.55[b]	4.83±0.45[a]	<0.001
Ferulic acid	193/134	11.80±0.98[a]	15.7±1.79[a,b]	15.25±0.94[b]	11.16±0.83[a]	0.002
Sinapic acid	223/164	0.99±0.18	1.25±0.19	1.43±0.2	1.18±0.26	0.091
Hydroxyphenylpropionic acids						
3-(3-Hydroxyphenyl) propionic acid	165/121	6.22±1.09[a]	7.13±1.26[a,b]	10.07±2.05[b]	4.70±0.87a	<0.001
3-(4-Hydroxyphenyl)propionic acid	165/121	287.44±27.16	371.63±45.16	389.2±39.36	313.3±36.76	0.06
Dihydrocaffeic acid	181/137	14.09±1.39[a,b]	16.22±1.75[a,b]	17.29±1.50[b]	12.87±1.51[a]	0.018
Flavan-3-ols [e]						
∑(Epi)catechin glucuronides[d]	465/289	9.42±1.58[a]	24.15±3.20[b]	26.61±5.39[b]	6.72±1.87[a]	<0.001
∑ (Epi)catechin sulfates[d]	369/289	3.04±0.49[a]	10.17±1.44[b]	10.69±1.66[b]	2.50±0.44[a]	<0.001
∑ Methyl(epi)catechin glucuronides[d]	479/303	3.76±0.93[a]	15.95±2.41[b]	13.84±2.74[b]	3.32±0.84[a]	<0.001
∑ Methyl(epi)catechin sulfates[d]	383/303	10.87±1.94[a]	25.27±2.92[b]	25.64±3.39[b]	7.33±1.9[a]	<0.001
Glycinates						
Vanilloylglycine	224/180	0.80±0.09[a]	1.41±0.26[b]	1.31±0.16[a,b]	0.80±0.13[a]	0.001
Feruloylglycine	250/100	9.23±1.05	11.27±1.38	11.24±1.31	8.88±1.35	0.14
Hydroxyphenylvalerolactones [e]						
DHPV 1	207/163	6.73±1.21[a]	13.80±2.78[b]	13.61±2.68[b]	3.67±0.77[a]	<0.001
DHPV 2	207/163	18.50±3.67[a]	34.20±5.59[b]	37.04±4.31[b]	7.80±1.97[c]	<0.001
∑ DHPV glucuronides [d]	383/207	70.62±14.54[a]	157.76±27.06[b]	177.49±24.62[b]	36.52±8.84[a]	<0.001
∑ DHPV sulfates[d]	287/207	527.13±55.87[a]	876.9±101.81[b]	913.43±114.12[b]	418.39±71.73[a]	<0.001
MHPV	221/162	ND	ND	ND	ND	
MHPV glucuronide [d]	397/221	23.81±4.43[a,c]	37.36±6.57[a,b]	38.43±7.08[b]	20.24±4.19[c]	<0.001
∑MHPV sulfates [d]	301/221	32.29±5.52[a,b]	38.42±6.12[a,b]	43.13±6.52[b]	24.9±4.26[a]	0.006
Stilbenes [d,f]						
Resveratrol Biomarker [g]	-	692.21±208.33[a]	5352.45±661.99[b]	5824.25±722.19[b]	238.00±84.61[a]	<0.001
∑Resveratrol Microbial Metabolites	-	506.39±107.97[a]	4208.95±430.76[b]	5230.62±508.44[b]	283.86±76.23[a]	<0.001
∑Total resveratrol metabolites	-	811.54±211.27[a]	6282.25±770.39[b]	7090.29±822.66[b]	306.08±90.44[a]	<0.001
Other polyphenols						
Enterolactone	297/254	8.73±1.10[a]	11.4±2.19[a,b]	14.81±3.40[b]	7.82±0.94[a]	0.001
Pyrogallol	125/69	1.96±0.43[a]	8.00±1.19[b]	8.08±1.78[b]	2.99±0.58[a]	<0.001

[a] BAS, baseline; DRW, dealcoholized red wine; DHPV, dihydroxyphenyl-γ-valerolactone; MHPV, methoxyhydroxyphenyl-γ-valerolactone; MRM, Multiple Reaction Monitoring; RW, red wine.

