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White Wine Phenolics Are Absorbed and Extensively Metabolized in Humans

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Despite the vast literature describing the biological effects of phenolic compounds, rather scarce data are available on their absorption from diet in humans. The present study focused on the absorption in humans of phenolic acids from white wine, particularly hydroxycinnamic acids and their esters with tartaric acid. The results obtained indicate that, following a single wine drink, hydroxycinnamic acids from white wine are absorbed from the gastrointestinal tract and circulate in the blood after being largely metabolized to the form of glucuronide and sulfate conjugates. Unmodified tartaric acid esters of hydroxycinnamic acids from wine are present in human plasma at low levels, if any. Wine hydroxycinnamic acids, although present in wine as conjugated forms, are still bioavailable to humans.

KEYWORDS: White wine; human plasma; phenolic acids; glucuronides; sulfates; caffeic acid; ferulic acid; *p*-coumaric acid; caftaric acid; fertaric acid; coutaric acid

INTRODUCTION

Oxidative stress is involved in the pathology of many diseases, such as atherosclerosis, diabetes, neurodegenerative diseases, aging, and cancer. Dietary antioxidants may afford protection against oxidative stress-related diseases.

Beverages account for a very high proportion of dietary antioxidant intake in the Mediterranean diet (*1*). Coffee is the main contributor, followed by wine, fruit juice, beer, tea, and milk.

Among dietary antioxidants, phenolics are by far the most abundant in most diets. Epidemiological studies have suggested associations between the consumption of phenolic-rich foods and the prevention of many human diseases associated with oxidative stress (*2*−*4*). On the basis of their daily intake, which greatly exceeds that of other antioxidants (vitamin E, vitamin C, β-carotene), phenolic compounds may be a major factor in assuring the antioxidant potential of the diet and may contribute in maintaining the endogenous redox balance in humans. A major class of phenolic compounds are phenolic acids, which are widely distributed in the diet, mostly in fruits, vegetables, coffee, wine, beer, and olive oil (*5*, *6*). They occur in food mainly in esterified forms with organic acids, sugars, and lipids. The average phenolic acid intake has been estimated to be on the order of 200 mg/day within a large range, depending on nutritional habits and preferences (*2*, *5*). Phenolic acid intake ranging from 6 to 987 mg/day has also been reported (*7*). For individuals regularly consuming wine, coffee, beer, and tea, these beverages will likely be the major sources of phenolics. Persons who drink coffee may ingest as much as 500−800 per day of hydroxycinnamic acids (*5*). However, the significance of this intake for metabolic effects and antioxidant status in vivo is affected by the bioavailability of these compounds.

Recently, some research has been done about the absorption of phenolic compounds (*8*), especially in rats. However, plasma levels of phenolic acids following phenolic-rich foods administration in humans are reported by a limited number of studies, as well as data on intestinal absorption. Indirect evidence of phenolics absorption through the gut barrier is the increase in plasma antioxidant capacity after the consumption of phenolic-rich foods. This has been observed for a wide array of foods such as red wine (*9*, *10*), beer (*11*), and coffee (*12*). Indeed, knowledge of the effective in vivo reachable concentrations is crucial to understand the significance of phenolic acid intake on human health. Moreover, phenolic acids undergo conjugation reactions in vivo with sulfate, glucuronate, *S*-adenosylmethionine, or a combination (*2*). Conjugation can dramatically alter the biological properties of the parent phenolic compounds (*13*−*15*). In this regard, a very limited number of human studies have been carried out in which the nature of the conjugates has been established.

Wine is a common beverage consumed at meals in Mediterranean countries. Mild to moderate wine consumption has been associated with beneficial healthy effects on the cardiovascular system (*16*, *17*). These beneficial effects have been related to both ethanol and polyphenols contents of wine. As widely described in recent literature, polyphenols are involved in the cardioprotective effect of wine (*18*−*21*). Ethanol, in addition

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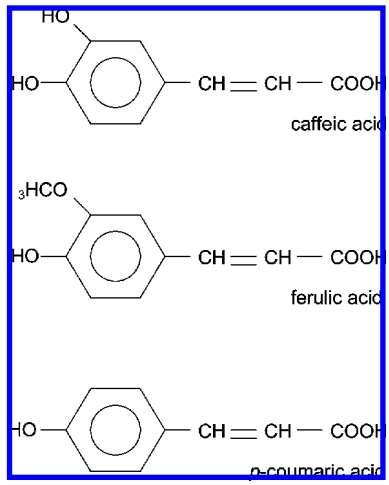

Figure 1. Chemical structures of the main hydroxycinnamic acids: caffeic acid, 3,4-dihydroxycinnamic acid; ferulic acid, 4-hydroxy-3-methoxycinnamic acid; *p*-coumaric acid, 4-hydroxycinnamic acid.

to its direct effect on platelet aggregation, HDL metabolism, and fibrinolysis (*22*), could play an important role also in the absorption of phenolic compounds (*23*).

However, data on the in vivo absorption of specific phenolic compounds of wine are rather scarce, with few studies carried out on rats (*24*, *25*) and humans (*26*−*33*). Microbial metabolites of wine polyphenols were identified in the urine of rats fed red wine polyphenols (*24*). Low molecular weight polyphenols from red wine are absorbed in the rat intestine and metabolized to their glucuronide conjugates (*25*). Among human studies, Pignatelli et al. (*26*) demonstrated that low molecular weight polyphenols (caffeic acid, resveratrol, cathechin) from red and white wine circulated in human plasma after 15 days of wine consumption. Dose-dependent concentration of plasma caffeic acid was also demonstrated in humans after red wine drinking (*31*).

Wine phenolics include both flavonoid and non-flavonoid compounds. Hydroxycinnamic acids (caffeic, ferulic, and *p*-coumaric acids) (**Figure 1**) and their tartaric esters (caftaric, fertaric, and coutaric acids) are the main class of non-flavonoid phenolics in red wines and the main class of phenolics in white wines (*34*).

Only a few studies specifically investigated the absorption and metabolism of phenolic compounds from white wine (*26*−*28*) and just one study accurately measured selected plasma phenolic compounds by HPLC-ECD (*26*).

In the present study we investigated the absorption in humans, after a single dose of white wine, of several hydroxycinnamic acids with related chemical structures and their derivatives with tartaric acid, focusing on the measurements of plasma levels of free (nonconjugated) and conjugated forms of hydroxycinnamic acids.

MATERIALS AND METHODS

Materials. β-Glucuronidase (EC 3.2.1.31, type IX A from *Escherichia coli*), sulfatase (type H-1 from *Helix pomatia*, containing β-glucuronidase), vanillic acid, caffeic acid, *p*-coumaric acid, ferulic acid, and isoferulic acid were from Sigma (St. Louis, MO). *o*-Coumaric acid was from Extrasynthese (Genay Cedex, France). Caftaric, fertaric, and coutaric acids were isolated from grape as reported below. Supelclean LC-SAX SPE cartridges (1 mL tubes) were from Supelco (Bellefonte, PA). All organic solvents were obtained from Carlo Erba (Milano, Italy). For HPLC analysis, ultrapure water from a Milli-Q system (Millipore, Bedford, MA) was used.

Stock solutions of standard phenolic acids were prepared in methanol (1 mg/mL), stored at −80 °C, and used within 1 week. Working standard solutions were prepared daily by dilution in sample buffer (1.25% glacial acetic acid, 7% methanol in water).

