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Absorption of Phenolic Acids in Humans after Coffee Consumption

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Despite extensive literature describing the biological effects of polyphenols, little is known about their absorption from diet, one major unresolved point consisting of the absorption of the bound forms of polyphenols. In this view, in the present work we studied the absorption in humans of phenolic acids from coffee, a common beverage particularly rich in bound phenolic acids, such as caffeic acid, ferulic acid, and *p*-coumaric acid. Coffee brew was analyzed for free and total (free + bound) phenolic acids. Chlorogenic acid (5′-caffeoylquinic acid), a bound form of caffeic acid, was present in coffee at high levels, while free phenolic acids were undetectable. After alkaline hydrolysis, which released bound phenolic acids, ferulic acid, *p*-coumaric acid, and high levels of caffeic acid were detected. Plasma samples were collected before and 1 and 2 h after coffee administration and analyzed for free and total phenolic acid content. Two different procedures were applied to release bound phenolic acids in plasma: β-glucuronidase treatment and alkaline hydrolysis. Coffee administration resulted in increased total plasma caffeic acid concentration, with an absorption peak at 1 h. Caffeic acid was the only phenolic acid found in plasma samples after coffee administration, while chlorogenic acid was undetectable. Most of caffeic acid was present in plasma in bound form, mainly in the glucuronate/sulfate forms. Due to the absence of free caffeic acid in coffee, plasma caffeic acid is likely to be derived from hydrolysis of chlorogenic acid in the gastrointestinal tract.

KEYWORDS: Chlorogenic acid; caffeic acid; coffee; polyphenols; human plasma

INTRODUCTION

Polyphenols have been reported to exert a variety of biological actions, such as free radical scavenging, metal chelation, and modulation of enzymatic activity (*1*−*4*), and, more recently, to affect signal transduction, activation of transcription factors, and gene expression (*5*−*9*). Epidemiological studies have suggested associations between the consumption of polyphenol-rich food and beverages and the prevention of many human diseases (*10*−*14*).

The total polyphenol intake has been reported to be in the order of 1 g/d, although large uncertainties remain due to the lack of comprehensive data on the content of some of the major polyphenol classes in food (*10*). For individuals regularly consuming wine, coffee, beer, and tea, these beverages will likely be the major sources of polyphenols. Despite extensive literature describing the effects of polyphenols, our knowledge about their absorption from diet is scarce, one major question arising on the absorption of bound forms of phenolic compounds (*10*).

A major class of phenolic compounds are hydroxycinnamic acids, which are widely present in fruits, vegetables, coffee, wine, and olive oil (*15*−*20*), mainly in esterified form with organic acids, sugars, and lipids (*1*, *20*). Caffeic acid is the major representative of hydroxycinnamic acids and occurs in foods mainly as chlorogenic acid (5-caffeoylquinic acid, an ester of caffeic acid with quinic acid). Fruits and coffee are the major sources of chlorogenic acid in human diet (*15*−*20*).

To fully understand the implications of dietary phenolic acids in human health, it is essential to determine their bioavailability in humans. In particular, due to the fact that phenolic acids are rarely present in foods in free forms, knowledge concerning the absorption of phenolic acids from their conjugated forms in humans is essential to evaluate possible in vivo effects. Therefore, in the present work we studied the absorption in humans of phenolic acids from coffee, a common beverage rich in bound hydroxycinnamic acid derivatives, particularly chlorogenic acid.

MATERIALS AND METHODS

Chemicals. β-Glucuronidase (EC 3.2.1.31, type HP-2, from *Helix pomatia*, 105000 units/mL, containing sulfatase activity, 4300 units/mL), ascorbic acid, caffeic acid, ethylenediaminetetraacetic acid (EDTA), 5′-caffeoylquinic acid (chlorogenic acid), ferulic acid, dihydroferulic acid, *p*-coumaric acid, syringic acid, and 3-(*p*-hydroxyphenyl)propionic acid were from Sigma (St. Louis, MO). *o*-Coumaric acid, *m*-coumaric acid, and isoferulic acid were from Extrasynthese (Genay Cedex, France). Dihydrocaffeic acid was from Avocado (Heysham, Lancashire, England). Stock solutions were prepared in methanol (1 mg/mL), stored at −80 °C, and used within 2 weeks. Working standard

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solutions were prepared daily by dilution in sample buffer (1.25% glacial acetic acid, 7% methanol in water).