[b] Results are expressed as mean ± SEM.
[c] Changes in variables were determined by using the ANOVA analysis for repeated measures. Means in a row with different superscript letters are significantly different, $P < 0.05$ (Bonferroni post hoc test).
[d] Metabolites determined in non-hydrolyzed samples.
[e] Identification of metabolites described previously by Boto-Ordoñez et al.[16]
[f] Data obtained from a previous study by Rotches-Ribalta et al.[15]
[g] Resveratrol Biomarker described by Zamora-Ros et al.[12].

Table 2. Fasting plasma concentrations of metabolites in 33 subjects at baseline and after the three intervention periods.[a]

Metabolites	MRM	Plasma samples (μmol/L) [b]				
		BAS	RW	DRW	GIN	*P* [c]
Hydroxybenzoic acids						
4-Hydroxybenzoic acid	137/93	3.26±0.14	3.47±0.12	3.44±0.09	3.02±0.17	0.22
3-Hydroxybenzoic acid	137/93	ND	ND	ND	ND	
2,4-Dihydroxybenzoic acid	153/109	ND	ND	ND	ND	
2,6-Dihydroxybenzoic acid	153/109	0.08±0.01	0.10±0.01	0.10±0.01	0.08±0.01	0.38
2,5-Dihydroxybenzoic acid	153/109	0.04±0.01	0.05±0.01	0.07±0.02	0.03±0.01	0.08
3,5-Dihydroxybenzoic acid	153/109	ND	ND	ND	ND	
Protocatechuic acid	153/109	1.74±0.06	1.74±0.08	1.86±0.08	1.69±0.07	0.73
Syringic acid	197/121	ND	ND	ND	ND	
4-Hydroxyhippuric acid	194/100	0.17±0.02	0.16±0.02	0.17±0.02	0.15±0.01	0.85
3-Hydroxyhippuric acid	194/150	0.85±0.53	1.03±0.45	1.21±0.62	0.69±0.32	0.89
Gallic acid metabolites						
Gallic acid	169/125	0.02±0.001[a]	0.04±0.005[b]	0.03±0.002[a,b]	0.02±0.002[a]	<0.001
Methylgallic acid [e]	167/108	0.04±0.004[a]	0.07±0.02[b]	0.06±0.01[a,b]	0.04±0.01[a]	0.037
Methylgallic sulfate [d,e]	263/183	0.002±0.0004[a]	0.02±0.004[b]	0.01±0.002[b]	0.001±0.0003[a]	<0.001
Ethylgallate metabolites						
Ethylgallate	197/169	0.03±0.01	0.04±0.01	0.09±0.06	0.03±0.02	0.93
Ethylgallate sulfate [d,e]	277/197	ND	ND	ND	ND	
Ethylgallate glucuronide 1 [d,e]	373/197	0.10±0.018.9	0.36±0.21	0.12±0.03	0.17±0.07	0.16
Ethylgallate glucuronide 2 [d,e]	373/197	0.09±0.011.63	0.11±0.02	0.10±0.01	0.09±0.02	0.45
Hydroxyphenylacetic acids						
Phenylacetic acid	135/91	0.37±0.03	0.38±0.03	0.38±0.03	0.36±0.04	0.76
3-Hydroxyphenylacetic acid	151/107	0.22±0.03[a]	0.38±0.06[b,c]	0.40±0.04[b]	0.24±0.04[a,c]	0.002
2-Hydroxyphenylacetic acid	151/107	0.08±0.01	0.10±0.01	0.10±0.01	0.08±0.01	0.33
3,4-Dihydroxyphenylacetic acid	167/123	0.03±0.01	0.03±0.01	0.03±0.01	0.02±0.01	0.80
Homovanillic acid	181/137	ND	ND	ND	ND	
Hydroxycinnamic acids						
p-Coumaric acid	163/119	0.005±0.002[a]	0.02±0.003[b]	0.02±0.003[b]	0.02±0.003[b]	<0.001

