

Bioavailability of chlorogenic acids in rats after acute ingestion of maté tea (*Ilex paraguariensis*) or 5-caffeoylquinic acid

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Abstract

Purpose Yerba maté is widely consumed in South America as different beverages, such as maté tea (roasted leaves) and *chimarrão* (green dried leaves), and linked to health benefits, mainly attributed to chlorogenic acids (CGAs). Health effects of CGAs depend on their bioavailability, but such data are scarce. The aim of this study was to investigate the distribution of CGAs and metabolites in tissues, hepatic and plasmatic kinetic profile and urinary excretion after ingestion of maté tea or 5-caffeoylquinic acid (5-CQA).

Methods Wistar rats ingested maté tea (MT) or 5-CQA (ST) and were killed after 1.5 h for tissue distribution analysis (pilot study) or at 0.5, 1, 2, 4 and 8 h for liver and plasma kinetics (main experiment). Urine was collected in metabolic cages. Biological samples were analyzed by UPLC-DAD-MS with and without incubation with β -glucuronidase and sulfatase.

Results CGAs and metabolites were detected in all tissues. Caffeic acid was the main compound in plasma up to 2 h after ingestion of maté tea, while 5-CQA predominated in ST group. Concentration of microbial metabolites increased 4 h after gavage and reached higher amounts in MT plasma and liver, when compared to ST group. Approximately 4.0 % of compounds ingested by MT and 3.3 % by ST were recovered in urine up to 8 h after the gavage.

Conclusion The study confirms that not only absorption, but also metabolization of CGAs begins in stomach. There were differences in compounds formed from maté tea or isolated 5-CQA, showing that CGAs profile in food may influence qualitatively and quantitatively the metabolites formed in the body.

Keywords Phenolic compounds · Chlorogenic acids · *Ilex paraguariensis* · Bioavailability · Biotransformation · UPLC-DAD-MS

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Introduction

Yerba maté (*Ilex paraguariensis*) is a plant from the subtropical region of South America, widely consumed in Brazil, Argentina, Paraguay and Uruguay as different beverages, such as maté tea (roasted leaves) and *chimarrão* (dried green leaves). This plant has been reported to have various biological activities, mostly attributed to the high content of chlorogenic acids, such as antioxidant, anti-inflammatory and chemopreventive properties, inhibition of glycation and atherosclerosis, improvement in glucose tolerance and anti-obesity effects, reviewed elsewhere [1, 2].

Chlorogenic acids (CGAs) are a family of non-flavonoid phenolic compounds, comprising esters of quinic acid with

one or more molecules of hydroxycinnamic acids such as caffeic, ferulic and *p*-coumaric acids [3]. CGAs occur in a diversity of plant-derived foods and are the main phenolic compounds in coffee, contributing significantly to daily dietary intake of phenolics [4, 5].

Although beneficial properties of CGAs and other plant phenolics are often attributed to antioxidant activities, emerging findings suggest a variety of potential mechanisms of action *in vivo*, beyond antioxidant functions [3, 6, 7]. As they pass along the gastrointestinal tract, phenolic compounds are extensively metabolized and it is their metabolites that probably reach the physiological targets, and may have different chemical, biological and functional properties from the compounds present in the food matrix [7–9]. Therefore, a deeper understanding of the bioavailability of phenolics is essential to link yerba maté and other foods containing these compounds to specific activities and mechanisms by which they may contribute to health [10, 11].

A limited number of studies have investigated the bioavailability of CGAs after ingestion of coffee or pure 5-caffeoylquinic acid (5-CQA). Following ingestion, a small part of CGAs may be absorbed intact or after cleavage of ester bonds by membrane [12, 13] or microbial esterases [14], appearing in plasma and urine mainly as phase II conjugates (methyl, glucuronide and sulfate derivatives) of CGAs, caffeic and ferulic acids [11, 15–19]. Late-appearing metabolites like hydroxyphenylpropionic, dihydrocaffeic, hippuric and hydroxybenzoic acids have been assumed to be absorbed and produced in the large intestine, after metabolization by the microbiota [5, 11, 20, 21]. In comparison with other phenolic compounds such as catechins and isoflavones, data on the biotransformation and extent of metabolism for the CGAs are still considered scarce and, in many cases, contradictory [22]. To our knowledge, there are no published papers describing the bioavailability of CGAs from yerba maté.

The purpose of this investigation was to explore the distribution of CGAs and their metabolites in Wistar rats tissues, kinetic profile in plasma and liver and urinary excretion after ingestion of maté tea, in comparison with the ingestion of isolated 5-caffeoylquinic acid. The presence of CGAs and several metabolites was investigated in plasma and tissues (liver, kidneys, skeletal muscle, stomach, small and large intestine) by ultra-performance liquid chromatography coupled to diode array detector and mass spectrometry (UPLC-DAD-MS). On a second experiment, concentration *versus* time profile in plasma and liver and urinary excretion of the compounds were evaluated.

Materials and methods

Chemicals and reagents

Standards of the phenolic acids 5-caffeoylquinic (5-CQA), caffeic, ferulic, isoferulic, hippuric, *p*-coumaric, *m*-coumaric, 3-hydroxybenzoic, dihydrocaffeic, vanillic and 3-hydroxyphenylpropionic acids, enzymes β -glucuronidase (Type IX from *E. coli*) and sulfatase (Type VIII from abalone entrails), MOPS, Na₂EDTA, sodium dithionite and ascorbic acid were purchased from Sigma-Aldrich (Buchs, Switzerland). Solid phase extraction cartridges (Oasis HLB, 1 cc 30 mg) were provided by Waters (Milford, Massachusetts, USA). Formic acid (98 %) and phosphoric acid (85 %) were obtained from Merck (Darmstadt, Germany) and HPLC grade organic solvents (acetonitrile and methanol) from Carlo Erba (Rodano, Italy). Deionized water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA).

Maté tea characterization

Instant maté tea (roasted *I. paraguariensis* aqueous spray-dried extract) produced by Leão Jr. (Curitiba-PR, Brazil) was purchased directly from the market. The phenolic compounds in maté tea were determined using ultra-performance liquid chromatography (1200SL, Agilent Technologies, Palo Alto, California, USA) coupled to a diode array detector (DAD) and a single-quadrupole mass spectrometer (6150B) (UPLC-DAD-MS). Instant maté tea was diluted with deionized water and injected into an Agilent Zorbax SB-C18 column (50 × 2.1 mm, 1.8 μ m). The mobile phase consisted of solvent A (100 % water + 0.1 % formic acid) and solvent B (methanol). Injections volume was 5 μ l. Gradient program was 15 % B from 0 to 1.8 min; 15–30 % B from 1.8 to 5 min; 30–35 % B from 5 to 6 min; held at 35 % B from 6 to 12 min; and back from 35 to 15 % from 12 to 13 min, at a flow rate of 320 μ l/min. MS parameters for the analysis were as follows: capillary voltage, –4 kV; nitrogen as nebulizer, 30 psi; drying, 12 L/min; and desolvation temperature, 350 °C. At first, a scan analysis was performed, so the deprotonated molecule and the most intense fragment ion of each compound were chosen for selected ion monitoring (SIM).

