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Effect of Simultaneous Consumption of Milk and Coffee on Chlorogenic Acids' Bioavailability in Humans

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ABSTRACT: Different studies have shown that milk may interact with polyphenols and affect their bioavailability in humans. The present study investigated the effect of the simultaneous consumption of coffee and milk on the urinary excretion of chlorogenic acids (CGA) and metabolites. Subjects were submitted to consumption of water, instant coffee (609 mmol of CGA) dissolved in water, and instant coffee dissolved in whole milk. Urine was collected for 24 h after consumption of each treatment for analysis of CGA and metabolites by HPLC/LC−MS. The amount of CGA and metabolites recovered after consumption of combined coffee−milk (40% ± 27%) was consistently lower in all subjects compared to that of coffee alone (68% ± 20%). Concluding, the simultaneous consumption of milk and coffee may impair the bioavailability of coffee CGA in humans.

KEYWORDS: chlorogenic acids, bioavailability, coffee, coffee and milk interaction, polyphenols

INTRODUCTION

In the past few years, coffee began to be considered by many as a functional food, owing to its high content of bioactive compounds, mainly the chlorogenic acids (CGA), which usually account for about 1−4% of roasted coffee composition.[1] These phenolic compounds are esters of hydroxycinnamic acids and quinic acid. The main subclasses in coffee are caffeoylquinic acids (CQA), dicaffeoylquinic acids (diCQA), and feruloylquinic acids (FQA) with at least three isomers per group.[2] Minor CGA compounds include *p*-coumaroylquinic acids (*p*-CoQA) and mixed esters such as caffeoylferuloylquinic acids and caffeoyltryptophan. A series of studies have shown that these compounds possess antioxidant,[3] antimicrobial,[4] hepatoprotective,[5] immunostimulating,[6] and hypoglycemic properties,[7] among others. CGA lactones (CGL) have also been shown to be bioactive in vivo.[8] As a consequence, various new coffee-based products are being created and massive research is being performed in the intent of combining the peculiar and appreciated flavor of coffee with its biological properties.[1]

Today, coffee is the most consumed beverage in the world, with the United States and Brazil as the main consumer countries. Regarding consuming habits, while in the United States adding cream to coffee is a regular practice, in Brazil, whole, skimmed, or semiskimmed milk accompanies coffee very often, in different amounts, according to the population segment or individual tastes. One of the most common consumption habits is adding a few grams or milliliters of instant or brewed coffee to a cup of whole milk.

We have recently shown that CGA are considerably bioavailable in humans.[9,10] However, previous studies have also shown that the bioavailability of polyphenols may be affected by the interaction with dietetic constituents, especially proteins.[11,12] Additionally, Muralidhara et al.[13] and Dupas et al.[14] showed using in vitro assays that albumin and casein, respectively, were able to bind CGA by both covalent and noncovalent interactions and suggested that the simultaneous consumption of coffee and milk could result in a negative impact on CGA absorption. However, studies investigating this hypothesis are scarce and inconclusive. Thus, the aim of the present study was to evaluate the effect of the simultaneous consumption of coffee and milk on the urinary excretion of CGA and metabolites.

EXPERIMENTAL PROCEDURES

Subjects. Five nonsmoking subjects (three female and two male), 24−35 years of age, were recruited at the Federal University of Rio de Janeiro (UFRJ). They were healthy as judged by a medical questionnaire, with normal blood values for hemoglobin and hematocrit, and were not taking any medication or nutritional supplements at the time of the study. The study protocol was approved by the Ethics Committee of Clementino Fraga Filho Hospital (UFRJ) and fully explained to the subjects, who gave their written informed consent prior to participation in the study.

Coffee Beverage Preparation. Samples of regular and instant coffee (*Coffea canephora* cv. Conillon) were kindly provided by the COCAM Co. (São Paulo, Brazil). The coffee beverage was prepared by mixing 4 g of commercial medium roast instant coffee with 200 mL of freshly hot water (60−70 °C). The coffee−milk beverage was prepared in the same way as the coffee beverage, with replacement of water by whole milk (200 mL) purchased in a local market and free of additives. The milk doses offered to the subjects contained 9.6 g of carbohydrates, 6.7 g of proteins, and 7.1 g of lipids. Both drinks were prepared on each day of the study, immediately before consumption.