m-Coumaric acid	163/119	ND	ND	ND	ND	
o-Coumaric acid	163/119	ND	ND	ND	ND	
Caffeic acid	179/135	0.09±0.01	0.09±0.01	0.11±0.01	0.08±0.01	0.19
Ferulic acid	193/134	1.63±0.06	1.65±0.06	1.69±0.06	1.55±0.04	0.56
Sinapic acid	223/164	ND	ND	ND	ND	
Hydroxyphenylpropanoic acids						
3-(4-Hydroxyphenyl)propionic acid	165/121	3.34±0.17	3.44±0.23	3.54±0.17	3.05±0.24	0.30
3-(3-Hydroxyphenyl)propionic acid	165/121	0.21±0.05	0.21±0.05	0.29±0.06	0.15±0.04	0.14
Dihydrocaffeic acid	181/137	ND	ND	ND	ND	
Flavan-3-ols [e]						
(Epi)catechin glucuronide[4]	465/289	0.02±0.01[a]	0.05±0.01[b]	0.04±0.01[b]	0.02±0.01[a]	<0.001
Methyl(epi)catechin glucuronide[4]	479/303	0.01±0.001[a,c]	0.03±0.01[b]	0.02±0.01[a,b]	0.01±0.001[c]	0.004
Glycynates						
Vanilloylglycine	224/180	0.03±0.001	0.03±0.001	0.03±0.001	0.03±0.001	0.24
Feruloylglycine	250/100	0.08±0.002	0.09±0.003	0.09±0.003	0.08±0.003	0.44
Hydroxyphenylvalerolactones [e]						
DHPV 1	207/163	0.07±0.02[a,c]	0.16±0.03[b]	0.10±0.02[a,b]	0.04±0.02[c]	<0.001
DHPV 2	207/163	0.17±0.04[a,c]	0.45±0.1[b]	0.29±0.06[a,b]	0.10±0.03[c]	<0.001
∑ DHPV glucuronides[d]	383/207	0.18±0.05[a,b]	0.46±0.13[b]	0.29±0.06[a,b]	0.14±0.08[a]	<0.001
∑ DHPV sulfates [d]	287/207	ND	ND	ND	ND	
MPHV	221/162	ND	ND	ND	ND	
MHPV glucuronide [d]	397/221	ND	ND	ND	ND	
∑ MPHV sulfates [d]	301/221	ND	ND	ND	ND	
Other polyphenols						
Enterolactone	297/254	0.01±0.002	0.01±0.002	0.02±0.01	0.01±0.002	0.18
Pyrogallol	125/69	ND	ND	ND	ND	

[a] BAS, baseline; DRW, dealcoholized red wine; DHPV, dihydroxyphenyl-c-valerolactone; MHPV, Methoxy-hydroxyphenyl-valerolactone; MRM, Multiple Reaction Monitoring; ND, no detected; RW, red wine. [b] Results are expressed as mean ± SEM. [c] Changes in variables were determined by using the ANOVA analysis for repeated measures. Means in a row with different superscript letters are significantly different, $P < 0.05$ (Bonferroni post hoc test). [d] Metabolites determined in non-hydrolyzed samples. [e] Identification of metabolites described previously by Boto-Ordoñez et al. [16]

Table 3. Urine and plasma metabolites in hydrolyzed and non-hydrolyzed samples selected by the stepwise logistic regression model for discriminating wine consumers obtained from the training set

	Coefficient	**Standard error**	***p* value**	**Coefficient CI 95%**
URINE				
Hydrolyzed samples				
2,4-Dihydroxybenzoic Acid	0.60	0.23	0.007	0.16, 1.05
Gallic Acid	0.84	0.24	<0.001	0.38, 1.31
Ethylgallate	0.48	0.18	0.009	0.12, 0.83
Constant	-4.47	0.94	<0.001	-6.31, -2.63
Non-Hydrolyzed samples				
Methylgallic Acid Sulfate	0.17	0.06	0.005	0.05, 0.29
Ethylgallate Sulfate	0.41	0.10	<0.001	0.21, 0.62
Constant	-4.19	0.91	<0.001	-5.98, -2.41
PLASMA				
Hydrolyzed samples				
3-Hydroxyphenylacetic Acid	2.38	1.02	0.020	0.39, 4.38
Gallic Acid	62.21	22.41	0.006	18.29, 106.12
p-Coumaric Acid	40.91	15.43	0.008	10.67, 71.14
Constant	-3.09	0.79	<0.001	-4.64, -1.54
Non-Hydrolyzed samples				
Methylgallic Acid Sulfate	525.00	118.68	<0.001	292.39, 757.61
Constant	-1.63	0.35	<0.001	-2.32, -0.94

Table 4. Threshold (cut-off), sensitivity, specificity, AUC and confidence interval of phenolic metabolite group biomarkers and the prediction model