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.

HPLC Instrumentation. 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 an electrochemical coulometric detector (Coulochem II, ESA, Bedford, MA). Turbochrom chromatography work station software was used for data processing. Operating conditions were as follows: column temperature, 30 °C; flow rate, 1 mL/min; injection volume, 50 μL; electrochemical detection at +600 mV; sensitivity range, 200 nA; filter, 2 s.

Chromatographic separations were performed on a Supelcosil LC-18 C_{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; solution B was absolute methanol. The following gradient was used: 0−25 min, from 93% A and 7% B to 76% A and 24% B, linear gradient; 26−45 min, 76% A and 24% B; 46−53 min, from 76% A and 24% B to 55% A and 45% B, linear gradient; 54−55 min, 55% A and 45% B; 56−86 min, 93% A and 7% 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 male nonsmoker moderate-coffee drinkers (2−4 cups per day) were asked to avoid coffee, wine, beer, tea, and fruit juices the day preceding the experiments. A standard amount (200 mL) of brewed coffee was administered, within 10 min from brewing, in fasting conditions. In another set of experiments, 200 mL of water was administered as the control drink, in fasting conditions. Blood was withdrawn into EDTA-containing vacutainers (1 mg/mL) just before coffee administration ($t = 0$) and 1 and 2 h after coffee administration. 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 18 μL of 4 N HCl and stored at −80 °C.

Coffee Brew Preparation and Treatment of Coffee Brew Samples. Coffee brew was prepared by using a commercial automatic brewing machine (60 g of roasted and ground coffee from an Italian brand per liter of water). Coffee brew samples were treated and analyzed for free and bound phenolic acid content according to the following procedures (*21*).

Nonhydrolyzed Coffee Brew Samples. Coffee brew (0.5 mL) was added with 0.1 mg of isoferulic acid as the internal standard and acidified with 1 N HCl to pH 3.0. After addition of 300 mg of NaCl, samples were extracted three times with ethyl acetate (×4 volumes) by vortexing for 5 min. After each extraction, samples were centrifuged (3000*g*, 10 min) and supernatants collected. The organic phase was dried under nitrogen flow. The residue was dissolved in a final volume of 5 mL of methanol, vortexed for 5 min, and then diluted 1 to 100 with sample buffer (1.25% glacial acetic acid, 7% methanol in water) prior to HPLC−ECD analysis. Extraction yields were calculated using isoferulic acid as the internal standard. Recovery experiments were performed by adding known amounts of pure chlorogenic acid and caffeic acid, as representative of coffee phenolic acids, to coffee brew. Recovery was found to be 97.2 ± 3.1% and 98.5 ± 4.4% for chlorogenic and caffeic acid, respectively.

Hydrolyzed Coffee Brew Samples. Coffee brew (0.5 mL) was subjected to alkaline hydrolysis in 1.8 N NaOH containing 10 mM EDTA and 1% ascorbic acid in 5 mL final volume at 30 °C for 30 min, in the presence of 0.1 mg of isoferulic acid as the internal standard. At the end of incubation, 0.5 mL samples were acidified to pH 3.0 with 4 N HCl, added with 300 mg of NaCl, and treated for extraction as reported above. The final residue was dissolved in 0.5 mL of methanol, vortexed for 5 min, and then diluted 1 to 100 or 1 to 1500 as specified with sample buffer prior to HPLC−ECD analysis. In these conditions, alkaline hydrolysis of known amounts of pure chlorogenic acid resulted in complete recovery of liberated caffeic acid (97.0 ± 2.8% of expected value). Moreover, addition of known amounts of pure chlorogenic acid to coffee brew resulted in total recovery of caffeic acid released upon hydrolysis (95.8 ± 3.5% of expected value). The hydrolytic procedure above-reported allows complete recovery of phenolic acids released upon hydrolysis. In fact, the recovery of standard phenolic acids (caffeic, *m*-coumaric, *o*-coumaric, *p*-coumaric, dihydrocaffeic, dihydroferulic, ferulic, homogentisic, homovanillic, isoferulic, sinapic, syringic, and vanillic acids) separately submitted to the above-reported hydrolytic procedure ranged from 94.3 ± 10.2% to 110.7 ± 12.2% (*21*).