Identification of 5-CQA and rutin was based on retention time, UV absorption and mass spectra compared to standards. Other phenolic compounds were tentatively identified based on UV spectrum (similarity with 5-CQA spectrum >95 %), MS spectrum and elution order, comparing with data previously published in the literature [15, 23–25]. Quantification

was performed with DAD data (wavelength 324 nm), using a five-point analytical curve with 5-CQA standard. Limit of detection (LOD) for 5-CQA was 1.74 nmol/ml and limit of quantification (LOQ) 5.33 nmol/ml.

Experimental design and sample collection

All animal procedures were in agreement with the Ethical Principles in Animal Research, adopted by the Brazilian College for Animal Experimentation, according to the American Psychological Association Guidelines for Ethical Conduct in the Care and Use of Animals. Study protocols were approved by the Ethical Committee of the Tropical Medicine Institute, University of São Paulo.

A total of 74 male Wistar rats were used. All animals were obtained at 5 weeks of age and housed in groups of four per cage in a controlled temperature (22 °C) room with a 12-/12-h light/dark cycle (lights on at 7:00 a.m.) with free access to water and a commercial feed for laboratory rats (Nuvilab CR1, Nuvital, Curitiba-PR, Brazil).

Pilot experiment

Pilot experiment was performed to evaluate whether the maté tea administered to the animals (same dose used in a previous study [26]) would be sufficient for the detection of phenolic acids in plasma and tissues and determine which compounds could be found. At nine weeks of age, six animals (280 ± 20 g body weight) were kept in fasting conditions for 16 h with access to water, weighed and randomly divided into 3 groups ($n = 2$ per group): MT—received instant maté tea (2 g/kg of body weight) diluted in water; ST—received 5-CQA standard (240 mg/kg of body weight) diluted in water; and control—received the correspondent amount of water. Beverages were administered by gavage. The amount of 5-CQA administered is equivalent to the total phenolic content of 2 g/kg of maté tea, determined by UPLC-DAD. After 1.5 h, rats were anesthetized with an association of ketamine and xylazine intraperitoneally and killed by exsanguination (cardiac puncture). Liver and kidney were excised after being perfused with saline solution. Stomach, small intestine, large intestine and *biceps femoris* (right paw) were removed and washed with saline solution. All samples were immediately frozen in liquid nitrogen and stored at -80 °C, and tissues were also freeze-dried for 12 h before analysis.

Main experiment

Animals (299 ± 33 g body weight) were treated as described for the pilot study: After 16-h fasting, these were randomly divided into MT group (30 rats), ST group (30 rats) or control (6 rats), receiving the same dosages of maté

tea and 5-CQA tested on the pilot study. After anesthesia, animals were euthanized at 0.5, 1, 2, 4 or 8 h after receiving maté tea or 5-CQA (6 rats/group/time) or 2 h after receiving water (control group, $n = 6$). Blood and liver were collected.

Animals killed at 2, 4 and 8 h were placed in metabolic cages for urine collection right after the gavage, with access to water. Urine volumes were measured and divided in 1-ml aliquots. Blood was centrifuged for 10 min at $3000 \times g$ (4 °C), and 40 μ l of an antioxidant solution (sodium acetate buffer 250 mM pH 5.0, 20 mg/mL ascorbic acid and 1 mg/mL Na_2EDTA) was added to each 1 ml of plasma and urine. All samples from pilot and main experiments were immediately frozen in liquid nitrogen and stored at -80 °C. Liver samples were freeze-dried for 12 h, with individual weights recorded before and after the freeze-drying process, so the results could be expressed per gram of fresh tissue.

Extraction of phenolic acids from fluids and tissues and liquid chromatography–mass spectrometry analyses

Sample preparation and liquid chromatography–mass spectrometry analyses were performed according to a validated method [27]. Briefly, triplicate samples of plasma, urine (200 μ l) or freeze-dried liver (30 mg) from each animal of the main experiment were incubated with or without β -glucuronidase and sulfatase, to estimate total (conjugated + not conjugated) and free (not conjugated) phenolic acids, and applied to solid-phase cartridges (Oasis HLB). Extraction solutions and procedures for all tissues from the pilot experiment were the same described before for liver [27]. However, blood and tissue samples from the pilot experiment were not subjected to enzymatic hydrolysis with β -glucuronidase and sulfatase, were evaluated qualitatively and screened for many compounds, including glucuronated and/or sulfated metabolites for which standards were not available. Compounds were tentatively identified based on m/z (SIM mode) selected from previously published literature that evaluated bioavailability of chlorogenic acids [15, 25, 28].

Samples were analyzed on an UPLC-DAD-MS system (Agilent Technologies, Palo Alto, California, USA). Separation was achieved on a Zorbax SB-C18 column (50×2.1 mm, 1.8 μ m) with a Zorbax SB-C8 guard column (12.5×2.1 mm, 5 μ m) (Agilent Technologies), operating at 45 °C. Mobile phases were constituted with water (solvent A) and acetonitrile (solvent B), both acidified with 0.1 % formic acid. The best separation was achieved with the gradient program: 6 % B from 0 to 0.5 min; 6–20 % B from 0.5 to 8.5 min; 20–90 % B from 8.5 to 9.5 min; 90 % B held from 9 to 12; back to 6 % B from 12 to 12.5 min;

and a 10-min post-run re-equilibration at 6 % B. Mass spectrometric data were collected between 0.5 and 8.5 min. The flow rate was 330 $\mu\text{L}/\text{min}$ and injection volume 5 μL . Data were acquired in SIM mode, and the deprotonated molecule and most intense fragment ion of each compound of interest were monitored. When possible, UV spectra were used for comparison with those of standards.

Quantification was performed for samples from the main experiment (plasma, urine and liver), using MS data. External calibration curves (minimum 5 points) for quantification of phenolic acids in plasma and liver were built by spiking control samples with standard solutions. Therefore, data are corrected for endogenous levels of hippuric and 3-hydroxyphenylpropionic acids, detected in very low levels in these control samples. Calibration curves for urine were built with standards diluted in mobile phase in order to quantify the studied compounds also in control group, as some of the phenolics (hippuric, ferulic and 3-hydroxyphenylpropionic acids) were present in control urine in relatively high concentrations. Isomers of 5-CQA (3- and 4-CQA) and feruloylquinic acids (FQA) were tentatively quantified using the calibration curves of 5-CQA. Concentrations of other compounds were estimated, respectively, to their own calibration curves (standards). All calibration curves presented $R^2 > 0.98$. Linearity range varied between 0.15 and 12 nmol/ml for curves used for plasma samples and from 0.10 to 5 nmol/g of fresh tissue in curves used for liver samples, for all compounds. Linearity range of curves used for urine samples varied from 0.15 to 150 nmol/ml for all compounds, except for hippuric acid (2–200 nmol/ml). Urine samples with hippuric acid concentrations higher than the highest concentration in the calibration curve were diluted accordingly, in order to fit the linearity range.