Study Design and Sample Collection. Subjects were instructed not to consume phenolic-containing foods during the 48 h prior to each experiment and during the 24 h of the day of the experiment. They were monitored to repeat the same diet for the three treatments. All the treatments were performed in a randomized crossover design with a minimum 7 day interval between tests. On separated days, after 10 h of overnight fasting, a standard amount of test beverage (200 mL) was offered to each subject. Urinary samples were collected for 4 h before (baseline) and at intervals of 0−4, 4−8, 8−12, and 12−24 h after each treatment into appropriate plastic containers, and aliquots for determination of CGA and metabolites were acidified with HCl 4 N and kept frozen at −80 °C until analysis.

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Table 1. Contents of the Main Chlorogenic Acids (CGA) and Caffeoyl-1,5-quinolactones (CGL) in the Instant Coffee Portion (4 g) Offered to the Subjects (n = 5) in Both Coffee Treatments[a]

compound	content (μmol/portion)
3-caffeoylquinic acid	151.2 ± 7.9
4-caffeoylquinic acid	149.2 ± 6.9
5-caffeoylquinic acid	172.5 ± 7.9
total caffeoylquinic acids	**472.9 ± 13.8**
3,4-dicaffeoylquinic acid	9.3 ± 0.4
3,5-dicaffeoylquinic acid	6.3 ± 0.3
4,5-dicaffeoylquinic acid	5.7 ± 0.2
total dicaffeoylquinic acids	**21.3 ± 0.1**
3-feruloylquinic acid	28.0 ± 1.6
4-feruloylquinic acid	21.2 ± 0.9
5-feruloylquinic acid	17.9 ± 1.2
total feruloylquinic acids	**67.1 ± 1.6**
5-*p*-coumaroylquinic acid	**6.76 ± 0.6**
caffeoylferuloylquinic acids[b]	**3.70 ± 0.2**
caffeoyltryptophan	**17.50 ± 2.3**
total CGA	**589.26 ± 17.6**
3-caffeoyl-1,5-quinolactone	11.28 ± 2.5
4-caffeoyl-1,5-quinolactone	9.08 ± 1.3
total CGL	**20.36 ± 1.2**
total CGA and CGL	**609.62 ± 12.8**

[a] Results are the mean ± SD of triplicate extraction. [b] Total of six isomers.

Blood Hemoglobin and Hematocrit Analyses. Hemoglobin was measured by the cyanomethmyoglobin method using a commercial kit (Bioclin, Quibasa, Brazil). Hematocrit was determined by conventional capillary centrifugation.

CGA Extraction in Brewed Coffee. CGA in coffee beverages were extracted according to Farah et al.[8] using methanol (40%) and Carrez solutions for clarification.

CGA and Metabolite Extraction in Urine Samples. GGA and metabolites were extracted in duplicate as described in detail by Monteiro et al.[9] using *Helix pomatia* (Sigma-Aldrich) extract containing β-glucoronidase and sulfatase activities for deconjugation of glucuronic acid conjugates and sulfated forms.

Analyses of CGA and Metabolites in Coffee and Urine. Analyses were performed according to Farah et al.[8] for coffee and Monteiro et al.[9] for urine using an HPLC-DAD (DAD = diode array detector) gradient system (SPD-M10A, Shimadzu, Japan) with a C18 Kromasil guard column and column (Akzo Nobel, New York) and a gradient with methanol and 0.3% formic acid running at 1 mL/min. Identification and quantification of CGA and metabolites in coffee and urine were performed by comparison of the retention time of investigated peaks with those of the respective standards. Peak identities were confirmed by LC−MS, according to Farah et al.[15]

Standards were injected as a pool. A mixture of 3-CQA, 4-CQA, and 5-CQA was prepared from 5-CQA using the isomerization method of Trugo and Macrae.[16] For diCQA, a mixture of 3,4-diCQA, 3,5-diCQA, and 4,5-diCQA was kindly donated by Professor Macrae (University of Reading, England). Standards of 5-CQA, caffeic acid, ferulic acid, isoferulic acid, *p*-coumaric acid, dihydrocaffeic acid, vanillic acid, gallic acid, syringic acid, sinapic acid, benzoic acid, 4-hydroxybenzoic acid, 2,4-dihydroxybenzoic acid, *trans*-3-hydroxycinnamic acid, 3-(4′-hydroxyphenyl)propionic acid, 3,4-dihydroxyphenylacetic acid, and hippuric acid were purchased from Sigma-Aldrich (St. Louis, MO).

Recovery determinations were made by considering the total number of equivalent moieties of cinnamic and quinic acids consumed in the coffee extracts and the total number of phenolic acid moieties recovered in urine collected for 24 h after each treatment.