Treatment of Plasma Samples. Aliquots of plasma samples (0.5 mL) from each subject were thawed and treated according to one of the three following procedures: no treatment, to detect free caffeic acid, β-glucuronidase treatment, and alkaline hydrolysis treatment to detect total (free + bound) caffeic acid. *o*-Coumaric acid was selected as the internal standard due to the absence of detectable amounts of this compound in human plasma samples before and after coffee administration, with or without β-glucuronidase or alkaline hydrolysis treatments.

No Treatment. The plasma sample was added with *o*-coumaric acid (100 ng) as the internal standard and deproteinized by addition of 3 volumes of ethanol. After vortexing, the sample was centrifuged at 17500*g* for 5 min at 4 °C. The protein pellet was resuspended twice in 1 volume of ethanol and centrifuged at 17500*g* for 5 min at 4 °C. The pooled ethanol phases were dried under nitrogen flow. The dried residue obtained was dissolved in 0.5 mL of distilled water and vortexed for 5 min. The pH was brought to 3.0 with 10 μL of 1 N HCl. After addition of 300 mg of NaCl, the sample was extracted three times with ethyl acetate (×4 volumes) by vortexing for 5 min. After each extraction, the sample was centrifuged (3000*g*, 5 min), and supernatants were collected. The organic phase was dried under nitrogen flow. The residue was dissolved in a final volume of 0.1 mL of methanol and vortexed for 5 min; then 0.4 mL of sample buffer was added, followed by 5 min of vortexing. The sample was centrifuged for 5 min at 17500*g* and filtered prior to HPLC−ECD analysis. Fifty microliters of the sample, corresponding to 0.05 mL of original plasma, was analyzed. Recovery experiments were performed by adding known amounts of pure chlorogenic acid and caffeic acid to the plasma samples. Recovery was found to be 100.7 ± 1.8% and 93.6 ± 5.6% for chlorogenic acid and caffeic acid, respectively.

β-Glucuronidase Treatment. The plasma sample was added with *o*-coumaric acid (100 ng) as the internal standard and deproteinized with ethanol as above reported. The dried residue obtained after deproteinization was dissolved in 0.5 mL of 0.1 M sodium acetate buffer, pH 5.0, and vortexed for 5 min. The sample was added with β-glucuronidase (4000 units) (containing sulfatase activity, 163 units) and incubated in a water bath at 37 °C for 2 h. At the end of incubation, the pH was brought to 3.0 with 52 μL of 1 N HCl. After addition of 300 mg of NaCl, the sample was extracted three times with ethyl acetate as above reported. The final residue was dissolved in a final volume of 0.1 mL of methanol and vortexed for 5 min; then 0.4 mL of sample buffer was added, followed by 5 min of vortexing. The sample was centrifuged for 5 min at 17500*g* and filtered prior to HPLC−ECD analysis. Fifty microliters of the sample, corresponding to 0.05 mL of the original plasma, was analyzed.

Alkaline Hydrolysis Treatment. The plasma sample was added with *o*-coumaric acid (500 ng) as the internal standard and deproteinized with ethanol as above reported. The dried residue obtained after deproteinization was dissolved in 0.5 mL of distilled water and vortexed for 5 min. The sample was then submitted to alkaline hydrolysis in the same experimental conditions above reported for coffee samples, 1.8 N NaOH, 10 mM EDTA, and 1% ascorbic acid in 1 mL final volume, and incubated in a water bath at 30 °C for 30 min. At the end of the incubation, the pH was brought at 3.0 with 0.44 mL of 4 N HCl. After addition of 600 mg of NaCl, the sample was extracted three times with ethyl acetate as above reported. The final residue was dissolved in a final volume of 0.5 mL of methanol and vortexed for 5 min; then 2.0 mL of sample buffer was added, followed by 5 min of vortexing. The sample was centrifuged for 5 min at 17500*g* and filtered prior to HPLC−ECD analysis. Fifty microliters of the sample, corresponding to 0.01 mL of the original plasma, was analyzed. Recovery experiments

Table 1. Phenolic Acid Content of Coffee Brew before and after Hydrolysis[a]

	chlorogenic acid (μg/mL)	caffeic acid (μg/mL)	*p*-coumaric acid (μg/mL)	ferulic acid (μg/mL)
nonhydrolyzed coffee	478.9 ± 23.2	nd	nd	nd
hydrolyzed coffee	nd	830.0 ± 69.9	14.0 ± 1.1	142.8 ± 12.3

[a] Values are means ± SD of four independent experiments.

were performed by adding known amounts of pure chlorogenic acid to the plasma samples. In these conditions, alkaline hydrolysis of chlorogenic acid resulted in complete recovery of released caffeic acid (101.0 ± 1.8% of expected value).