Extraction of Hydroxycinnamic Acids. Partially unripe grape berries grown in the vineyard of the Edmund Mach Foundation (*Vitis vinifera* cv. Rhine Riesling, 40 kg) were pressed in the presence of ascorbic acid (1 g/kg) and sodium metabisulfite (1 g/kg). The juice (27 L) was treated with bentonite (1 g/L, 12 h at 4 °C) and centrifuged (4000 rpm, 15 min) to remove proteins. Hydroxycinnamic acids were then adsorbed by stirring for 1 h with activated carbon and diatomaceous earth (each 10 g/L) following the method of Singleton (*35*). The adsorbent was washed out with water (3 × 1 L), whereas hydroxycinnamic acids were eluted with (3 × 500 mL) methanol/acetic acid (99:1), brought to dryness in a rotary evaporator, and redissolved in 400 mL of methanol. The raw methanolic extract was enriched in the grape hydroxycinnamic acids, mainly 83.4% *trans*-caftaric acid with a lower amount of *trans*-fertaric (9.1%) and *trans*-*p*-coutaric acid (7,5%). It was shielded all of the time from direct light, to avoid trans−cis isomerization.

Purification of Hydroxycinnamic Acids. The purification of the three hydroxycinnamic acids from all other major constituents was obtained with a single step by flash chromatography on TSK gel Toyopearl HW-40S (Tosoh Corp., Tokyo, Japan). The preparative flash column (37 mm diameter, 150 mL capacity) was preconditioned with acidic methanol (0.5% acetic acid) and acidic water (0.5% acetic acid). An aliquot of 20 mL of methanolic extract was brought to dryness, redissolved in 3 mL of acidic water (0.5% acetic acid), loaded on the column, and eluted with the mixture (1:5) of methanol and acidified water (0.5% acetic acid). The fractions containing the purified hydroxycinnamates, eluting from 500 to 800 mL, were collected, brought to dryness, redissolved in 40 mL of diethyl ether, stabilized with copper gauze and crystallized with the slow addition of 200 mL of *n*-hexane. Diethyl ether was removed by rotavapor; the remaining solution was cooled at 4 °C and filtered on a Buchner funnel with a GVWP filter (0.22 μm), leading to a purified grape hydroxycinnamic acid mixture. The crystals were immediately put in desiccator under vacuum, because they are highly hygroscopic. The recoveries from grape were estimated to be 73.7, 69.2, and 34.5% for *trans*-caftaric acid, *trans*-*p*-coutaric acid, and *trans*-fertaric acid, respectively.

The final isolation of each compound from the purified hydroxycinnamic acids fraction was obtained by reversed-phase preparative HPLC with a Shimadzu SCL-10 AVP equipped with a Shimadzu SPD-10 AVP UV−vis detector, 8A pumps, and Class VP software (Shimadzu Corp., Kyoto, Japan). Each isolated acid was dissolved in diethyl ether and crystallized with *n*-hexane, as described above.

The purity of the standards used for the study was checked by UV spectroscopy. Two solutions in MeOH were prepared containing between 5 and 20 mg/L of each compound, thus obtaining final concentrations in the range of (3.3−7.6) × 10^{-5} mol/L. The spectral characteristics of the known solutions were recorded on a Hitachi U-2000 UV−vis spectrophotometer using quartz cells with a 10 mm optical path. The average experimental values of the molar absorptivities of each standard, measured in MeOH both at λ_{max} and at the wavelength used for the HPLC analysis (320 nm) were obtained: UV $\lambda_{max,MeOH}$ (log ε) *trans*-caftaric acid, 330.5 (4.28), *trans*-*p*-coutaric acid, 314.0 (4.40), *trans*-fertaric acid, 326.5 (4.29), *trans*-caffeic acid, 323.0 (4.21), *trans*-*p*-coumaric acid, 308.0 (4.28) and *trans*-ferulic acid, 319.0 (4.24); UV $\lambda_{320,MeOH}$ (log ε) *trans*-caftaric acid (4.23), *trans*-*p*-coutaric acid (4.37), *trans*-fertaric acid (4.26), *trans*-caffeic acid (4.20), *trans*-*p*-coumaric acid (4.14), and *trans*-ferulic acid (4.24).

HPLC Instrumentation. Phenolic acids in food and human plasma extracts are routinely detected in our laboratory by HPLC-ECD (*36*−*38*). The HPLC consists of a Perkin-Elmer Series 4 liquid chromatograph (Perkin-Elmer, Norwalk, CT) with a gradient pump, column thermoregulator, and autosampling injector (Gilson, Beltline, Middleton, WI) equipped with electrochemical coulometric detector (Coulochem II, ESA, Bedford, MA). Operating conditions were as follows: column temperature, 30 °C; flow rate, 1 mL/min; injection volume, 100 μL; electrochemical detection at +600 mV; sensitivity range, 200 nA; filter, 2 s.

For the detection of the phenolic acids caffeic acid, ferulic acid, and *p*-coumaric acid the chromatographic separations were performed on a Supelcosil LC-18 column (5.0 μm particle size, 250 × 4.6 mm i.d.) including a guard column (C_{18}, 5.0 μm particle size, 20 × 4.0 mm i.d.; both Supelco, Bellefonte, PA). For gradient elution, mobile phases A and B were employed. Solution A contained 1.25% glacial acetic acid in water, and solution B was absolute methanol. The following gradient was used: 0−35 min, from 98% A, 2% B to 86% A, 14% B, linear gradient; 36−45 min, 86% A, 14% B; 46−65 min, from 86% A, 14% B to 80% A, 20% B, linear gradient; 66−75 min, from 80% A, 20% B to 76% A, 24% B, linear gradient; 76−80 min, from 76% A, 24% B to 55% A, 45% B, linear gradient; 81−83 min, 55% A, 45% B; 84−114 min, 98% A, 2% B.

For the detection of the esters of hydroxycinnamic acids (caftaric acid, coutaric acid, and fertaric acid), the chromatographic separations were performed on a Luna C-18 column (5.0 μm particle size, 250 × 3.0 mm i.d., Phenomenex, Torrance, CA) including a guard column. For gradient elution mobile phases A and B were employed. Solution A contained 0.5% formic acid in water, and solution B was 0.5% formic acid in methanol. The following gradient was used: 0−20 min, from 98% A, 2% B to 80% A, 20% B, linear gradient; 21−40 min, from 80% A, 20% B to 60% A, 40% B, linear gradient; 41−50 min, 60% A, 40% B; 51−80 min, 98% A, 2% B.

Prior to HPLC analysis, all samples were filtered using Millex-HV filters (Millipore, Bedford, MA) with 0.45 μm pore size.

Study Design and Sample Collection. The study was approved by the Ethical Committee of the National Institute for Food and Nutrition Research. Ten healthy subjects (8 males, 2 females) aged between 23 and 49 years, with a body mass index between 18 and 29 kg/m^2, were recruited. Subjects were nonsmokers, either nondrinkers or social drinkers (<28 and <14 g ethanol per day, for males and females, respectively), and they were not taking dietary antioxidant supplements. They were asked to avoid coffee, wine, beer, tea, and fruit juices the day preceding the experiment. Fasting subjects received in the morning 250 mL of white wine in combination with 6.7 g of crackers. Crackers were given to avoid effects of ethanol in fasting conditions. The white wine used in this study was an Italian variety (Greco di Tufo) containing 12.0% ethanol, of the vintage 2005. This autochtonous grape variety, widely cultivated in southern Italy, was selected on the basis of its high content of hydroxycinnamic acids. The grape was cultivated in the vineyards of the Azienda Agricola Di Marzo (Tufo, Avellino, Italy), whereas the wine was produced in the Azienda Agricola Pojer e Sandri (Faedo, Trento, Italy), fermenting the juice extracted from the grape in positive pressure and under nitrogen in a pneumatic press (Vaslin Bucher, France). This technique, now widely used in Italy, was chosen because it is particularly suited for preventing the oxidation processes, thus recovering the maximal amount of intact (non oxidized) grape hydroxycinnamates into the wine (*39*). The young wine was aged for 4 months on its own yeast lees in used, sealed oak barrels (300 L), filtered, bottled, and stored at cellar temperature.