Statistical Analysis. Results are presented as means with the corresponding SD. The comparison between the urinary concentrations of CGA and metabolites was performed using analysis of variance (ANOVA) factorial and one-way ANOVA, with the post-test of Fisher. All statistical analyses were performed using GraphPad Prism software version 4.0. Differences were considered significant at $p < 0.05$.

RESULTS

CGA in Brewed Coffee Beverages. Nineteen CGA and CGL compounds were quantified in the 4 g portion of instant coffee (in 200 mL of water or milk) offered to subjects in both coffee treatments (Table 1). CQA represented most of CGA composition (77.5%), followed by FQA (∼11%), diCQA and CGL (∼3.5% each), caffeoyltryptophan (∼3%), 5-*p*-CoQA (∼1%), and caffeoylferuloylquinic acids (∼0.5%). The mean total amount of CGA in the instant coffee portion was 0.61 mmol, which corresponded to 1.19 mmol in equivalent moieties of cinnamic and quinic acids.

Subject Characterization. Three female and two male subjects participated in this study. All hemoglobin and hematocrit values were normal compared to reference data. There was no significant difference among the anthropometric and biochemical parameters observed in all three treatments (Table 2).

Urinary Excretion of CGA Compounds and Metabolites after Water, Coffee, and Coffee−Milk Consumption. Six CGA (3-CQA, 4-CQA, 5-CQA, 3,4-diCQA, 3,5-diCQA, and 4,5-diCQA) and 16 phenolic compounds (caffeic, vanillic, ferulic, isoferulic, *p*-coumaric, gallic, 4-hydroxybenzoic, dihydrocaffeic, syringic, sinapic, 2,4-dihydroxybenzoic, hippuric, 3,4-dihydroxyphenylacetic, 3-(4′-hydroxyphenyl)propionic, *trans*-3-hydroxycinnamic, and benzoic acids) were identified in the baseline urine and in all intervals for 24 h after the consumption of all three treatments.

Baseline CGA values ranged from 46.5 to 922 μmol with variations from 1- to 9-fold for the same individual intraday. Total phenolic acids ranged from 330.1 to 4823.2 μmol, with variations from 1- to 14-fold for the same individual intraday.

The total urinary excretion of phenolic compounds in the water treatment was 1.3 ± 0.8 mmol. Higher amounts of CGA and metabolites were excreted after coffee (3.3 ± 1.4 mmol, $p = 0.004$) and coffee−milk (2.2 ± 0.6 mmol, $p = 0.006$) consumption. While after water consumption the highest average excretion of phenolic compounds occurred between 4 and 8 h, after coffee and coffee−milk consumption, the highest average excretion occurred between 8 and 12 h (Figure 1). Although a large interindividual variation was observed in the urinary excretion of CGA and metabolites in all three treatments, varying from 0.33 to 4.8 mmol, subjects showed a similar pattern of excretion up to 24 h after the consumption of the three treatments regarding the predominance of compounds. Hippuric, 3,4-dihydroxyphenylacetic, dihydrocaffeic, vanillic, and gallic acids were the main compounds identified after the beverage consumption, except for in water treatment in which 3,4-dihydroxyphenylacetic acid was a minor compound. Together, these five compounds were responsible for about 96% and 97% of all compounds excreted in water and coffee treatments, respectively.

Hippuric acid (*N*-benzoylglycine) excretion after water, coffee, and coffee−milk consumption represented respectively 88%,

Table 2. Average Anthropometric and Biochemical Characterization of Subjects (n = 5) on the Days of Water, Coffee, and Coffee−Milk Treatments

	mean ± SD	min−max	reference values
age (years)	26.5 ± 0.71	24−35	
BMI (kg/m^2)	22.45 ± 3.18	19.32−27.70	18.5−25.0[a]
hematocrit (%)	44.0 ± 2.97	38.3−58.6	40−45[b]
hemoglobin concentration (g/dL)	12.16 ± 1.19	11.32−16.6	11.5−13.5[b]

[a] World Health Organization (WHO). [b] Reference 48.

80%, and 80% of the total phenolic compounds identified up to 24 h after consumption. Higher amounts of this compound were observed in urine after coffee (2.5 ± 0.4 mmol) and coffee−milk (1.7 ± 0.3 mmol) consumption when compared to water consumption (1.1 ± 0.4 mmol). 3,4-Dihydroxyphenylacetic acid was the second major compound excreted for up to 24 h and followed the same pattern as hippuric acid, with a higher excretion in coffee treatment (0.5 ± 0.3 mmol), followed by coffee−milk (0.2 ± 0.1 mmol) and water (0.01 ± 0.02 mmol) treatments. High levels of dihydrocaffeic, vanillic, and gallic acids were also present in the urine of subjects after consumption of both coffee beverages, being together responsible for 7% of total urinary excretion of phenolic compounds (Figure 2).