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 500 ng/mL) were analyzed. The 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 means ± standard deviation. Statistical analysis was performed using a one-factor analysis of variance (ANOVA, Scheffe's method) for multiple comparison or paired *t*-test as specified. A probability of $p < 0.05$ was considered statistically significant.

RESULTS

In this study, coffee brew was used as a dietary phenolic acid source because of its high content of bound phenolic acids, particularly chlorogenic acids. Chlorogenic acids are a family of esters formed between certain hydroxycinnamic acids and quinic acid. The chlorogenic acids found in coffee are derivatives of caffeic acid, ferulic acid, and *p*-coumaric acid, the caffeic acid derivatives being the most abundant (*22*). Since 5′-caffeoylquinic acid is by far the dominant isomer in coffee and also the only chlorogenic acid isomer commercially available, we used the generic name of chlorogenic acid to indicate this isomer in the following of this study.

Table 1 shows the content of free (nonhydrolyzed coffee) and total (free + bound, hydrolyzed coffee) phenolic acids and their derivatives in coffee brew. Chlorogenic acid was present in nonhydrolyzed coffee samples at high concentrations, while free caffeic acid, *p*-coumaric acid, and ferulic acid were undetectable. After hydrolysis, ferulic acid, *p*-coumaric acid, and high levels of caffeic acid were detected. The amount of caffeic acid released upon hydrolysis, higher than the amount expected from hydrolysis of chlorogenic acid on the basis of 1 to 1 stoichiometry, is explained by the fact that coffee also contains dicaffeoylquinic acid derivatives and different isomers of caffeoylquinic acids besides 5′-caffeoylquinic acid, the one detected in our experiments (*23*). From the data in **Table 1**, a cup of coffee (200 mL) contained 95.8 ± 4.6 mg of chlorogenic acid (5′-caffeoylquinic acid). This value is in agreement with data from the literature (*24*). After hydrolytic treatment, the total phenolic acid content of a cup of coffee was as follows: caffeic acid, 166.0 ± 14.0 mg; *p*-coumaric acid, 2.8 ± 0.2 mg; ferulic acid, 28.6 ± 2.5 mg.

To study the absorption of coffee phenolic acids, plasma samples were collected before and after coffee administration and analyzed for content of both free and total (free + bound) phenolic acids. Two different procedures were used to release phenolic acids from bound forms. In the first procedure, β-glucuronidase was used to selectively hydrolyze glucuronidated forms of hydroxycinnamic acids. In the second procedure, an alkaline hydrolytic treatment was used to liberate phenolic acids from bound complexes.

Figure 1A shows the chromatographic profile obtained in our experimental conditions of a standard mixture containing several common phenolic acids and/or their derivatives. Typical chromatograms obtained from human plasma 1 h after coffee brew consumption are shown in **Figure 1B−D**. A peak (peak 3) was identified as caffeic acid by both retention time and coelution with standard caffeic acid in all plasma samples. The amount of caffeic acid was significantly higher in β-glucuronidase-treated (**Figure 1C**) and alkaline hydrolysis treated samples (**Figure 1D**) with respect to nontreated samples (**Figure 1B**), taking into account the amount of the injected sample, corresponding to 0.05 mL of the original plasma in **Figure 1B,C** and to 0.01 mL of the original plasma in **Figure 1D**. Despite the high levels of chlorogenic acid present in coffee brew, we failed to find a detectable amount of this compound in our samples, as no peak coeluting with standard chlorogenic acid was detected. Also, we failed to find a detectable amount of ferulic acid and *p*-coumaric acid.

Peaks with retention times of 25.5 and 34.9 min in untreated plasma samples at 1 h (**Figure 1B**) were present also at time 0 at the same extent. Peaks at 25.5, 29.6, 33.6, 35.2, and 53.0 min in β-glucuronidase-treated samples at time 1 h (**Figure 1C**) were also present at the same level at time 0. Components with retention times of 25.5, 33.6, and 35.0 min in hydrolyzed plasma at 1 h (**Figure 1D**) were similarly present at time 0. None of these peaks coeluted with known standards of phenolic acids.