Crackers (wheat flour, brewer's yeast, salt, sodium bicarbonate) were from an Italian brand and did not contain added fat. The content of phenolic acids of crackers was not relevant with respect to the content of phenolic acids of wine, being 0.02% for caffeic acid, 0.1% for *p*-coumaric acid, and 10.1% for ferulic acid of the total ingested phenolic acids. Blood was collected into EDTA-containing vacutainers (1 mg/mL) just before ($t = 0$) and 30 and 60 min after the ingestion of wine plus crackers. Plasma was immediately prepared by centrifugation at 1000*g* for 20 min at 4 °C. Plasma aliquots (0.5 mL) were acidified at pH 3.0 with 4 N HCl and stored at −80 °C.

Wine and Cracker Analyses. Analytical characterization of hydroxycinnamic acids and their derivatives in white wine was obtained by HPLC-VIS (*40*). Total polyphenols were measured as already described (*41*). Crackers were smashed and powdered in a Waring Blendor. Aliquots of 250 mg of powdered crackers were suspended in 1 mL of distilled water and treated for free and total phenolic acids content (*36*).

Treatment of Plasma Samples. Plasma samples were treated for phenolic acid extraction essentially as already described (*38*), with minor modifications concerning the enzymes used to hydrolyze conjugated phenolic acids. Plasma samples (1 mL aliquots) from each subject were thawed and treated according to one of the three following procedures: no hydrolytic treatment, to detect free, nonconjugated phenolic acids; β-glucuronidase plus sulfatase treatment, to hydrolyze glucuronide and sulfate conjugates; and alkaline hydrolysis treatment to hydrolyze conjugated phenolic acids. *o*-Coumaric acid was selected as internal standard due to the absence of detectable amounts of this compound in human plasma samples before and after wine plus crackers administration, with or without hydrolytic treatments.

No Hydrolysis. Plasma sample was added with *o*-coumaric acid (150 ng) and treated as already described for free phenolic acid extraction (*38*). After deproteinization and extraction, the residue was dissolved in 0.5 mL of water, vortexed for 5 min, and then processed for solid phase extraction (SPE) as described below. This procedure allows the detection of free, nonconjugated phenolic acids in plasma sample.

Enzymatic Hydrolysis. Plasma samples were submitted to sequential enzymatic hydrolysis with β-glucuronidase, followed by sulfatase treatment. Briefly, plasma sample was added with *o*-coumaric acid (150 ng) and deproteinized with ethanol as previously described (*38*). The dried residue obtained was suspended in 0.5 mL of 0.2 M potassium phosphate buffer, pH 6.8, added with 50 μL (776 IU) of β-glucuronidase dissolved in the same buffer and incubated at 37 °C for 1 h. At the end of incubation, the pH was brought to 5.0 with 1 N HCl, and sample added with 50 μL sulfatase (containing 70 IU sulfatase and 2325 U of β-glucuronidase). After 1 h of incubation at 37 °C, the pH was brought to 3.0 with 1 N HCl, 300 mg of NaCl was added, and the sample was extracted with ethyl acetate as described (*38*). The final residue was dissolved in 0.5 mL of water, vortexed for 5 min, and then processed for SPE as reported below. This procedure allows the detection of total phenolic acids (nonconjugated, glucuronides, sulfates, and possible mixed sulfate/glucuronide conjugates).

Alkaline Hydrolysis. o-Coumaric acid (150 ng) was added to plasma sample, and the mixture was hydrolyzed for total phenolic acid detection as already described (*38*). After deproteinization and extraction, the residue was dissolved in 0.5 mL of water, vortexed for 5 min, and then processed for SPE as described below. This procedure allows the detection of total (conjugated and nonconjugated) phenolic acids in plasma sample.

Solid Phase Extraction. SPE of treated plasma samples was carried out as follows. The residue dissolved in 0.5 mL of water obtained as above-described was brought to pH 7−8 with 0.1 N NaOH and passed through the LC-SAX tube preconditioned with 1 mL of absolute methanol and 2 mL of water. The tube was then washed with 1 mL of water. Phenolic acid elution was obtained with 1 mL of buffer containing 1 N acetic acid/MeOH (90:10). The eluant was immediately brought to pH 3 with 6 μL of 4 N NaOH and filtered, and an aliquot (50 μL) was injected into the HPLC system.

In a separate set of experiments for the detection of caftaric, fertaric, and coutaric acids in unhydrolyzed samples and in sample subjected to enzymatic hydrolysis, the elution of the LC-SAX tube was performed with 1 mL of buffer containing 1 N formic acid/MeOH (90:10) instead of the acetic acid containing buffer.

Recovery. The overall procedures allow an almost complete recovery of all phenolic acids under study, as found by recovery experiments performed by adding known amounts of pure compounds to plasma samples. In particular, the recovery was in the range from 86.2 ± 8.6 ot 103.8 ± 5.1% for all hydroxycinnamic acids and esters tested (caffeic acid, *p*-coumaric acid, ferulic acid, *o*-coumaric acid, caftaric acid, coutaric acid, and fertaric acid) ($n = 4$).

Data Evaluation, Quantitation, and Statistical Analysis. For the calibration curve, appropriate volumes of the stock standard solutions were diluted with sample buffer. Three replicates of standards at four concentration levels (20, 100, 200, and 400 ng/mL) were analyzed. A calibration curve was determined on each day of analysis. For quantitative determination, peak areas in the sample chromatograms were correlated with the concentrations according to the calibration curve.

Data presented are mean ± standard error. Statistical analysis was performed by repeated measures ANOVA test. A probability of $p < 0.05$ was considered to be statistically significant.

Table 1. Content of Hydroxycinnamic Acids and Their Derivatives in White Wine[a]

compound	mg/L
caftaric acid	164.4
fertaric acid	4.7
coutaric acid	31.0
caffeic acid	2.8
ferulic acid	0.1
p-coumaric acid	0.5
GRP[b]	1.3
total polyphenols[c]	416.0

[a] Hydroxycinnamic acids and their derivatives were measured by HPLC-VIS (*40*). Total polyphenols were measured as previously described (*41*). [b] GRP, *trans*-2-*S*-glutathionylcaftaric acid, expressed as caffeic acid equivalents. [c] Expressed as (+)-catechin equivalents.

RESULTS

Table 1 shows the content of hydroxycinnamic acids and their derivatives in the white wine Greco di Tufo, which was 1 year old at the moment of the in vivo study. In agreement with data from the literature, in white wine hydroxycinnamic acids occur distinctively as conjugates with tartaric acid (*42*). Caftaric acid (the ester of caffeic acid with tartaric acid) is by far the most abundant hydroxycinnamic acid derivative, followed by coutaric and ferulic acids, the esters of, respectively, *p*-coumaric acid and ferulic acid with tartaric acid. The free, unbound forms of hydroxycinnamic acids (caffeic, *p*-coumaric, and ferulic acid) are present at very low levels, if any. Overall, hydroxycinnamic acids and their derivatives, being present at 204.8 mg/L, explained 50% of total polyphenol content of this white wine.