Limits of detection ranged from 0.05 (5-CQA) to 0.36 nmol/ml (hippuric acid) in plasma, 0.08 (5-CQA, caffeic and dihydrocaffeic acids) to 0.12 nmol/g of fresh tissue (isoferulic acid) in liver and from 0.04 (5-CQA) to 0.34 nmol/ml (isoferulic acid) in urine. Limits of quantification ranged from 0.15 (5-CQA) to 1.12 nmol/ml (hippuric acid) in plasma, 0.14 (5-CQA) to 0.30 nmol/g of fresh tissue (isoferulic acid) in liver and from 0.14 (5-CQA) to 1.04 nmol/ml (isoferulic acid) in urine [27].

Data analysis

Body weight and total amount of phenolics ingested were analyzed by one-way ANOVA followed by Tukey's test. Group differences for other variables were assessed using nonparametric statistical tests (Mann–Whitney or Kruskal–Wallis followed by post hoc Dunn's test) since data did not show normal distribution or homoscedasticity. All

statistical analysis was carried out using Statistica software (version 11.0, Stat Soft Inc., Tulsa, OK, USA). Values for $p < 0.05$ were considered significant.

Results

Phenolic compounds in maté tea

DAD ($\lambda = 324 \text{ nm}$) and MS chromatograms of maté tea are shown in Fig. 1a, b, respectively. Retention times, deprotonated molecule $[\text{M}-\text{H}]^-$, most intense fragment ions (m/z) and estimated concentration (expressed as 5-CQA equivalents) of phenolic compounds are given in Table 1.

Besides 5-CQA, identified based on its own commercial standard, two isomers of monocaffeoylquinic acids (CQAs) were tentatively identified as 3-CQA and 4-CQA (peaks 1 and 4), respectively, based on similarity of UV and MS spectra with those of 5-CQA standard and elution order of CQAs compared to previously published literature [24, 25], taking in consideration that Jaiswal et al. [24] reported 3-, 4- and 5-CQA as the most intense CQAs found in yerba maté, after performing LC-MSⁿ analysis. Tentative identification of other chlorogenic acids which have been previously reported in yerba maté beverages [23, 24] was based on similarity to 5-CQA UV spectrum (>95 %), MS spectrum and elution order from previous reports [15, 23–25].

A total of 11 phenolic compounds were identified/tentatively identified and quantified: 4 monocaffeoylquinic acids (CQAs), 2 caffeoylshikimic acids or caffeoylquinic acid lactones, 1 feruloylquinic acid, 3 di-caffeoylquinic acids (DiCQAs) and 1 rutin. Caffeoylquinic acids (CQAs) represented 64 % of the total phenolics. Three isomers of di-caffeoylquinic acids (DiCQAs) represented 34 % of the total phenolics, and the sum of the other compounds represented 2 % of the total phenolics in maté tea.

Regarding the amount of phenolic compounds ingested by the animals, MT group received an absolute amount of $613 \pm 62 \text{ mg}$ of maté tea, corresponding to $75 \pm 8 \text{ mg}$ ($210 \pm 21 \mu\text{mol}$) of chlorogenic acids (as 5-CQA equivalents), and ST group received $72 \pm 8 \text{ mg}$ ($203 \pm 22 \mu\text{mol}$) of 5-CQA standard. Body weights and total amount of phenolic compounds ingested did not differ statistically among groups (ANOVA and Tukey's post hoc).

One cup (200 ml) of maté tea, prepared as recommended on the label (1 g in 200 ml), contains approximately 120 mg (343 μmol) of phenolic compounds (Table 1). For comparison, one cup of coffee (200 ml) may contain 70–350 mg of chlorogenic acids [4, 11, 15–19, 29], and the daily ingestion of phenolic compounds in human diet may vary between 100 mg and 2 g [30].

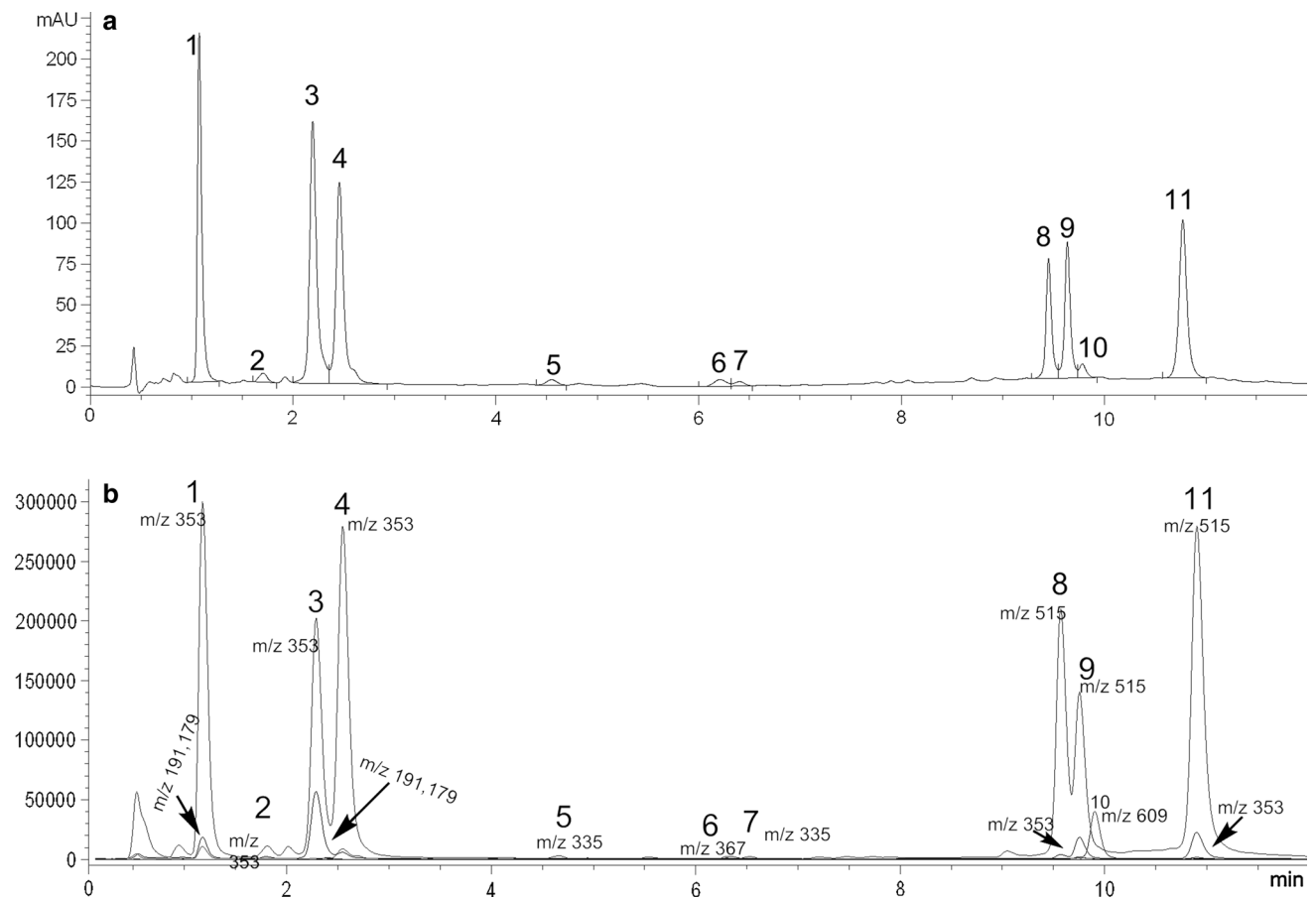


Fig. 1 Phenolic compounds in maté tea. **a** UPLC-DAD chromatogram ($\lambda = 324$ nm) **b** UPLC-MS chromatogram (selected ion monitoring)