Figure 2 shows the plasma levels of caffeic acid measured before and at 1 and 2 h after coffee brew consumption in 10 volunteers. At 1 h after coffee brew consumption a significant increase in free caffeic acid plasma levels was found in untreated plasma samples in respect to time 0 ($p < 0.05$, paired *t*-test) (**Figure 2A**, **Table 2**). For one subject (subject 3) the plasma caffeic acid level was higher at 2 h than at 1 h, while for three other subjects (subjects 4, 6, and 10) very similar levels were detected at 1 and 2 h after coffee consumption (**Figure 2A**). In all of the remaining subjects, plasma caffeic acid levels at 2 h were lower in respect to levels measured at 1 h. After β-glucuronidase treatment, total caffeic acid levels were found to be significantly higher at both 1 and 2 h after coffee administration with respect to time 0 ($p < 0.05$, paired *t*-test, **Table 2**), with a maximum absorption peak at 1 h for all subjects (91.1 ± 33.2 ng/mL) (**Figure 2B**). Alkaline hydrolysis treatment of plasma samples gave similar results, with significantly higher levels of total caffeic acid at 1 and 2 h with respect to time 0 ($p < 0.05$, paired *t*-test, **Table 2**) and a maximum absorption peak at 1 h (**Figure 2C**). As summarized in **Table 2**, both β-glucuronidase and alkaline hydrolysis treatments released a considerable amount of caffeic acid at 1 and 2 h after coffee consumption. No significant differences were observed between caffeic acid values obtained with the two hydrolytic procedures (ANOVA, Scheffe's method). Caffeic acid levels at time 0 were not statistically different in untreated samples and in β-glucuronidase-treated and alkaline hydrolysis treated samples (ANOVA, Scheffe's method).

Administration of the control drink (water) did not result in any significant increase of caffeic acid levels at both 1 h and 2 h with respect to time 0 in untreated, β-glucuronidase-treated, and hydrolyzed plasma samples.

DISCUSSION

Phenolic acids are present in all plant-derived foods and in most diets. The average phenolic acid intake of men and women has been reported to be in the order of 200 mg/d within a large range, depending on nutritional habits and preferences (*24*).