To study the absorption of hydroxycinnamic acids and their derivatives from white wine, plasma samples were collected before and after wine plus crackers administration and analyzed for the content of both free (nonconjugated) and total (nonconjugated plus conjugated) hydroxycinnamic acids. To estimate total phenolic acids, two different procedures were used to release phenolic acids from conjugated forms. In the first procedures, enzymatic hydrolysis with β-glucuronidase and sulfatase was used to selectively hydrolyze glucuronide and sulfate derivatives of hydroxycinnamic acids. In the second procedure, an alkaline hydrolytic treatment was used to liberate hydroxycinnamic acids from bound complexes.

As shown in **Figure 2**, white wine plus crackers administration resulted in a fast increase of the plasma levels of hydroxycinnamic acids. **Figure 2A** shows the levels of caffeic acid before and 30 and 60 min after administration. The plasma levels of nonconjugated, free caffeic acid (no hydrolysis) increased significantly at 30 and 60 min after wine administration (3.3 $\pm$ 0.7 ng/mL plasma at 60 min). Similarly, a 2−3-fold increase in total plasma caffeic acid concentration was observed after either enzymatic or alkaline hydrolysis, at both 30 and 60 min with respect to time 0, with absorption peak at 60 min after wine administration (16.0 $\pm$ 3.8 ng/mL enzymatic hydrolysis, 15.6 $\pm$ 4.0 ng/mL alkaline hydrolysis), indicating that a significant absorption and conjugation of caffeic acid occur after wine administration. Most of the caffeic acid was present in plasma as the conjugated form, the free, nonconjugated form amounting to 22.6, 27.0, and 21.1% of total caffeic acid (measured after enzymatic hydrolysis) at time 0, 30, and 60 min, respectively. The levels of total caffeic acid measured after enzymatic hydrolysis were similar to those obtained after alkaline hydrolysis. **Figure 2B** shows the plasma levels of ferulic acid measured before and 30 and 60 min after white wine plus crackers consumption. A significant increase in both nonconjugated (no hydrolysis) and total ferulic acid (enzymatic or alkaline hydrolysis) was observed 30 and 60 min after administration with respect to time 0. The absorption peak was found at 30 min for total ferulic acid (6.0 $\pm$ 1.8 ng/mL enzymatic

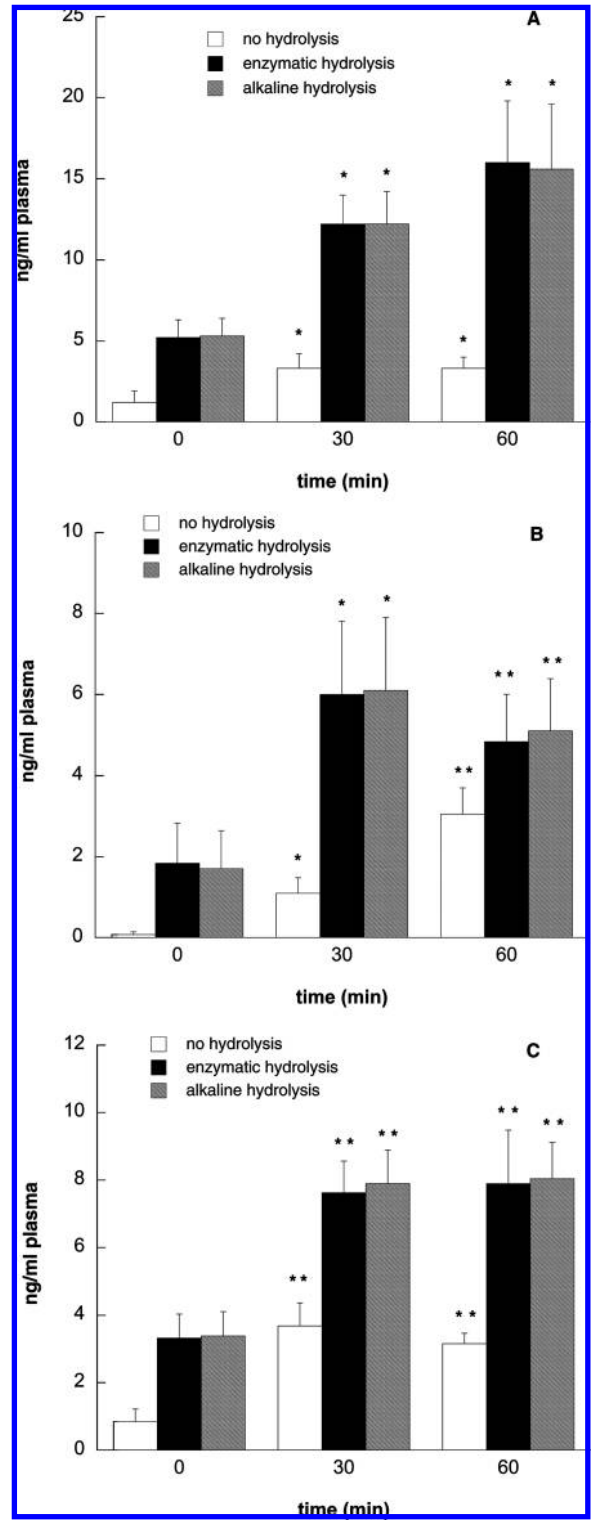

Figure 2. Plasma phenolic acid levels before and after wine plus crackers consumption: **A**, caffeic acid; **B**, ferulic acid; **C**, *p*-coumaric acid. Plasma samples from 10 different subjects separated from blood collected just before (time 0) or after (30 and 60 min) wine plus crackers administration were analyzed for nonconjugated (no hydrolysis) and conjugated (enzymatic and alkaline hydrolysis) phenolic acids as reported under Materials and Methods. Values are means $\pm$ SE; *, $p \leq 0.05$ with respect to time 0 by repeated measures ANOVA test; **, $p \leq 0.01$ with respect to time 0 by repeated measures ANOVA test.

hydrolysis, 6.1 ± 1.8 ng/mL alkaline hydrolysis), whereas nonconjugated ferulic acid peaked at 60 min after administration (3.0 ± 0.6 ng/mL). The bulk of ferulic acid circulated in plasma as the conjugated form, mainly glucuronate and sulfate derivatives, the nonconjugated form amounting to 4.1 and 18.2% of total ferulic acid (measured after enzymatic hydrolysis) before and 30 min after administration, respectively. Nevertheless, a slight prevalence of nonconjugated form with respect to total ferulic acid (measured after enzymatic hydrolysis or alkaline hydrolysis) was observed 60 min after administration. The levels of total ferulic acid measured after enzymatic or alkaline hydrolysis were similar. As shown in **Figure 2C**, the levels of *p*-coumaric acid significantly increased 30 and 60 min after wine plus crackers administration with respect to time 0. *p*-Coumaric acid was present in plasma as both conjugated and nonconjugated forms, the nonconjugated forms amounting to 24.2, 48.7, and 39.2% of total *p*-coumaric acid measured after enzymatic hydrolysis at time 0, 30, and 60 min, respectively. Absorption peak was at 60 min after wine plus crackers administration for total *p*-coumaric acid (7.9 ± 1.6 ng/mL enzymatic hydrolysis, 8.0 ± 1.1 ng/mL alkaline hydrolysis) and at 30 min for free, nonconjugated *p*-coumaric acid (3.7 ± 0.7 ng/mL). No significant differences were observed in total *p*-coumaric acid levels measured with either enzymatic or alkaline hydrolysis, as previously for caffeic and ferulic acids, suggesting that hydroxycinnamic acids circulate in plasma mainly as glucuronate and sulfate derivatives.

Administration of crackers alone did not result in any significant increase of plasma hydroxycinnamic acids levels at both 30 and 60 min after administration with respect to time 0.