	Threshold [a]	Sensitivity (%)	Specificity (%)	AUC (%)	AUC 95% CI (%)
URINE					
Hydrolyzed samples					
Gallic Acid Metabolites	4.89	88.89	77.80	87.75	81.97, 93.53
DHPV Metabolites	18.17	83.33	66.70	81.15	74.14, 88.17
Phenolic Acid Metabolites	207.2	69.44	62.50	71.95	64.03, 80.34
Ethylgallate	0.69	93.06	84.72	92.35	87.73, 96.97
Training Set		94.74	91.23	96.24	92.73, 99.76
Validation Set		93.33	93.33	96.00	89.24, 100.0
All population		**91.66**	**91.66**	**96.14**	**93.12, 99.16**
Non-hydrolyzed samples					
(Epi)catechin Metabolites	39.84	75.00	83.33	86.32	80.38, 92.27
DHPV Metabolites	695.53	66.70	73.60	76.33	68.70, 83.96
Ethylgallate Metabolites	152.31	98.60	84.70	93.67	89.49, 97.86
Methylgallic Sulfate	5.49	87.50	86.11	93.23	89.31, 97.15
Resveratrol Biomarker	1966.05	91.67	95.83	96.45	93.38, 99.52
Resveratrol Microbial Metabolites	1424.19	95.83	93.06	98.77	97.46, 100.0
Training Set		94.74	96.49	98.68	97.13, 100.0
Validation Set		100.0	93.33	96.44	89.32, 100.0
All population		**95.83**	**94.44**	**98.40**	**96.80, 100.0**
PLASMA					
Hydrolyzed samples					
Gallic Acid Metabolites	0.06	68.18	57.58	64.10	54.66, 73.53
DHPV Metabolites	0.14	68.18	62.12	68.37	59.31, 77.42
Phenolic Acid Metabolites	0.24	72.73	65.15	68.02	58.88, 77.16
Training Set		74.07	76.92	80.13	71.75, 88.51
Validation Set		75.00	100.0	88.10	74.20, 100.0
All population		**75.76**	**74.24**	**81.18**	**73.86, 88.49**
Non-hydrolyzed samples					
(Epi)catechin Metabolites	0.03	69.70	75.76	76.92	68.76, 85.07
DHPV Metabolites	0.06	72.73	62.12	71.12	62.37, 79.87
Methylgallic Sulfate	0.002	84.85	77.27	87.50	81.65, 93.35
Training Set		85.19	76.92	86.89	80.13, 93.66
Validation Set		91.67	78.57	91.07	80.22, 100.0
All population		**84.85**	**77.27**	**87.50**	**81.65, 93.35**

DHPV, dihydroxyphenyl-γ-valerolactone; ROC, receiver operating characteristic.
[a] Urine (μmol/24-h) or plasma (μmol/L).

Table 5. Spearman's correlations between fasting plasma and 24-h urine samples for individual, phenolic metabolite groups and the prediction model

	R	*P*
Hydrolyzed samples		
Gallic acid	0.338	<0.001
3-Methylgallic acid	0.441	<0.001
Gallic acid group	0.451	<0.001
2,5-Dihydroxybenzoic acid	0.411	<0.001
Protocatechuic acid	0.174	0.046
3-(3-Hydroxyphenyl)propionic acid	0.402	<0.001
Ferulic acid	0.253	0.003
4-Hydroxyhippuric acid	0.310	<0.001
3-Hydroxyphenylacetic acid	0.444	<0.001
2-Hydroxyphenylacetic acid	0.204	0.019
Enterolactone	0.503	<0.001
p-Coumaric acid	0.370	<0.001
Phenolic acid group	0.442	<0.001
DHPV1	0.321	<0.001
DHPV2	0.336	<0.001
DHPV group	0.348	<0.001
Prediction model score	0.565	<0.001
Non-hydrolyzed samples		
Methylgallic acid sulfate	0.587	<0.001
(Epi)catechin glucuronide 3	0.342	<0.001
Methyl (epi)catechin glucuronide 2	0.294	0.001
Flavan-3-ol group	0.382	<0.001
DHPV1 Glucuronide	0.321	<0.001
DHPV2 Glucuronide	0.342	<0.001
DHPV Group	0.356	<0.001
Prediction model score	0.599	<0.001