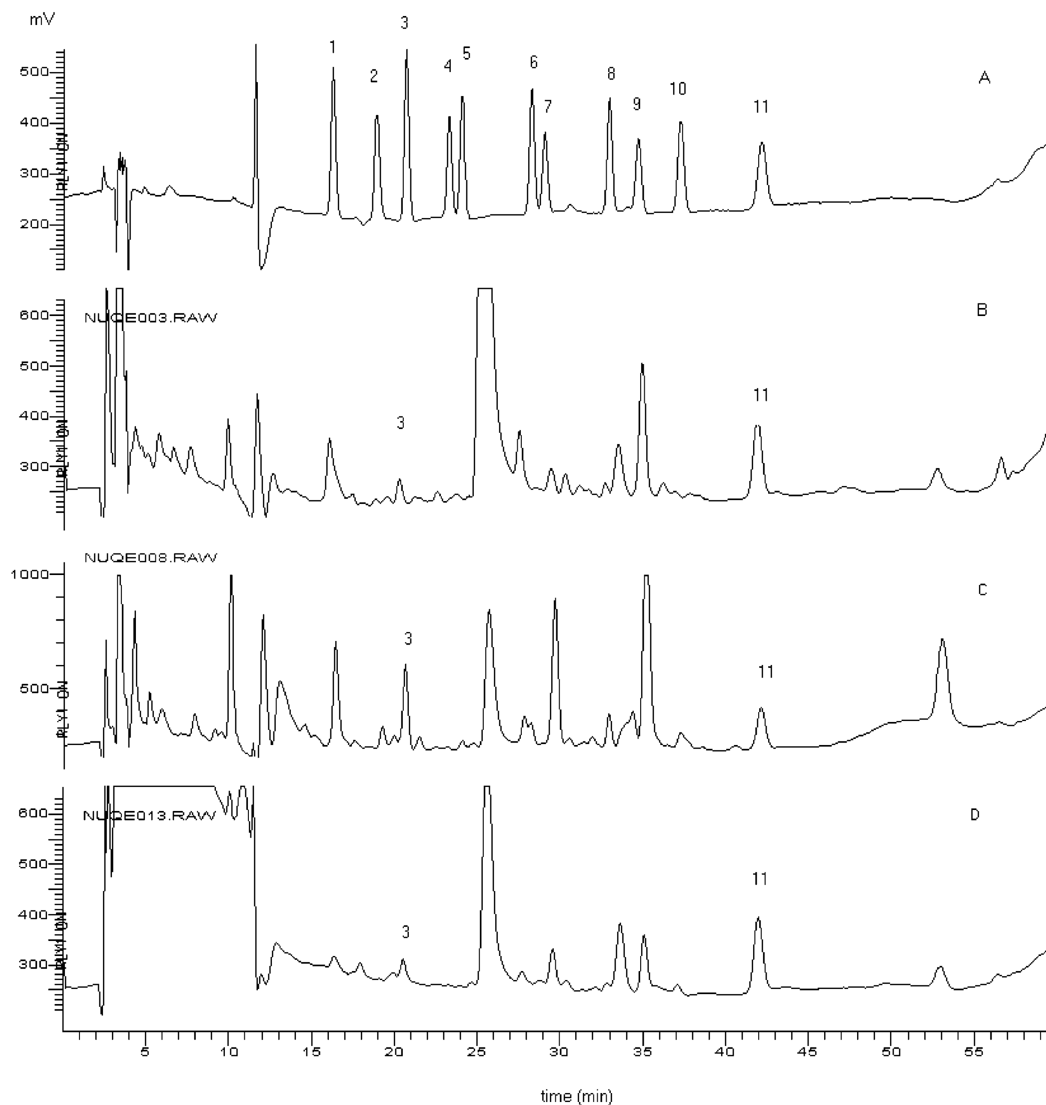

Figure 1. High-performance liquid chromatograms of (A) a standard mixture of phenolic acids (range 2.5–10 ng), (B) a nontreated plasma sample, (C) a β-glucuronidase-treated plasma sample, and (D) a hydrolyzed plasma sample. Chromatograms correspond to 0.05 mL of original plasma in (B) and (C) and to 0.01 mL of original plasma in (D). The operating conditions are reported in the Materials and Methods section. Peak identification: 1, dihydrocaffeic acid; 2, chlorogenic acid; 3, caffeic acid; 4, homovanillic acid; 5, syringic acid; 6, 3-(*p*-hydroxyphenyl)propionic acid; 7, *p*-coumaric acid; 8, ferulic acid; 9, *m*-coumaric acid; 10, isoferulic acid; 11, *o*-coumaric acid.

Most of the phenolic acids present in the diet are ester-linked to sugars, quinic acid, and other compounds.

In this study, coffee brew was chosen as the dietary phenolic acid source because of its high content of bound phenolic acids, particularly chlorogenic acid (5′-caffeoylquinic acid), while free phenolic acids (caffeic acid, ferulic acid, *p*-coumaric acid) are absent. Moreover, a previous study reported the presence of specific caffeic acid metabolites in human urine after coffee consumption (*26*).

In the present study we were able to demonstrate a significant rise in both free and bound plasma caffeic acid levels, with a maximum absorption peak at 1 h after coffee brew administration. Due to the absence of free caffeic acid in coffee brew (**Table 1**), it can be argued that coffee chlorogenic acid is the source of plasma caffeic acid. Moreover, we failed to detect chlorogenic acid in plasma samples after coffee consumption. However, we cannot completely exclude that traces of chlorogenic acid could be present in our samples at concentrations under the detection limit of our instrument (about 200 pg of chlorogenic acid injected, corresponding to a theoretical plasma concentration of about 10 nM). Also, we failed to find detectable amounts of ferulic and *p*-coumaric acid in plasma samples, although they might be present at very low concentration, under the detection limit of our instrument.

The absence of chlorogenic acid simultaneously with an increase in caffeic acid levels in human plasma after coffee consumption can be explained on the basis of two different possible mechanisms.