To study in depth the absorption of esterified forms of hydroxycinnamic acids from white wine (caftaric acid, fertaric acid, and coutaric acid), we attempted to measure these compounds by HPLC-ECD in plasma samples of five subjects after wine plus crackers administration, both in nonhydrolyzed samples and in sample subjected to enzymatic hydrolysis with β-glucuronidase and sulfatase. We measured fertaric acid in nonhydrolyzed plasma 60 min after wine plus crackers administration in one subject (2.1 ng/mL plasma) and in plasma submitted to enzymatic hydrolysis 60 min after administration in another subject (3.0 ng/mL plasma); in the remaining three subjects fertaric acid was undetectable in our experimental conditions. Caftaric acid was measured in nonhydrolyzed plasma samples 30 min after wine plus crackers administration in one subject (2.9 ng/mL plasma) and 60 min after administration in another subject (9.3 ng/mL plasma). Similar values of plasma caftaric acid were obtained after enzymatic hydrolysis for these two subjects. In the remaining three subjects caftaric acid was undetectable. Coutaric acid remained undetectable in all plasma samples. However, it must be emphasized that the detector sensitivity for coutaric acid in our experimental conditions was remarkably lower with respect to those of caftaric and fertaric acids (detection limits for injection in our experimental conditions: 0.08 ng of caftaric acid, 0.05 ng of fertaric acid, 0.7 ng of coutaric acid). Moreover, the detector sensitivity for caftaric, fertaric, and coutaric acids was lower with respect to the sensitivity observed for their respective free hydroxycinnamic acids (0.03 ng of caffeic acid, 0.01 ng of ferulic acid, 0.03 ng of *p*-coumaric acid).

DISCUSSION

In grapes and wines, hydroxycinnamic acids occur distinctively as conjugates with tartaric acid (caftaric, fertaric, and coutaric acids), as first reported by Ribéreau-Gayon (*42*). In the white wine Greco di Tufo used in the present investigation, free caffeic acid amounted to only 1.7% of caftaric acid (its ester with tartaric acid) (**Table 1**). Therefore, the esters of hydroxycinnamic acids with tartaric acid were actually assumed with white wine drinking.

In the present investigation we demonstrated a significant rise in both free and conjugated (glucuronates and sulfates) hydroxycinnamic acid levels in human plasma at 30 and 60 min following wine plus crackers administration (**Figure 2**). Due to the almost absolute absence of the free forms of hydroxycinnamic acids in white wine, plasma hydroxycinnamic acids are likely derived by in vivo hydrolysis of their esterified forms present in wine. In this regard, hydroxycinnamic acids have been recently described to be absorbed from their bound forms present in the human diet (*38*, *43*−*45*).

Moreover, after wine plus crackers administration, the tartaric acid esters of hydroxycinnamic acids are present in human plasma only at low concentrations, if any. Small amounts of caftaric acid were detected in nonhydrolyzed plasma from two of five subjects, at 30 and 60 min after wine administration. Similar values were measured in the same samples after enzymatic hydrolysis, indicating that neither glucuronidation nor sulfatation occurred to a detectable extent in vivo for caftaric acid, as previously described (*46*). Also, low amounts of fertaric acid were measured in plasma samples from two of five subjects at 60 min after wine administration. Coutaric acid remained undetectable in all subjects under study. These scattered data may be partially due to both the low plasma concentrations (near the detection limit of the instrument) and interindividual variability.

Nevertheless, the rapid appearance of hydroxycinnamic acids and their glucuronate and sulfate conjugates in human plasma observed in the present investigation indicates that the absorption and hydrolysis of hydroxycinnamic acid esters from wine likely takes place in the stomach and in the proximal part of the gastrointestinal tract. Gastric absorption has been recently demonstrated in rats for various hydroxycinnamic acids and related compounds, such as caftaric acid (*46*) and chlorogenic acid (5′-caffeoylquinic acid) and (*47*, *48*). Monocarboxylic acid transporter has been suggested to be involved in both gastric and intestinal absorption of phenolic acids, such as ferulic and *p*-coumaric acids, in rats, and in human intestine Caco-2 cells (*49*−*51*). Dihydroxy derivatives of phenolic acids, such as caffeic acid, with low affinity for monocarboxylic acid transporter, are mainly absorbed by paracellular pathway (passive diffusion) and, to a lesser extent, by the monocarboxylic acid transporter (*52*−*54*). Esterified phenolic acids, such as chlorogenic and rosmarinic acids, are absorbed by paracellular diffusion, due to the negative effect of the ester group on the interaction with monocarboxylic acid transporter (*51*−*54*).

The low level, if any, of tartaric acid esters of hydroxycinnamic acids recovered in human plasma seems to indicate that these compounds are efficiently and rapidly hydrolyzed upon absorption. It has been suggested that esters of hydroxycinnamic acids, such as chlorogenic acid, are hydrolyzed in the gut before absorption (*53*), by the reported presence of tissue esterases in the upper intestinal mucosa (*44*, *55*) and by the microbial esterases in the lower intestinal tract (*56*, *57*). Esterases with the ability to hydrolyze hydroxycinnamate esters at appreciable rates have been described in human and rats (*44*, *55*). The cinnamoyl esterase activity is distributed all along the small and large intestine and is present both in the mucosa cells and in the lumen. Enterocyte-like differentiated human Caco-2 cells exhibited hydrolase activity toward esters of the major hydroxy-

cinnamates and diferulates (*58*). Bacteria in the gastrointestinal tract of mammals are also capable of releasing free phenolic acids from bound complexes into the gastrointestinal tract (*45, 59, 60*). Indeed, an esterase activity able to hydrolyze chlorogenic acid and release caffeic acid has been demonstrated in human colonic microflora (*56, 61*). However, in our study, plasma concentrations of hydroxycinnamic acids peaking at 30−60 min after wine plus crackers administration seem to indicate that hydrolysis of hydroxycinnamic acid esters from wine occurs early in the gastrointestinal tract.

In our study the total circulating plasma levels of hydroxycinnamic acids (caffeic acid > *p*-coumaric acid > ferulic acid) reflect the concentration of their respective precursors in white wine (caftaric acid > coutaric acid > fertaric acid). However, the plasma level of caffeic acid was only about 3-fold higher at peak time with respect to ferulic acid and about 2-fold higher with respect to *p*-coumaric acid, whereas the level of its precursor caftaric acid in wine was about 5-fold higher than that of coutaric acid (the precursor of *p*-coumaric acid) and 35-fold higher than that of fertaric acid (the precursor of ferulic acid). Indeed, caftaric acid seems to be less efficiently absorbed with respect to fertaric and coutaric acids. However, it must be considered that, following absorption, a portion of phenolic acids is quickly metabolized and in particular methylated into ferulic and isoferulic acids, a reaction catalyzed by catechol-*O*-methyltransferase (*62*).

Altogether, our results show that, following wine plus crackers administration, hydroxycinnamic acid esters from wine are absorbed and rapidly metabolized, and parent hydroxycinnamic acids circulate in blood mainly as glucuronate and sulfate conjugates. *p*-Coumaric acid was present as free, unconjugated form at levels slightly higher than those observed for ferulic and caffeic acids. These two phenolic acids were present in plasma mainly as conjugated forms. Similar results have been reported after ingestion of ferulic acids in rats, with plasma conjugated ferulic acid amounting to 76% of the total ferulic acid (*63*) after coffee (*38*) and beer drinking (*36*) in humans.

Glucuronidation and sulfation are two well-known ways of detoxification, leading to increased solubility of compounds, which promote their excretion in urine and bile.