With respect to minor phenolic compounds, such as *p*-coumaric, syringic, sinapic, benzoic, 4-hydroxybenzoic, 2,4-dihydroxybenzoic, *trans*-3-hydroxycinnamic, and isoferulic acids, together they contributed to less than 5% of total urinary excretion without significant differences among the three treatments. With respect to CGA compounds, 3-CQA, 4-CQA, and 5-CQA isomers were found in urine samples, resulting in less than 1% of total urinary excretion. Although a decrease of 23% was observed in the urinary excretion of CQA in coffee and coffee−milk treatments (from 4.37 ± 3.9 to 3.36 ± 2.02 μmol), it was not statistically significant. DiCQA were also identified in the urine of subjects, but it was not possible to quantify them because the values were below the detection limit (LOD = 0.002 μmol/L for 5-CQA). No FQA, *p*-CoQA, caffeoyltryptophan, or CGL was identified in the evaluated urine samples.

Regarding the urinary recovery of phenolic metabolites and CGA up to 24 h after the consumption of coffee containing on average 0.61 mmol of CGA—which corresponded to 1.20 mmol of cinnamic and quinic acid equivalents—subjects excreted from 0.41 to 1.1 mmol (average of 68%) of consumed CGA equivalents in coffee treatment. On the other hand, after coffee−milk consumption, only 0.31−0.64 mmol (average of 40%) of ingested CGA equivalents was excreted. This represents an average decrease of 28% compared to coffee alone.

Considering the total urinary excretion of intact CGA compounds, only from 0.3% to 1.2% (average of 0.7%) of total CGA was recovered in urine after coffee consumption, whereas from 0.2% to 0.7% (average of 0.5%) was recovered after coffee−milk consumption, an average decrease of 19% compared to coffee alone.

DISCUSSION

The CGA contents in both plain coffee and coffee−milk beverages (200 mL) used in the present study are in agreement with contents reported in the literature for coffee brews.[8,15−17]

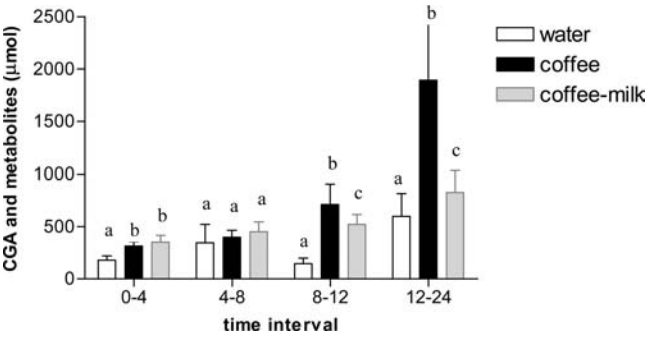

Figure 1. Total of CGA and metabolites excreted in urine of subjects for 24 h after water, coffee, and coffee−milk consumption at the following time intervals: 0−4, 4−8, 8−12, and 12−24 h. Different letters in the same time interval indicate statistical difference (one-way ANOVA, with Fisher post-test, $p < 0.05$).