First, chlorogenic acid from administered coffee is not absorbed as such, but it undergoes hydrolysis in the gastrointestinal tract by the action of cytosolic esterases in the gut mucosa or gut microflora. Released caffeic acid is then absorbed and enters the vascular system. Acid hydrolysis of chlorogenic acid in the stomach is unlikely to occur due to the stability of chlorogenic acid at pH 2, which is the pH found in the stomach. Further, chlorogenic acid has been reported to be stable in artificial digestive juice (*27*). Esterases with the ability to hydrolyze hydroxycinnamate esters at appreciable rates have been recently described in humans and rats (*28*). The cinnamoyl esterase activity is distributed all along the small and large

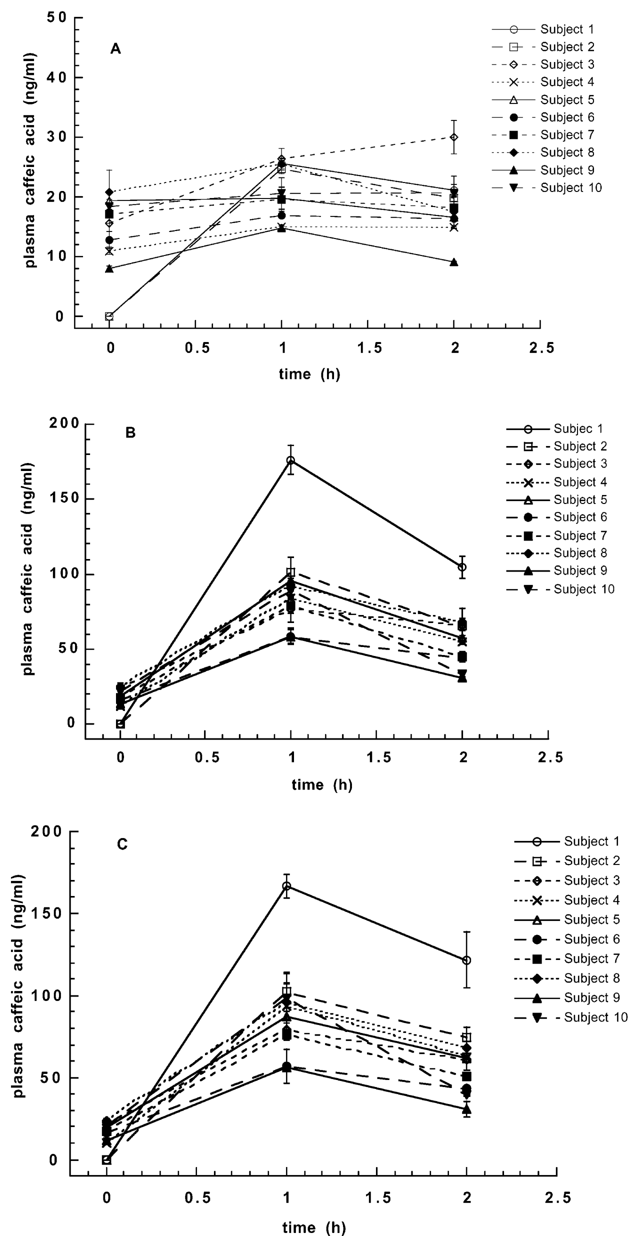

Figure 2. Plasma caffeic acid levels in 10 different subjects before and after coffee consumption. Plasma samples separated from blood collected just before (time 0) or after (1 h and 2 h) coffee administration were analyzed for free and total caffeic acid as reported in the Materials and Methods section. (A) untreated samples; (B) β-glucuronidase-treated samples; (C) alkaline hydrolysis treated samples. Values are means ± SD of three determinations.

intestine and is present both in the mucosa cells and in the lumen. Moreover, small amounts of caffeic acid, besides chlorogenic acid, were found in the small intestine after oral administration of chlorogenic acid in rats, suggesting that hydrolysis of chlorogenic acid occurs early in the gastrointestinal tract (*29*). Bacteria in the gastrointestinal tract of mammals are also capable of releasing free phenolic acids from bound complexes into the gastrointestinal tract (*30*−*33*). Indeed, an esterase activity able to hydrolyze chlorogenic acid and release caffeic acid has been demonstrated in human colonic microflora (*34*, *35*). However, in our study, plasma concentrations of caffeic acid peaking at about 1 h after coffee ingestion seem to indicate that hydrolysis of chlorogenic acid occurs early in the gastrointestinal tract.