Table 1 Identification and concentration of phenolic compounds (mean \pm SD) in instant maté tea

Compound	Peak ^a	RT ^b (min)	[M–H] ^c (m/z)	Fragment ion(s) (m/z) ^c	Concentration ^d (μ mol/g)
3-Caffeoylquinic acid (3-CQA)	1	1.1	353	191, 179	65.28 \pm 0.21
Caffeoylquinic acid	2	1.7	353	191, 179	2.83 \pm 0.15
5-Caffeoylquinic acid (5-CQA)	3	2.2	353	191	80.42 \pm 0.34
4-Caffeoylquinic acid (4-CQA)	4	2.4	353	179, 191	68.68 \pm 0.29
Caffeoylshikimic acid or caffeoylquinic acid lactone	5	4.5	335	161	1.96 \pm 0.03
Feruloylquinic acid	6	6.2	367	173	3.10 \pm 0.01
Caffeoylshikimic acid or caffeoylquinic acid lactone	7	6.4	335	161	1.47 \pm 0.03
Di-caffeoylquinic acid	8	9.4	515	353	28.97 \pm 0.60
Di-caffeoylquinic acid	9	9.6	515	353	33.44 \pm 0.16
Rutin	10	9.8	609	301	3.60 \pm 0.05
Di-caffeoylquinic acid	11	10.8	515	353	53.41 \pm 0.27
Total phenolic compounds					343.17 \pm 0.64

^a Refer to Fig. 1

^b Retention time of UPLC-DAD

^c Mass spectrometer data used for identification

^d Values are mean \pm SD ($n = 3$) expressed as 5-CQA equivalents by UPLC-DAD ($\lambda = 324$ nm)

Table 2 UPLC-MS detection of phenolic acids^a in Wistar rats tissues and plasma 1.5 h after ingestion of aqueous solutions of maté tea (MT) (2 g/kg of body weight), 5-CQA (ST) (240 mg/kg of body weight) or water (CT)

Compound	<i>m/z</i>	RT (min)	Liver			Kidney			Stomach			Small intestine		
			CT	MT	ST	CT	MT	ST	CT	MT	ST	CT	MT	ST
<i>Chlorogenic acids (present in maté tea)</i>														
5-Caffeoylquinic acid (5-CQA)	353; 191	3.4	–	+	+	–	+	+	–	+	+	–	+	+
3-Caffeoylquinic acid (3-CQA) ^b	353; 191	1.8	–	+	–	–	+	–	–	+	–	–	+	–
4-Caffeoylquinic acid (4-CQA) ^b	353; 191	3.7	–	+	–	–	+	–	–	+	–	–	+	–
Di-caffeoylquinic acid (Di-CQA1) ^b	515; 353	8.7	–	–	–	–	–	–	–	+	–	–	+	–
Di-caffeoylquinic acid (Di-CQA2) ^b	515; 353	9.0	–	–	–	–	–	–	–	+	–	–	+	–
Di-caffeoylquinic acid (Di-CQA3) ^b	515; 353	9.9	–	–	–	–	–	–	–	+	–	–	+	–
<i>Free phenolic acids</i>														
Caffeic acid	179; 135	3.7	–	+	+	–	+	+	–	+	+	–	+	+
Hippuric acid	178; 134	3.3	+	+	+	+	+	+	+	+	+	+	+	+
Ferulic acid	193; 178	6.7	–	+	+	–	+	+	+	+	+	–	+	+
Isoferulic acid	193; 134	7.2	–	–	–	–	–	–	–	+	–	–	–	–
<i>m</i> -Coumaric acid	163; 119	6.8	–	–	–	–	–	–	–	–	–	–	–	–
<i>p</i> -Coumaric acid	163; 119	5.6	–	–	–	–	–	–	–	+	–	–	+	–
Dihydrocaffeic acid	181; 137	3.2	–	–	–	+	+	+	+	+	+	–	+	+
3-Hydroxybenzoic acid	137; 93	3.6	–	–	–	–	–	–	–	–	–	–	–	–
3-Hydroxyphenylpropionic acid	165; 121	5.9	–	–	–	–	+	–	–	+	–	–	–	–
Vanillic acid	167; 152	3.7	–	–	–	–	–	–	–	–	–	–	–	–
Feruloylquinic acid (FQA1) ^b	367; 353	5.7	–	–	–	–	+	–	–	+	–	–	+	–
Feruloylquinic acid (FQA2) ^b	367; 353	5.9	–	–	+	–	+	+	–	+	+	–	+	+
Feruloylquinic acid (FQA3) ^b	367; 353	6.3	–	–	+	–	+	+	–	+	+	–	+	+
<i>Conjugated phenolic acids^b</i>														
CQA glucuronide	529; 353	3.0	–	+	+	–	+	+	–	+	–	–	+	+
CQA sulfate	433; 353	3.3	–	+	+	–	–	–	–	–	–	–	–	–
CQA glucuronide-sulfate	609; 529	7.6	–	+	–	–	–	–	–	+	+	–	+	–
Ferulic/isoferulic acid glucuronide	369; 193	4.6/6.1	–	+	–	–	–	–	–	–	–	–	–	–
Ferulic/isoferulic acid sulfate	273; 193	3.7/4.5	–	+	+	–	+	+	–	+	+	–	+	+
Ferulic/isoferulic acid glucuronide-sulfate	449; 369	6.1	–	–	+	–	+	+	–	+	+	–	+	+
Caffeic acid glucuronide	355; 179	3.2/3.4	–	+	+	–	+	+	–	+	+	–	+	+
Caffeic acid sulfate	259; 179	2.2/3.6	–	+	+	–	+	+	–	+	–	–	+	+
Compound	<i>m/z</i>	RT (min)	Large intestine			Muscle (<i>biceps femoris</i>)			Plasma					
			CT	MT	ST	CT	MT	ST	CT	MT	ST			
<i>Chlorogenic acids (present in maté tea)</i>														
5-Caffeoylquinic acid (5-CQA)	353; 191	3.4	–	+	+	–	+	+	–	+	+	–	+	+
3-Caffeoylquinic acid (3-CQA) ^b	353; 191	1.8	–	+	–	–	+	–	–	+	–	–	+	–
4-Caffeoylquinic acid (4-CQA) ^b	353; 191	3.7	–	+	–	–	+	–	–	+	–	–	+	–
Di-caffeoylquinic acid (Di-CQA1) ^b	515; 353	8.7	–	+	–	–	–	–	–	–	–	–	–	–
Di-caffeoylquinic acid (Di-CQA2) ^b	515; 353	9.0	–	+	–	–	–	–	–	–	–	–	–	–
Di-caffeoylquinic acid (Di-CQA3) ^b	515; 353	9.9	–	+	–	–	–	–	–	–	–	–	–	–
<i>Free phenolic acids</i>														
Caffeic acid	179; 135	3.7	–	+	+	–	+	–	–	+	–	–	+	+
Hippuric acid	178; 134	3.3	–	–	–	+	+	+	+	+	+	+	+	+
Ferulic acid	193; 178	6.7	–	+	–	–	–	–	–	–	–	–	+	–
Isoferulic acid	193; 134	7.2	–	–	–	–	–	–	–	–	–	–	–	–
<i>m</i> -Coumaric acid	163; 119	6.8	–	–	–	–	–	–	–	–	–	–	–	–