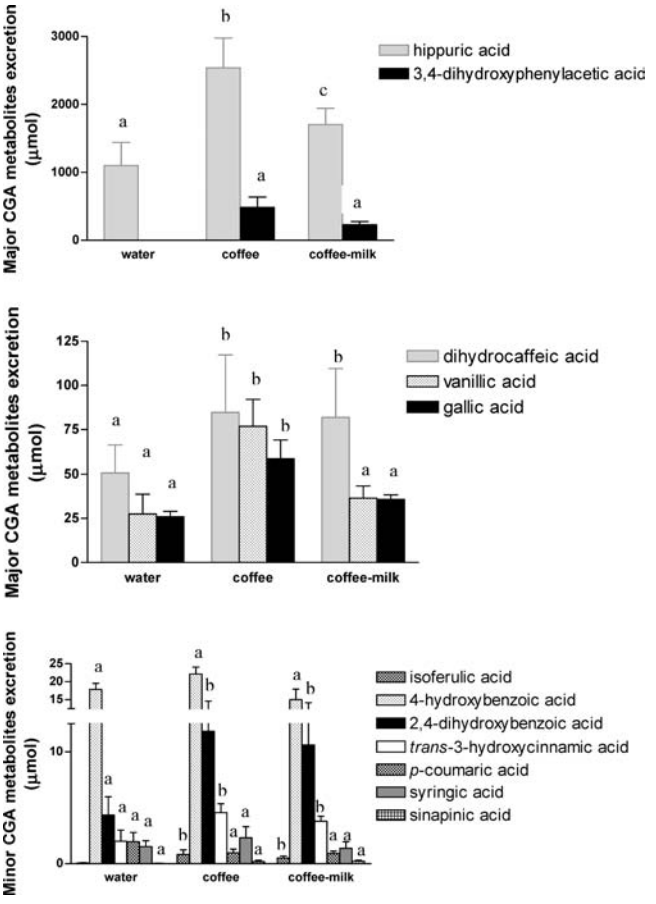

Figure 2. Major (A, B) and minor (C) phenolic compounds excreted for 24 h after water, coffee, and coffee−milk consumption. Different letters in the same time interval indicate statistical difference (one-way ANOVA, with Fisher post-test, $p < 0.05$).

This is the first study to quantify CGA and metabolites in human urine after the consumption of water, coffee, and instant coffee added to milk. The large interindividual variability observed in the urinary excretion of CGA and metabolites in all treatments has been commonly observed in studies evaluating the bioavailability of polyphenols[9−11,18,19] and is probably due to

differences in the subjects' capacity to absorb and metabolize these compounds, among other factors.

Regarding baseline and water treatment results, Nurmi et al.[18] have previously identified 10 phenolic compounds (averaging 95 μmol) in human baseline urine samples after a 7 day phenolic-free diet. In the present study, although subjects were monitored not to consume food sources of phenolic compounds during the 48 h prior to each experiment and throughout the 24 h of the days of the experiment, the presence of phenolic compounds not only in baseline urine intervals but for 24 h after water consumption is not surprising considering the results from Nurmi et al.[18] after a 7 day phenolic-free diet as well as the large amounts of CGA identified in freshly secreted human digestive fluids after 12 h of fasting.[17] This reinforces the hypotheses of the long half-life of these compounds through enterohepatic recycling[17,20] and, less probably, partial storage and slow release of CGA in the human body, earlier proposed by Booth et al.[20] Because the excretion patterns of phenolic compounds in urine after water and coffee treatments were very different along the 24 h collection, the water treatment values were not used as a blank for coffee treatments.

Since studies evaluating the 24 h urinary excretion of CGA and metabolites after coffee consumption are scarce, it is difficult to make comparisons. Monteiro et al.[9] observed that human subjects excreted about 0.12 mmol of CGA compounds up to 6 h after consumption of a decaffeinated coffee brew containing 3.4 mmol of CGA. In the present study, although the CGA content in both coffee beverages offered to the subjects was 6 times lower than that used by Monteiro et al.,[9] the total amount of metabolites excreted by our subjects up to 8 h after coffee consumption was 6.4 times higher (757 mmol) than those reported by Monteiro et al.[9] Moreover, Farah et al.[10] reported excretion of 0.25 mmol of CGA compounds up to 8 h after oral administration of green coffee extract capsules containing in total 0.45 mmol of CGA, a 3 times lower amount than that observed in the present study. The higher amount of CGA metabolites identified in the present study may be explained by the fact that Monteiro et al.[9] and Farah et al.[10] identified 12 compounds in urine whereas 22 compounds (including a few major compounds) were quantified in the present study. On the other hand, Olthof et al.[21] reported excretion of 9.3 mmol of phenolic compounds in 24 h urine after a 7 day oral administration of a supplement containing 5.5 mmol of 5-CQA. The higher CGA dose consumed by the subjects, the higher number of identified compounds (60), and the longer period of intervention (7 consecutive days) used by Olthof et al.[21] probably justify the higher excretion observed compared with that of the present study.

The major hippuric acid excretion after the intake of dietary polyphenols in humans has previously been reported.[21−24] This acid could partially derive from the action of microorganisms in the colon, which are able to hydrolyze 5-CQA to caffeic and quinic acids,[24] or from hydrolysis and/or absorption of 5-CQA in the small intestine.[7,9,10] According to the metabolic pathway proposed by Olthof et al.,[24] subsequently, the caffeic acid molecule would be absorbed and then β-oxidized to benzoic acid, whereas the molecule of quinic acid would originate the cyclohexanecarboxylic acid, which would then be aromatized to benzoic acid by the action of colonic microflora and then absorbed by the tissues. In the kidney, benzoic acid would be conjugated with glycine and then excreted in the urine as hippuric acid. Thus, each molecule of 5-CQA would be able to give rise to two molecules of hippuric acid. Additionally, this acid may also be formed from other food components such as the aromatic amino acids tryptophan, tyrosine, and phenylalanine[22] and benzoic acid derivatives, widely used as food preservatives in industrial products such as canned foods.[25]

Differently from hippuric acid, 3,4-dihydroxyphenylacetic acid, the second major compound excreted in the 24 h urine of all subjects after coffee beverage consumption, is considered to be a marker of polyphenol metabolism, more specifically the flavonoid class of compounds.[20,23,25] This compound is also known to derive from the action of intestinal bacteria on a molecule of caffeic acid[18,25] and, for the first time, was identified in the urine of humans after ingestion of brewed coffee.