Table 2. Plasma Caffeic Acid Levels (ng/mL) before and after Coffee Consumption[a]

treatment	time 0	time 1 h	time 2 h
no treatment	12.6 ± 7.4	20.9 ± 4.4[b]	18.4 ± 5.3
	(0–20.8)[c]	(14.8–26.4)	(9.1–30.0)
β-glucuronidase	14.5 ± 8.8	91.1 ± 33.2[b]	57.0 ± 21.4[b]
	(0–24.7)	(58.3–176.3)	(30.8–104.7)
alkaline hydrolysis	13.9 ± 8.4	91.3 ± 31.1[b]	61.2 ± 25.1[b]
	(0–23.3)	(56.3–166.7)	(30.9–121.5)

[a] Plasma samples separated from blood collected just before (time 0) and after (1 and 2 h) coffee administration were analyzed for free and total caffeic acid as reported in Materials and Methods. Data presented are means ± SD of 10 different subjects and are expressed as ng/mL of plasma. [b] $p < 0.05$ from time 0 level (paired *t*-test). [c] Values in parentheses represent the range.

Second possible mechanism is that chlorogenic acid from administered coffee is absorbed as such and rapidly hydrolyzed and metabolized in human plasma. However, data from the literature do not support this hypothesis. In fact, in an in vitro model using the small intestine from rats only very little absorption of chlorogenic acid (0.1%) was found (*36*). Moreover, a recent study reported no evidence of enzymic hydrolysis of chlorogenic acid by human plasma (*34*).

Our results are in agreement with previous studies on rats demonstrating that, after ingestion of chlorogenic acid, caffeic acid, but not intact chlorogenic acid, was found in the plasma (*29*, *37*), and no chlorogenic acid was found in urine (*38*). Moreover, caffeic acid was present in urine after consumption of chlorogenic acid in humans (*39*). Further, after consumption of chlorogenic acid containing fruits, caffeic acid metabolites, but not intact chlorogenic acid, were found in human urine (*40*). A recent study (*41*) reported that chlorogenic acid is absorbed at some extent in humans after oral supplementation (1 g of chlorogenic acid ingested). However, in this study the reported absorption was calculated as the difference between the amount of chlorogenic acid ingested and the amount excreted in ileostomy fluid in subjects without a colon, while direct measurements of chlorogenic acid in blood were not performed. Therefore, it cannot be excluded that part of the supplemented chlorogenic acid was lost in the gastrointestinal tract. Moreover, the amount of chlorogenic acid administered in this study was 10-fold higher than the amount of chlorogenic acid present in a cup of coffee, as used in our study.

Our results, obtained using two different hydrolytic procedures, indicated that, following coffee brew administration, caffeic acid is present in plasma mainly in bound forms. Moreover, the fact that similar values of total plasma caffeic acid were obtained with the two above-reported procedures would exclude the presence of bound forms of caffeic acid different from glucuronate/sulfate conjugates. The glucuronidation is likely to occur in the liver (*42*) but also possibly in the small intestine (*36*) and colon, where UDP-glucuronyltransferase activity has also been reported (*43*). A previous study reported that, following caffeic acid perfusion, the major products transferred across the rat intestinal epithelium were glucuronides (*36*).

On the basis of caffeic acid content of coffee brew measured after hydrolytic treatment, an intake of about 166 mg of caffeic acid can be calculated for a cup of coffee. The total plasma caffeic acid concentration measured 1 h after coffee consumption ranged from 0.32 to 0.98 μM after β-glucuronidase treatment (from 0.31 to 0.92 μM after alkaline hydrolysis). Our data are consistent with data from the literature concerning the absorption

of polyphenols. In fact, the plasma concentration of an individual molecule has been reported rarely to exceed 1 μM after the consumption of 10−100 mg of a single compound (*1*). However, it must be taken into account that, due to the absence of free caffeic acid in coffee brew, the level of plasma caffeic acid obtained in our study represents the amount of caffeic acid absorbed following the in vivo release of free caffeic acid from bound complexes present in coffee.

In conclusion, our study suggests that caffeic acid, even if present in the diet in bound forms, such as chlorogenic acids, is still bioavailable to humans. Further studies will be necessary to investigate the absorption of other phenolic acids, such as ferulic and *p*-coumaric acids, from their bound forms.

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