Table 2 continued

Compound	<i>m/z</i>	RT (min)	Large intestine			Muscle (<i>biceps femoris</i>)			Plasma			
			CT	MT	ST	CT	MT	ST	CT	MT	ST	
<i>p</i> -Coumaric acid	163; 119	5.6	–	–	–	–	–	–	–	+	–	
Dihydrocaffeic acid	181; 137	3.2	–	–	–	–	–	–	–	+	–	
3-Hydroxybenzoic acid	137; 93	3.6	–	–	–	–	–	–	–	–	–	
3-Hydroxyphenylpropionic acid	165; 121	5.9	–	–	+	–	–	–	–	–	–	
Vanillic acid	167; 152	3.7	–	–	–	–	–	–	–	–	–	
Feruloylquinic acid (FQA1) ^b	367; 353	5.7	–	+	–	–	–	–	–	+	–	
Feruloylquinic acid (FQA2) ^b	367; 353	5.9	–	+	+	–	–	+	–	+	+	
Feruloylquinic acid (FQA3) ^b	367; 353	6.3	–	+	+	–	–	+	–	+	+	
<i>Conjugated phenolic acids^b</i>												
CQA glucuronide	529; 353	3.0	–	+	+	–	+	+	–	+	+	
CQA sulfate	433; 353	3.3	–	–	–	–	–	–	–	–	–	
CQA glucuronide-sulfate	609; 529	7.6	–	+	–	–	–	–	–	–	–	
Ferulic/isoferulic acid glucuronide	369; 193	4.6/6.1	–	–	–	–	+	+	–	–	–	
Ferulic/isoferulic acid sulfate	273; 193	3.7/4.5	–	+	–	–	+	+	–	+	+	
Ferulic/isoferulic acid glucuronide-sulfate	449; 369	6.1	–	–	–	–	–	–	–	–	–	
Caffeic acid glucuronide	355; 179	3.2/3.4	–	+	+	–	+	+	–	+	+	
Caffeic acid sulfate	259; 179	2.2/3.6	–	–	–	–	+	+	–	–	–	

^a (+) Presence of compound detected (–) compound not detected

^b Tentatively identified based on elution order, MS and DAD spectra

Presence of phenolic compounds in tissues and plasma (Pilot experiment)

Table 2 shows the compounds detected in plasma and tissues of Wistar rats after ingestion of maté tea (MT), 5-CQA standard (ST) or water (CT). Due to differences in mobile phase composition and gradient program with those used for analysis of maté tea, retention times of compounds differed between maté tea and biological samples.

Among the phenolic compounds that were present in the ingested beverages, mono-CQAs were detected in the free form (not metabolized) in all analyzed tissues and plasma of animals from group MT (3-, 4- and 5-CQA) and ST (5-CQA). Three compounds with *m/z* 515 and 353 were tentatively identified as isomers of DiCQAs (here denominated DiCQA1, DiCQA2 and DiCQA3), and three other compounds with *m/z* 367 and 193 were tentatively identified as isomers of feruloylquinic acid (here denominated FQA1, FQA2 and FQA3), based on UV (>95 % similar to 5-CQA) and MS spectra, as detailed before for maté tea analysis, and on previously published literature that reported FQAs in human plasma and urine after ingestion of coffee [15]. DiCQAs were detected only in stomach, small and large intestine samples from MT group. The three FQAs were found in digestive tissues and kidney of MT group, while only FQA2 and FQA3 were detected in all tissues and plasma of ST group. Small quantities of a

single FQA isomer were found in maté tea, and none was present in the beverage administered to ST group. Therefore, the presence of three FQA isomers in MT samples and 2 isomers in ST samples indicates that FQAs were formed by methylation of CQAs.

Other metabolites found in plasma and/or tissues include the hydroxycinnamates caffeic, ferulic and isoferulic acid in free form or as phase II metabolites (glucuronated and/or sulfated). Caffeic acid glucuronide-sulfate (*m/z* 435; 179) was not found in any of the samples. Some compounds formed after metabolization of chlorogenic acids by intestinal microbiota were also found (hippuric, dihydrocaffeic and 3-hydroxyphenylpropionic acids), while others were searched but not detected in any of the samples (*m*-coumaric, vanillic and hydroxybenzoic acids).

Plasma and liver kinetics of phenolic compounds

Concentration–time profile of phenolic compounds in rat plasma after oral administration of maté tea (MT) or 5-CQA solution (ST) is illustrated in Figs. 2 and 3, respectively. Compounds can be divided in two groups, according to their time of appearance in plasma and liver: intact CQAs and its primary metabolites or microbial metabolites, which appeared at later time points.

The most abundant CQA isomers found in maté tea (3-, 4- and 5-CQA) were present in MT group plasma in higher