The major excretion of dihydrocaffeic and vanillic acids after coffee brew consumption has already been reported in the literature.[9,10,20,21,26] The fact that dihydrocaffeic acid is considered to be a primary metabolite of caffeic acid by Booth et al.[20] and Farah et al.,[10] among other studies, is consistent with a higher urinary excretion of this compound in the first 12 h after coffee consumption compared to the period between 12 and 24 h. Vanillic acid is, on the other hand, known as a secondary metabolite of molecules of quinic and caffeic acids,[10,21,26] which explains a higher excretion of this compound in the period between 12 and 24 h after consumption of both coffee beverages. The major excretion of gallic acid after coffee consumption had already been reported by Monteiro et al.[10] Its excretion has also been identified by Olthoff[21,24] after 5-CQA consumption. Like vanillic acid, this compound is also known as a secondary metabolite of caffeic, ferulic, and quinic acid molecules.

Different studies[9,10,27,28] suggest that ferulic, isoferulic, vanillic, and dihydrocaffeic acids are the main metabolites of caffeic acid identified in plasma or urine of humans. Additionally, the urinary excretion of 3-(4′-hydroxyphenyl)propionic, 3-*trans*-hydroxycinnamic, and benzoic acids, identified for the first time in urine after coffee consumption, has previously been reported in the literature after the consumption of other polyphenol-rich foods or beverages.[18,20,23,27] Figure 3 presents the urinary metabolites identified in the present study as well as in previous studies evaluating the metabolism of CGA and hydroxycinnamates.

The total percentage of CGA and metabolites, as well as the nature of the metabolites, recovered in urine for 24 h after coffee consumption indicates that an average of 68% ± 20% of CGA ingested were absorbed in the whole digestive tract. This includes absorption of intact CGA or primary CGA metabolites (such as caffeic and ferulic acids) by the stomach and small intestine, as reported in the literature,[9,10,22,23] and absorption of primary and secondary metabolites in the large intestine after colonic action, as previously reported in various studies evaluating the metabolism of phenolic acids and CGA.[18−31] The lack of data on the urinary recovery of CGA and metabolites after coffee consumption in the literature makes comparisons difficult. The present results are in accordance with the recovery of 67% of Olthoff et al.[24] in ileostomy fluids for 24 h after consumption of 2.8 mmol of 5-CQA. Farah et al.[10] reported that on average 26% of total CGA were recovered in the urine of subjects up to 8 h after consumption of decaffeinated green coffee extract containing 451 μmol of CGA, when expressed in absolute values. In the present study, when we calculate the urinary recovery up to 8 h after ingestion of coffee alone, the average recovery was 16.2% (13.7−19.1%), lower than the percentage reported by Farah et al.[10] This lower percentage may be explained by the fact that the highest excretion in the present study occurred from 8 to 24 h after coffee consumption. Stalmach et al.[32] reported an average recovery of 29% of glucuronated and sulfated forms of CGA and