Glucuronidation has been reported to occur in the liver (*64, 65*), small intestine (*66, 67*), and colon (*68*). Recently, glucuronidation activity was found throughout the length of the human gastrointestinal tract, from stomach to colon (*69*). Evidence for sulfation and glucuronidation of various hydroxycinnamic acids and their esters by human Caco-2 cells has also been reported (*58*). Moreover, Spencer and co-workers, using the perfusion analysis of isolated intestine, reported that flavonoids and hydroxycinnamates were glucuronidated during transfer across the rat jejunum and ileum (*66*).

Phenolic acids such as caffeic acid have been reported to inhibit human sulfotransferase activity in in vitro experiments (*70*). Our results indicated that in our experimental conditions, there was sufficient sulfotransferase activity to extensively conjugate ingested wine phenolics. Our study indicates that, once absorbed, dietary phenolic acids are extensively conjugated to sulfate and glucuronate. Differences in the degree of conjugation are likely related to the different chemical features of the phenolic acids under study and may reflect differences in the activity and specificity of both glucuronosyltransferase and sulfotransferase families toward different phenolic acids.

A relevant conclusion of our study is that the plasma concentrations of conjugated forms of ferulic and caffeic acids largely exceed those of nonconjugated forms. Thus, at least for these two compounds, phenolic metabolites will likely be the molecules responsible for any biological activity in vivo.

The antioxidant activity and biological effects of caffeic, ferulic, and *p*-coumaric acids have been widely studied and described in the literature in the past decade. However, scarce or no information is available concerning the potential activity of the metabolites of phenolics. In fact, glucuronidation and sulfation may modify the biological activity of the parent, nonconjugated molecules. The conjugation of the catechol unit might result in a partial lost of biological activity for those phenolics bearing a 3,4-dihydroxy unit. For quercetin, one of the most abundant flavonoids in the human diet, it has been described that the conjugated forms quercetin glucuronides and quercetin 3-*O*-sulfate retain antioxidant activity, although to a lesser extent with respect to quercetin (*13*). The antioxidant activity of ferulic acid glucuronide, which has not only the hydrophobic ferulic acid moiety but also a hydrophilic sugar moiety, is stronger than that exhibited by ferulic acid (*14*). Even if glucuronidation and sulfation are the most important pathways, the presence of other metabolic forms in vivo cannot be completely ruled out.

In the present study, total plasma hydroxycinnamic acids reached approximately 0.09 μM concentration for caffeic acid, 0.03 μM for ferulic acid, and 0.05 μM for *p*-coumaric acid at peak time after wine plus crackers administration. In a previous human study, plasma concentrations on the order of 0.18 μM were measured for total caffeic acid in individuals drinking 300 mL/day of white wine for 15 days of followup (*26*). Although the concentrations of phenolic acids measured in the present investigation seem to be quite low, it must be considered that they were obtained after a single dose of 250 mL of white wine, containing about 30 mg in total of the hydroxycinnamic acids under study (calculated as free forms on the basis of **Table 1**). This value is quite far from the average total daily intake of phenolic acids, which has been calculated to be on the order of 200 mg/day or higher (*2, 5, 7*). Synergistic effects might also occur in vivo. Moreover, an accumulation of phenolic acids and their metabolites might occur in many tissues (lung, heart, liver) as described for 3-palmitoylcatechin, epigallocatechin gallate, and resveratrol (*71−73*).

In conclusion, our study demonstrated that hydroxycinnamic acids, present in the wine as tartaric acid esters, are still bioavailable to humans. They are quickly absorbed from the gastrointestinal tract and circulate in the blood after being largely metabolized to the form of glucuronide and sulfate conjugates. The circulating plasma levels of unmodified tartaric acid esters of hydroxycinnamic acids are correspondingly very low or undetectable. Further research will be accomplished to establish the antioxidant and biological activity of the different metabolites of hydroxycinnamic acids with respect to the parent compounds and possible synergistic in vivo effects.

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LITERATURE CITED

(1) Pulido, R.; Hernandez-Garcia, M.; Saura-Calixto, F. Contribution of beverages to the intake of lipophilic and hydrophilic antioxi-