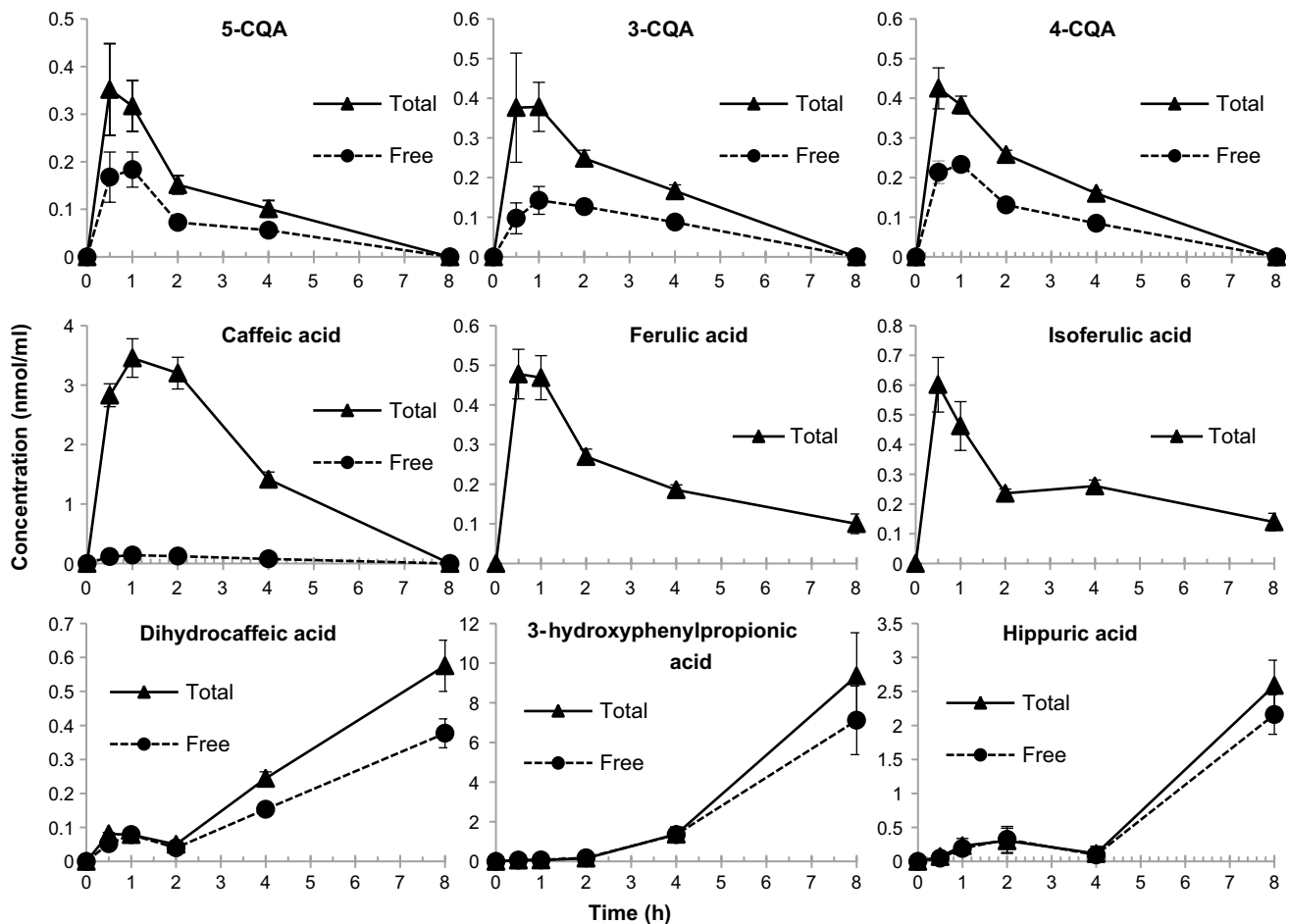


Fig. 2 Plasmatic concentration–time curves of phenolic acids in rats after oral administration of maté tea (2 g/kg of body weight). Free (samples not treated with β -glucuronidase and sulfatase)

and total (free + conjugated phenolic acids, samples treated with β -glucuronidase and sulfatase) phenolic acids, expressed as mean \pm SE ($n = 6$ rats per time point, each analyzed in triplicate)

concentrations 0.5 and 1 h after ingestion (~ 0.4 nmol/ml), with lower amounts in samples from animals killed in subsequent periods. Kinetics of caffeic, ferulic and isoferulic acids followed a similar trend. The main compound present in plasma in the first 4 h after ingestion of maté tea was caffeic acid, with $C_{\max 1h}$ 3.45 nmol/ml (Total). As expected, concentration of microbial metabolites dihydrocaffeic, hippuric and 3-hydroxyphenylpropionic acids began to increase after 4 h, with the higher concentrations observed 8 h after gavage. DiCQAs were not detected in any sample.

For ST group, the main compound found in plasma was 5-CQA, reaching a $C_{\max 0.5h}$ 5.24 nmol/ml, followed by FQA2 ($C_{\max 1h}$ 1.46 nmol/ml) and FQA3 ($C_{\max 1h}$ 1.31 nmol/ml). FQAs were found in amounts $<LOQ$ (limit of quantitation) in plasma from MT group. Caffeic acid was present in amounts lower than in MT group, reaching the maximum concentration (1.21 nmol/ml) 2 h after gavage. Kinetics of microbial metabolites dihydrocaffeic and 3-hydroxyphenylpropionic acids followed the same pattern

observed in MT group. Hippuric, ferulic and isoferulic acids were detected below the limit of quantification.

In both groups, approximately 50 % of the CQAs were present in plasma in free form, while more than 90 % of caffeic acid was present as glucuronated and/or sulfate conjugates. Ferulic and isoferulic acids could only be quantified in samples subjected to enzymatic treatment. The two FQA isomers quantified in ST group were also mostly in conjugated form, with only 31 % of FQA2 and 24 % of FQA3 in free form. However, compounds that appeared later in plasma were present mainly in the free form: dihydrocaffeic (72 % free), 3-hydroxyphenylpropionic (91 % free) and hippuric acid (97 % free, only in MT group), as observed in Figs. 2 and 3. The relative amount of each compound in the free form (%) did not differ between MT and ST groups (Mann–Whitney test).

Concentration–time curves of phenolic acids in liver are shown in Fig. 4 (a) for MT group and (b) for ST group, only for samples treated with β -glucuronidase and