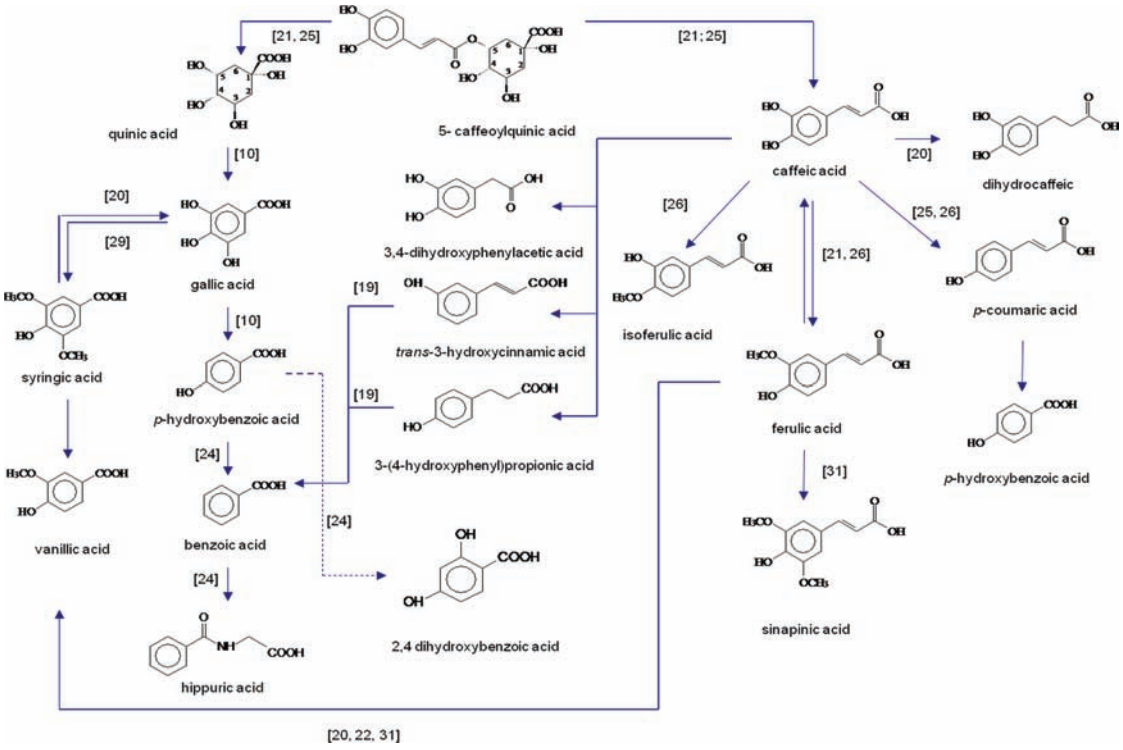

Figure 3. Simplified scheme of metabolites of 5-ceffeoylquinic acid 5-CQA—excluding conjugated forms with glucuronic acid and sulfate—as a representative of the chlorogenic acid class, based on results from previous studies as well as from the present study.

primary metabolites in healthy subjects after consumption of 412 μmol of CGA. Comparing the recoveries from Farah et al.[10] and Stalmach et al.[32] with the total phenolic compounds recovered in the present study (68% $\pm$ 20%), the higher urinary recovery percentages obtained in the present study may be explained by the compounds derived from the metabolism of colonic bacteria as well as other compounds which were only accounted for in this study. Excluding from recovery calculations the compounds 3-hydroxyphenylacetic, (3,4-dihydroxyphenyl)propionic, 2,4-dihydroxybenzoic, and *trans*-3-hydroxycinnamic acids, which are known as exclusively colonic metabolites, only 10.5−23% of total CGA consumed were recovered in urine in the form of metabolites after coffee, which is in accordance with the literature.[9,10,21,24]

Lower total recovery values (averaging 51%) would be obtained if water treatment excretions were used as a blank. However, there is no way to guarantee that the amount of phenolic compounds excreted during fasting would still be excreted as a baseline even after phenolic-containing food consumption. This is corroborated by the different excretion profiles of water and coffee treatments cited above.

The range of urinary concentrations of CGA and metabolites recovered in the present study is of the same order of magnitude (μmol) as those from different studies evaluating the metabolism of other polyphenols,[9,10,19,24] even though recoveries (%) varied considerably in such studies according to the metabolites accounted for, analytical methodologies applied, study designs, and so forth. For example, in a review from Manach et al.,[34] urinary recoveries of 1.1% and 30.2% of naringenin from orange and grapefruit juice, respectively, were reported after consumption of 23 mg of naringenin equivalents. On the same line, urinary recovery of 0.1% of metabolites was observed after consumption of epigallocatechin gallate through green tea (2 mg/kg of body weight), while 55% recovery was observed after consumption of 2 g of pure cathechin. Similar variations have been observed in studies investigating isoflavone bioavailability. While Kano et al.[35] reported a urinary recovery of 6.8% of total isoflavones after consumption of 0.6 mg of daidzein and 1 mg of genistein from soy beverage, Setchell et al.[36] reported a recovery of 54.5% after consumption of 0.55 mg of daidzein and 0.15 mg of genistein from soy germ.

The low recovery of intact CGA compounds (less than 1%) is in accordance with previous studies in humans evaluating the metabolism of CGA and other hydroxycinamates,[9,10,24,29] supporting evidence that urine is not the preferential excretion route of intact CGA compounds,[29] which are excreted in free and conjugated forms in digestive fluids and reabsorbed in the intestinal tract after breakdown of conjugates by the intestinal microflora.[7] Exceptions are the two studies from Stalmach et al.[32,33] evaluating ileostomized and healthy patients in which 24 h urinary recovery of sulfated and glucuronated forms of CGA after coffee consumption were equivalent to 8−10% of the consumed CGA amount. Considering the different analytical methodologies used as well as the lower amounts consumed in these studies compared to the present study as well as other studies, comparisons are difficult. Dose−response studies using the same methodologies are required for further discussion.