dants in the Spanish diet. *Eur. J. Clin. Nutr.* **2003**, *57*, 1275–1282.
(2) Scalbert, A.; Williamson, G. Dietary intake and bioavailability of polyphenols. *J. Nutr.* **2000**, *130*, 2073S–2085S.
(3) Block, G.; Patterson, B.; Subar, A. Fruit, vegetables and cancer prevention: a review of the epidemiological evidence. *Nutr. Cancer* **1992**, *18*, 1–30.
(4) Renaud, S.; deLorgeril, M. Wine, alcohol, platelets and the French paradox for coronary heart disease. *Lancet* **1992**, *339*, 1523–1526.
(5) Clifford, M. N. Chlorogenic acids and other cinnamates—nature, occurrence, dietary burden, absorption and metabolism. *J. Sci. Food Agric.* **2000**, *80*, 1033–1043.
(6) Manach, C.; Scalbert, A.; Morand, C.; Rémésy, C.; Jiménez, L. Polyphenols: food sources and bioavailability. *Am. J. Clin. Nutr.* **2004**, *79*, 727–747.
(7) Radtke, J.; Linseisen, J.; Wolfram, G. Phenolic acid intake of adults in a Bavarian subgroup of the national food composition survey. *Z. Ernahrungswiss* **1998**, *37*, 190–197.
(8) Manach, C.; Williamson, G.; Morand, C.; Scalbert, A.; Remesy, C. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *Am. J. Clin. Nutr.* **2005**, *81*, 230S–242S.
(9) Simonetti, P.; Gardana, C.; Pietta, P. Plasma levels of caffeic acid and antioxidant status after red wine intake. *J. Agric. Food Chem.* **2001**, *49*, 5964–5968.
(10) Serafini, M.; Maiani, G.; Ferro-Luzzi, A. Alcohol-free red wine enhances plasma antioxidant capacity in humans. *J. Nutr.* **1998**, *128*, 1003–1007.
(11) Ghiselli, A.; Natella, F.; Guidi, A.; Montanari, L.; Fantozzi, P.; Scaccini, C. Beer increases plasma antioxidant capacity in humans. *J. Nutr. Biochem.* **2000**, *11*, 76–80.
(12) Natella, F.; Nardini, M.; Giannetti, C.; Dattilo, C.; Scaccini, C. Coffee drinking influences plasma antioxidant capacity in humans. *J. Agric. Food Chem.* **2002**, *50*, 6211–6216.
(13) Manach, C.; Morand, C.; Crespy, V.; Demigné, C.; Texier, O.; Regerat, F.; Remesy, C. Quercetin is recovered in human plasma as conjugated derivatives which retain antioxidant properties. *FEBS Lett.* **1998**, *426*, 331–336.
(14) Ohta, T.; Nakano, T.; Egashira, Y.; Sanada, H. Antioxidant activity of ferulic acid beta-glucuronide in the LDL oxidation system. *Biosci., Biotechnol., Biochem.* **1997**, *61*, 1942–4943.
(15) Spencer, J. P.; Kuhnle, G. G.; Williams, R. J.; Rice-Evans, C. Intracellular metabolism and bioactivity of quercetin and its *in vivo* metabolites. *Biochem. J.* **2003**, *372*, 173–181.
(16) St Leger, A. S.; Cochrane, A. L.; Moore, F. Factors associated with cardiac mortality in developed countries with particular reference to the consumption of wine. *Lancet* **1979**, *1*, 1017–1020.
(17) Rinaud, D.; De Lorgeril, M. Wine, alcohol, platelets and the French paradox for coronary hearth disease. *Lancet* **1992**, *339*, 1523–1523.
(18) Das, D. K.; Sato, M.; Ray, P. S.; Maulik, G.; Engelman, R. M.; Bertelli, A. A.; Bertelli, A. Cardioprotection of red wine: role of phenolic antioxidants. *Drugs Exp. Clin. Res.* **1999**, *25*, 115–120.
(19) Agewall, S.; Wright, S.; Doughty, R. N.; Whalley, G. A.; Duxbury, M.; Sharpe, N. Does a glass of red wine improve endothelial function? *Eur. Heart J.* **2000**, *21*, 74–78.
(20) Frankel, E. N.; Kanner, J.; German, J. B.; Parks, E.; Kinsella, J. E. Inhibition of oxidation of human low density lipoprotein by phenolic substances in red wine. *Lancet* **1993**, *341*, 454–457.
(21) Nigdikar, S. V.; Williams, N. R.; Griffin, B. A.; Howard, A. N. Consumption of red wine polyphenols reduces the susceptibility of low-density lipoproteins to oxidation *in vivo*. *Am. J. Clin. Nutr.* **1998**, *68*, 258–265.
(22) Marques-Vidal, P.; Cambou, J. P.; Nicaud, V.; Luc, G.; Evans, A.; Arveiler, D.; Bingham, A.; Cambien, F. Cardiovascular risk factors and alcohol consumption in France and Northen Ireland. *Atherosclerosis* **1995**, *115*, 225–232.
(23) Ghiselli, A.; Natella, F.; Guidi, A.; Montanari, L.; Fantozzi, P.; Scaccini, C. Beer increases plasma antioxidant capacity in humans. *J. Nutr. Biochem.* **2000**, *11*, 76–80.
(24) Gonthier, M. P.; Cheynier, V.; Donovan, J. L.; Manach, C.; Morand, C.; Mila, I.; Lapierre, C.; Remesy, C.; Scalbert, A. Microbial aromatic acid metabolites formed in the gut account for a major fraction of the polyphenols excreted in urine of rats fed red wine polyphenols. *J. Nutr.* **2003**, *133*, 461–467.
(25) Yamashita, S.; Sakane, T.; Harada, M.; Sugiura, N.; Koda, H.; Kiso, Y.; Sezaki, H. Absorption and metabolism of antioxidative polyphenolic compounds in red wine. *Ann. N.Y. Acad. Sci.* **2002**, *957*, 325–328.
(26) Pignatelli, P.; Ghiselli, A.; Buchetti, B.; Carnevale, R.; Natella, F.; Germanò, G.; Fimognari, F.; Di Santo, S.; Lenti, L.; Violi, F. Polyphenols synergistically inhibit oxidative stress in subjects given red and white wine. *Atherosclerosis* **2006**, *188*, 77–83.
(27) Whelan, A. P.; Sutherland, W. H.; McCormick, M. P.; Yeoman, D. J.; de Jong, S. A.; Williams, M. J. Effects of white and red wine on endothelial function in subjects with coronary artery disease. *Intern. Med. J.* **2004**, *34*, 224–228.
(28) Williams, M. J.; Sutherland, W. H.; Whelan, A. P.; McCormick, M. P.; de Jong, S. A. Acute effect of drinking red and white wines on circulating levels of inflammation-sensitive molecules in men with coronary artery disease. *Metabolism* **2004**, *53*, 318–323.
(29) Vitaglione, P.; Sforza, S.; Galaverna, G.; Ghidini, C.; Caporaso, N.; Vescovi, P. P.; Fogliano, V.; Marchelli, R. Bioavailability of *trans*-resveratrol from red wine in humans. *Mol. Nutr. Food Res.* **2005**, *49*, 495–504.
(30) Bitsch, R.; Netzel, M.; Frank, T.; Strass, G.; Bitsch, I. Bioavailability and biokinetics of anthocyanins from red grape juice and res wine. *J. Biomed. Biotechnol.* **2004**, *2004*, 293–298.
(31) Simonetti, P.; Gardana, C.; Pietta, P. Plasma levels of caffeic acid and antioxidant status after red wine intake. *J. Agric. Food Chem.* **2001**, *49*, 5964–5968.
(32) Caccetta, R. A.; Croft, K. D.; Beilin, L. J.; Puddey, I. B. Ingestion of red wine significantly increases plasma phenolic acid concentrations but does not acutely affect *ex vivo* lipoprotein oxidazibility. *Am. J. Clin. Nutr.* **2000**, *71*, 67–74.
(33) Simonetti, P.; Gardana, C.; Pietta, P. Caffeic acid as biomarker of red wine intake. In *Methods in Enzymology. Flavonoids and Other Polyphenols*; Packer, L., Ed.; Academic Press: San Diego, CA, 2001; Vol. 335, pp 122−130.
(34) Waterhouse, A. L. Wine phenolics. *Ann. N.Y. Acad. Sci.* **2002**, *957*, 21–36.
(35) Singleton, V. L.; Timberlake, C. F.; Lea, A. G. H. The phenolic cinnamates of white grapes and wine. *J. Sci. Food Agric.* **1978**, *29*, 403–410.
(36) Nardini, M.; Natella, F.; Scaccini, C.; Ghiselli, A. Phenolic acids from beer are absorbed and extensively metabolized in humans. *J. Nutr. Biochem.* **2006**, *17*, 14–22.
(37) Ghiselli, A.; Serafini, M.; Maiani, G.; Azzini, E.; Ferro-Luzzi, A. A fluorescence-based method for measuring total plasma antioxidant capability. *Free Radical Biol. Med.* **1995**, *18*, 29–36.
(38) Nardini, M.; Cirillo, E.; Natella, F.; Scaccini, C. Absorption of phenolic acids in humans after coffee consumption. *J. Agric. Food Chem.* **2002**, *50*, 5735–5741.
(39) Mattivi, F.; Versini, G.; Vrhovsek, U.; Pojer, M.; Zanoni, M. Un metodo innovativo di pressatura in pressione positiva sotto gas inerte. *L'Enologo* **2005**, *3*, 77–91.
(40) Spagna, G.; Pifferi, P. G.; Rangoni, C.; Mattivi, F.; Nicolin, G.; Palmonari, R. The stabilization of white wines by absorption of phenolic compounds on chitin and chitosan. *Food Res. Int.* **1996**, *29*, 241–248.
(41) Rigo, A.; Vianello, F.; Clementi, G.; Rossetto, M.; Scarpa, M.; Vrhovsek, U.; Mattivi, F. Contribution of proanthocyanidins to the peroxy radical scavenging capacity of some Italian red wines. *J. Agric. Food Chem.* **2000**, *48*, 1996–2002.
(42) Ribéreau-Gayon, P. Vinifera. *C.R. Hebd. Seances Acad. Sci.* **1965**, *260*, 341–343.
(43) Rechner, A. R.; Spencer, J. P. E.; Kuhnle, G.; Hahn, U.; Rice-Evans, C. Novel biomarkers of the metabolism of caffeic acid derivatives *in vivo*. *Free Radical Biol. Med.* **2001**, *30*, 1213–1222.