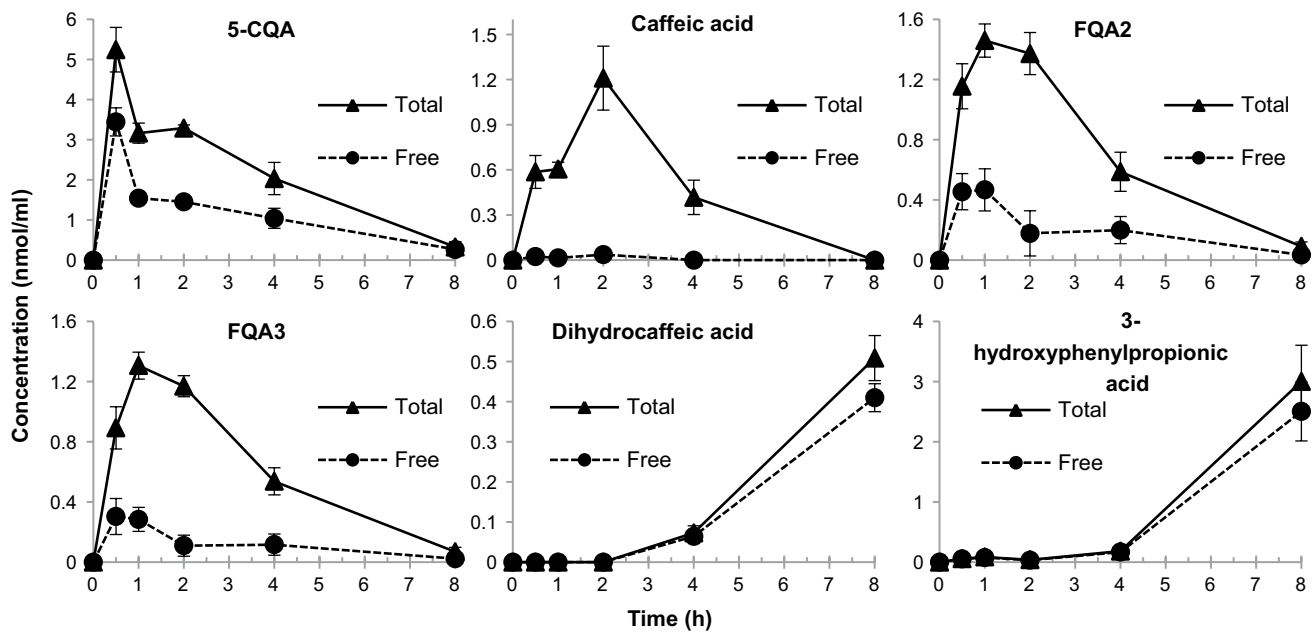


Fig. 3 Plasmatic concentration–time curves of phenolic acids in rats after oral administration of 5-CQA (240 mg/kg of body weight). Free (samples not treated with β -glucuronidase and sulfatase)

and total (free + conjugated phenolic acids, samples treated with β -glucuronidase and sulfatase) phenolic acids. Values are mean \pm SE ($n = 6$ rats per point, each analyzed in triplicate)

sulfatase, as quantities of phenolic compounds in free form were below the LOQ. This is consistent with the major role liver plays in phase II metabolism. Kinetics of phenolic compounds were very similar to plasma, in both groups: compounds present in beverages administered to animals (CQAs) and products of direct cleavage (Caffeic acid) or phase II metabolism of CQAs (FQAs) were found in higher concentrations 0.5–2 h after gavage, while compounds that depend on microbiota to be formed (hippuric and 3-hydroxyphenylpropionic acids) were present in higher concentrations after 4 h. Appearance of some compounds was different from plasma: FQAs, below LOQ in MT plasma, showed clear peaks of concentration in MT liver, with FQA2 achieving $C_{\max 0.5h}$ of 0.27 nmol/g tissue and FQA3 0.71 nmol/g tissue. Ferulic, isoferulic and dihydrocaffeic acids were <LOQ in MT and ST groups.

Urinary excretion

Quantitative data of phenolic acids in total volume of urine collected are given in Table 3. Excretion of CQAs, in free or conjugated form—including their methylated forms, FQAs—was observed early in urine collected 0–2 h after ingestion of maté tea or 5-CQA. In MT group, apparently all CQAs and FQAs circulating in the body were excreted within 4 h, as there were no statistical differences in the amounts of these compounds excreted between 0 and 4 or 0 and 8 h. For ST group, however, quantities excreted 0–8 h

were significantly higher than those excreted during 0- to 4-h interval. The same behavior and time-related difference between MT and ST groups were observed for total caffeic acid and its methylated derivatives, ferulic and isoferulic acids.

Regarding microbial metabolites, hippuric and 3-hydroxyphenylpropionic acids were present in small amounts in control urine and 0–2 h samples from MT and ST groups, while *m*-coumaric and dihydrocaffeic acids were not detected in CT or 0–2 h samples in both MT and ST groups. Concentration of microbial metabolites was significantly higher in MT and ST urine collected up to 4 h compared to 0- to 2-h interval and significantly higher in samples collected 0–8 h than both previous intervals.

Comparing compounds found in both MT and ST groups within the same collection period, amounts of 5-CQA, FQA2 and FQA3 were higher in ST group in all intervals, while amounts of caffeic, ferulic, isoferulic acids and microbial metabolites were higher in MT samples, which is consistent with plasma results. Hippuric acid was the most abundant phenolic acid excreted at the end of 8-h period, representing 69 % of all compounds quantified in MT group and 42 % in ST group, followed by 3-hydroxyphenylpropionic (16 %) and caffeic acids (4 %) in MT group and by FQA2 (21 %), FQA3 (18 %), 3-hydroxyphenylpropionic (8 %) and 5-CQA (6 %) in ST group.

Considering the total phenolic acids excreted in urine up to 8 h after oral administration of maté tea

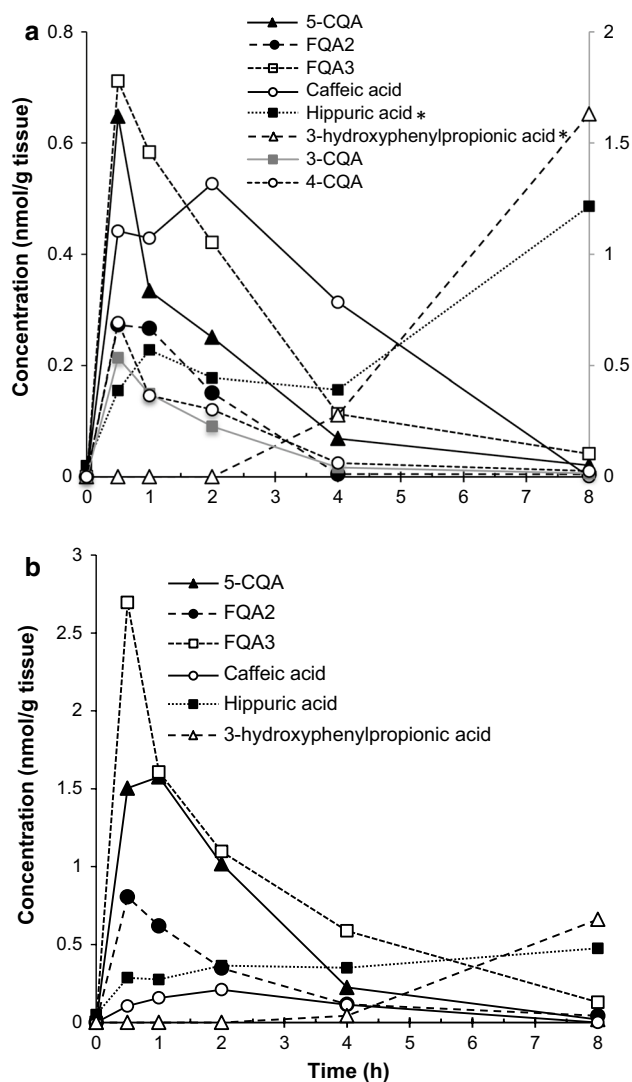


Fig. 4 Concentration–time curves of phenolic acids in rat liver after oral administration of **a** maté tea (2 g/kg of body weight) or **b** 5-CQA (240 mg/kg of body weight). Samples treated with β -glucuronidase and sulfatase. Data expressed as means ($n = 6$ rats per time point). *refers to right Y axis

(8701 ± 261 nmol) or 5-CQA (6798 ± 812 nmol) and the amount of chlorogenic acids ingested by each animal (MT group 210 ± 21 μ mol; ST group 203 ± 22 μ mol), the urinary recovery was 4.0 % of the total phenolics ingested (mol/mol) by MT group and 3.3 % for ST group, with no statistical difference between groups. Approximately 0.04 % of the 5-CQA ingested by MT group was recovered in urine (0.02 % in free form), while the recovery of 5-CQA in ST group was 0.21 % (0.07 % in free form). Sum of all phenolic acids excreted did not differ between CT and 0- to 2-h collection period of MT and ST groups, neither between MT and ST in each time interval, but was significantly higher in larger intervals within the same group (0–8 > 0–4 > 0–2 h and CT).