Comparing the total 24 h excretion of polyphenols after both coffee treatments, as the subjects ingested the same amount of CGA in both of them, an average reduction of 28% on the excretion of CGA and metabolites after the consumption of coffee−milk treatment indicates that adding coffee to milk quantitatively altered the absorption and/or the metabolism of CGA from coffee in all subjects. This decrease was mainly driven by the differences in the colonic metabolites hippuric acid and 3,4-dihydroxyphenylacetic acid. The lower excretion of these two

compounds when comparing coffee and coffee−milk treatments was also observed by Urpi-Sarda et al.[19] in investigating the effect of simultaneous consumption of cocoa powder and milk in humans. Although the 23% decrease in the urinary recovery of CGA compounds in coffee−milk treatment compared to coffee alone was not significant probably due to the small number of subjects as well as the differences among them, the 28% decrease in total urinary excretion demonstrates that adding large amounts of milk to coffee decreased the bioavailability of these compounds. This was consistently observed in all subjects.

It is noteworthy to mention that the amount of milk used in this study is commonly suggested by instant coffee manufacturers in Brazil, but the proportion of coffee and milk used by consumers may vary considerably according to cultural habits and individual preferences around the world. It is possible that lower amounts of milk added to coffee would not interfere in CGA absorption and/or metabolism. In fact, Renoulf et al.[37] reported that the addition of 20% whole milk to coffee did not affect CGA bioavailability in humans.

Studies investigating the effect of simultaneous consumption of coffee and other dietary components on the absorption and metabolism of CGA in the human body are scarce. Nonetheless, in addition to agreeing with the results from Urpi-Sarda[19] cited above, the present results are consistent with the findings of Mullen et al.,[38] who observed in humans a significant decrease in the urinary excretion of flavonoid metabolites caused by the addition of milk to a cocoa drink. According to the authors, this result seems to be a direct consequence of interactions between milk constituents and flavonoids present in chocolate, which probably would have caused changes in the mechanism of transport of these compounds through the intestinal wall into the bloodstream. These findings, as well as those observed in the present study, corroborate the results from Dupas et al.,[14] who observed that at least 40% of 5-CQA was associated with milk proteins, especially casein in an in vitro digestion system. From this amount, about 17% of 5-CQA remained complexed to milk proteins and peptides to the end of digestion, modifying, hypothetically, its absorption. Also, in addition to showing that ferulic, caffeic, and gallic acids, as well as 5-CQA, may interact with whey proteins such as β-lactoglobulin, Rawel et al.[39] observed in an in vitro digestion system that these complexes may adversely affect their susceptibility to proteolysis by gastrointestinal enzymes such as trypsin, chymotrypsin, pepsin, and pancreatin. This event could, therefore, hinder the release of phenolic compounds from the protein complex and consequently their absorption.

Additional studies have investigated the effect of the simultaneous consumption of milk on polyphenol bioactivity. Serafini et al.[40,41] demonstrated that adding 26% whole milk to black/green teas and blueberry juice affected the antioxidant status in human plasma. Similar results were observed by Reddy et al.[42] in a study in which 20% whole milk was associated with black tea. In line with these results, Lorenz et al.[43] demonstrated that adding 10% whole milk to black tea counteracted the favorable health effects of tea cathechins on vascular function. The three studies attributed the milk interference on the bioactivity of polyphenols to the negative impact of its proteins on polyphenol absorption. On the other hand, three additional studies evaluating the effect of simultaneous consumption of milk and polyphenols from green tea,[44,45] black tea,[44,46] and blueberry juice[47] on plasma antioxidant activity did not observe differences among treatments, although the authors acknowledged the possibility of formation of polyphenol−protein complexes.

In conclusion, considering the results from the present study as well as from previous studies, the simultaneous consumption of milk and coffee may produce a negative effect on CGA bioavailability in humans. This effect seems to depend on the proportion of milk to coffee used. In addition to dose−response studies evaluating CGA bioavailability, more studies are needed to determine the minimum proportion of milk to coffee that will impair CGA bioavailability after coffee consumption.

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NOTE ADDED AFTER ASAP PUBLICATION

Figure 2 has been revised from the original online publication of June 15, 2011. The version published online on June 23, 2011, is correct.