(44) Andreason, M. F.; Kroon, P.; Williamson, G.; Garcia-Conesa, M. T. Esterase activitable to hydrolyze dietary antioxidant hydroxycinnamates is distributed along the intestine of mammals. *J. Agric. Food Chem*. **2001**, *49*, 5679–5684.
(45) Andreason, M. F.; Kroon, P. A.; Williamson, G.; Garcia-Conesa, M. T. Intestinal release and uptake of phenolic antioxidant diferulic acids. *Free Radical Biol. Med*. **2001**, *31*, 304–314.
(46) Vanzo, A.; Cecotti, R.; Vrhovsek, U.; Torres, A. M.; Mattivi, F.; Passamonti, S. The fate of *trans*-caftaric acid administered into the rat stomach. *J. Agric. Food Chem*. **2007**, *55*, 1604–1611.
(47) Konishi, Y.; Zhao, Z.; Shimizu, M. Phenolic acids are absorbed from the rat stomach with different absorption rates. *J. Agric. Food Chem*. **2006**, *54*, 7539–7543.
(48) Lafay, S.; Gil-Izquierdo, A.; Manach, C.; Morand, C.; Besson, C.; Scalbert, A. Chlorogenic acid is absorbed in its intact form in the stomach of rats. *J. Nutr*. **2006**, *136*, 1192–1197.
(49) Konishi, Y.; Shimizu, M. Transepithelial transport of ferulic acid by monocarboxylic acid transporter in Caco-2 cell monolayers. *Biosci., Biotechnol., Biochem*. **2003**, *63*, 856–862.
(50) Konishi, Y.; Kobayashi, S.; Shimizu, M. Transepithelial transport of *p*-coumaric acid and gallic acid by monocarboxylic acid transporter in Caco-2 cell monolayers. *Biosci., Biotechnol., Biochem*. **2003**, *67*, 2317–2324.
(51) Konishi, Y.; Kobayashi, S. Transepithelial transport of chlorogenic acid caffeic acid and their colonic metabolites in intestinal Caco-2 cell monolayers. *J. Agric. Food Chem*. **2004**, *52*, 2518–2526.
(52) Konishi, Y.; Kubo, K.; Shimizu, M. Structural effects of phenolic acids on the transepithelial transport of fluorescein in Caco-2 cell monolayers. *Biosci., Biotechnol., Biochem*. **2003**, *67*, 2014–2017.
(53) Lafay, S; Morand, C.; Manach, C.; Besson, C.; Scalbert, A. Absorption and metabolism of caffeic acid and chlorogenic acid in the small intestine of rats. *Br. J. Nutr*. **2006**, *96*, 39–46.
(54) Konishi, Y.; Hitomi, Y.; Yoshida, M.; Yoshioka, E. Pharmacokinetic study of caffeic and rosmarinic acids in rats after oral administration. *J. Agric. Food Chem*. **2005**, *53*, 4740–4746.
(55) Azuma, K.; Ippoushi, K.; Nakyama, M.; Ito, H.; Higashio, H.; Terao, J. Absorption of chlorogenic acid and caffeic acid in rats after oral administration. *J. Agric. Food Chem*. **2000**, *48*, 5496–5500.
(56) Couteau, D.; McCartney, A. L.; Gibson, G. R.; Williamson, G.; Faulds, C. B. Isolation and characterization of human colonic bacteria able to hydrolyse chlorogenic acid. *J. Appl. Microbiol*. **2001**, *90*, 873–881.
(57) Gonthier, M. P.; Verny, M. A.; Besson, C.; Remesy, C.; Scalbert, A. Chlorogenic acid bioavailability largely depends on its metabolism by the gut microflora in rats. *J. Nutr*. **2003**, *133*, 1853–1859.
(58) Kern, S. M.; Bennet, R. N.; Needs, P. W.; Mellon, F. A.; Kroon, P. A.; Garcia-Conesa, M. T. Characterization of metabolites of hydroxycinnamates in the in vitro model of human small intestinal epithelium Caco-2 cells. *J. Agric. Food Chem*. **2003**, *51*, 7884–7891.
(59) Kroon, P. A.; Faulds, C. B.; Ryden, P.; Robertson, J. A.; Williamson, G. Release of covalently bound ferulic acid from fiber in human colon. *J. Agric. Food Chem*. **1997**, *5*, 661–667.
(60) Buchanan, C. J.; Wallace, G.; Fry, S. C. In vivo release of ^{14}C-labeled phenolic groups from intact dietary spinach walls during passage through the rat intestine. *J. Sci. Food Agric*. **1996**, *71*, 459–469.
(61) Plumb, G.; Garcia-Conesa, M. T.; Kroon, P.; Rhodes, M.; Saxon, R.; Williamson, G. Metabolism of chlorogenic acid by human plasma, liver, intestine and gut microflora. *J. Sci. Food Agric*. **1999**, *79*, 390–392.
(62) Mannisto, P. T.; Kaakkola, S. Catechol-*O*-methyltransferase (COMT): biochemistry, molecular biology, pharmacology, and clinical efficacy of the new selective COMT inhibitors. *Pharmacol. Reb*. **1999**, *51*, 593–628.
(63) Rondini, L.; Peyrat-Maillard, M. N.; Marsset-Baglieri, A.; Berset, C. Sulfated ferulic acid is the main in vivo metabolite found after short-term ingestion of free ferulic acid in rats. *J. Agric. Food Chem*. **2002**, *50*, 3037–3041.
(64) Scheline, R. R. Metabolism of acids, lactones and esters. In *Mammalian Metabolism of Plant Xenobiotics*; Schline, R. R., Ed.; Academic Press: New York, 1978; pp 170−228.
(65) Mateos, R.; Gova, L.; Bravo, L. Uptake and metabolism of hydroxycinnamic acids (chlorogenic, caffeic, and ferulic acids) by HepG2 cells as a model of the human liver. *J. Agric. Food Chem*. **2006**, *54*, 8724–8732.
(66) Spencer, J. P. E.; Chowrimootoo, G.; Choudhury, R.; Debnam, E. S.; Srai, S. K.; Rice-Evans, C. A. The small intestine can both absorb and glucuronidate luminal flavonoids. *FEBS Lett*. **1999**, *458*, 224–230.
(67) Kuhnle, G.; Spencer, J. P.; Schroeter, H.; Shenov, B.; Debnam, E. S.; Srai, S. K.; Rice-Evans, C.; Hahn, U. Epicatechin and catechin are *O*-methylated and glucuronidated in the small intestine. *Biochem. Biophys. Res. Commun*. **2000**, *277*, 507–512.
(68) Cheng, Z.; Radominska-Pandya, A.; Tephly, T. R. Studies on the substrate specificity of human intestinal UDP-glucuronosyltransferase 1A8 and 1A10. *Drug Metab. Dispos*. **1999**, *27*, 1165–1170.
(69) Sabolovic, N.; Humbert, A. C.; Radominska-Pandva, A.; Magdalou, J. Resveratrol is efficiently glucuronidated by UDP-glucuronosyltransferases in the human gastrointestinal tract and in Caco-2 cells. *Biopharm. Drug Dispos*. **2006**, *27*, 181–189.
(70) Yeh, C. T.; Yen, G. C. Effects of phenolic acids on human phenolsulfotransferases in relation to their antioxidant activity. *J. Agric. Food Chem*. **2003**, *51*, 1474–1479.
(71) Das, N. P.; Griffiths, L. A. Studies of flavonoid metabolism. Metabolism of (+)-[^{14}C]catechin in rat and guinea pig. *Biochem. J*. **1969**, *115*, 831–836.
(72) Suganuma, M.; Okabe, S.; Oniyama, M.; Tada, Y.; Ito, H.; Fujiki, H. Wide distribution of [3H](-)epigallocatechin gallate, a cancer preventive tea polyphenol, in mouse tissue. *Carcinogenesis* **1998**, *19*, 1771–1776.
(73) Bertelli, A. A.; Giovannini, L.; Stradi, R.; Bertelli, A.; Tillement, J. P. Plasma, urine and tissue levels of *trans*- and *cis*-resveratrol (3,4′,5-trihydrostilbene) after short-term or prolonged administration of red wine to rats. *Int. J. Tissue React*. **1996**, *18*, 67–71.

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