Discussion

The present study aimed to evaluate the absorption and metabolic fate of chlorogenic acids from maté tea (*I. paraguariensis*). Wistar rats received oral maté tea, 5-CQA standard or water, and the presence of several phenolic acids, which had been described in the metabolic pathway of chlorogenic acids in the literature [14–16, 20, 28, 31], was investigated in plasma and tissues. On a second experiment, plasma and liver concentration–time profiles and urinary excretion of phenolic acids were assessed. Typical UPLC-MS (SIM mode) chromatograms obtained with the urine samples from MT group are illustrated in Fig. 5. Figure 6 illustrates the suggested metabolic route of chlorogenic acids, based on our results.

CQAs were detected in all tissues, plasma and urine of MT group (3-, 4- and 5-CQA) and ST group (5-CQA). Their presence in gastric tissue (Table 2) and in plasma and liver 0.5 min after ingestion of maté tea or 5-CQA standard (Figs. 2, 3, 4) indicates that absorption begins in stomach, as demonstrated before [28, 32].

Caffeic acid was detected in all tissues from MT and ST groups (Table 2) and was the most abundant compound found early in MT plasma (0.5, 1 and 2 h after maté tea ingestion) and liver and also present, in smaller quantities, in ST plasma and liver (Figs. 2, 3, 4). This compound is the product of CQAs and DiCQAs cleavage by esterases. Ferulic and isoferulic acids methylated derivatives of caffeic acid and were also found in MT tissues and fluids, while FQAs are methylated forms of CQAs [15] and were among the compounds found in higher concentrations in ST plasma and in liver of both groups, and were also detected in all analyzed tissues and in urine. The presence of 3-, 4- and 5-FQA in human plasma and urine after coffee ingestion had been reported previously [11, 15, 16, 19]. In these two studies, concentrations of FQAs in biological fluids were higher or similar to the concentration of CQAs, although CQAs were present in higher quantities in the ingested coffee, and it was hypothesized [15] that the rate of FQAs absorption should be much higher than CQAs. However, the results of the present experiment indicate that the FQAs found in fluids and tissues after maté tea ingestion are probably formed mostly by the methylation of CQAs, reaction carried out by catechol-O-methyltransferase enzymes (COMT), because only small quantities of a single FQA isomer were present in maté tea (Table 1) and no FQA was administered to the ST group, although three FQA isomers were found in MT and two isomers in ST tissues and fluids.

Therefore, the appearance of caffeic, ferulic, isoferulic and/or FQAs early in plasma (both in free form or conjugated with glucuronic acid/sulfates) and stomach (Table 2) indicates that not only the absorption, but also the

Table 3 Urinary excretion of phenolic acids (nmol) by Wistar rats in different time intervals following ingestion of maté tea (2 g/kg of body weight—MT), 5-CQA (240 mg/kg body weight—ST) or water (CT)

Compound	CT	0–2 h		0–4 h		0–8 h		
		MT	ST	MT	ST	MT	ST	
5-Caffeoylquinic acid	Free	nd	2.1 ± 0.7 ^a	23.4 ± 6.8 ^b	11.4 ± 1.3 ^b	73.9 ± 23.4 ^c	11.4 ± 3.1 ^b	146 ± 54 ^d
	Total	nd	4.9 ± 1.2 ^a	43.9 ± 9.9 ^b	31.8 ± 5.7 ^{bd}	203 ± 34 ^c	20.4 ± 4.5 ^d	393 ± 120 ^e
3-Caffeoylquinic acid [†]	Free	nd	2.3 ± 1.6 ^a	nd	9.9 ± 1.6 ^b	nd	11.7 ± 4.1 ^b	nd
	Total	nd	3.9 ± 0.6 ^a	nd	26.6 ± 5.3 ^b	nd	20.6 ± 4.6 ^b	nd
4-Caffeoylquinic acid [†]	Free	nd	3.5 ± 0.9 ^a	nd	17.9 ± 2.5 ^b	nd	21.3 ± 6.7 ^b	nd
	Total	nd	5.6 ± 1.1 ^a	nd	37.6 ± 7.6 ^b	nd	29.8 ± 8.0 ^b	nd
Caffeic acid	Free	nd	13.9 ± 6.9 ^a	6.8 ± 4.8 ^a	35.8 ± 4.9 ^b	24.5 ± 15.8 ^b	41.3 ± 7.3 ^b	35.7 ± 14.2 ^b
	Total	nd	97.1 ± 17.6 ^a	20.1 ± 5.6 ^b	587 ± 146 ^c	105 ± 15.8 ^{ad}	371 ± 87 ^c	194 ± 38 ^d
Ferulic acid	Free	2.4 ± 0.9 ^a	35.9 ± 13.0 ^b	1.4 ± 0.4 ^a	32.8 ± 10.7 ^b	9.3 ± 2.7 ^c	158.5 ± 56.4 ^d	31.1 ± 6.9 ^b
	Total	3.5 ± 1.4 ^a	79.5 ± 17.1 ^b	6.4 ± 1.0 ^a	319 ± 59 ^c	26.8 ± 2.4 ^d	288 ± 78 ^c	62.8 ± 8.2 ^b
Isoferulic acid	Free	nd	20.8 ± 7.9 ^a	1.7 ± 0.3 ^b	56.9 ± 8.3 ^c	10.5 ± 1.3 ^d	81.4 ± 18.2 ^e	15.9 ± 1.6 ^d
	Total	nd	34.3 ± 7.5 ^a	2.9 ± 0.6 ^b	151 ± 28 ^c	12.4 ± 1.3 ^d	156 ± 39 ^c	26.8 ± 3.2 ^a
<i>m</i> -Coumaric acid	Free	nd	nd	nd	6.7 ± 1.7 ^a	nd	50.6 ± 18.2 ^b	31.9 ± 9.3 ^b
	Total	nd	nd	nd	9.0 ± 2.6 ^a	nd	67.2 ± 28.7 ^b	46.9 ± 12.4 ^b
<i>p</i> -Coumaric acid	Free	nd	7.0 ± 1.9 ^a	nd	24.3 ± 3.8 ^b	nd	27.7 ± 6.3 ^b	5.4 ± 1.4 ^a
	Total	nd	9.0 ± 2.9 ^a	nd	27.7 ± 4.6 ^b	nd	30.6 ± 7.6 ^b	6.6 ± 1.6 ^a
Dihydrocaffeic acid	Free	nd	nd	nd	48.5 ± 11.6 ^a	6.1 ± 1.7 ^b	85.8 ± 28 ^c	30.3 ± 8.6 ^a
	Total	nd	nd	nd	66.9 ± 16.9 ^a	8.1 ± 2.3 ^b	92.19 ± 31.3 ^c	50.2 ± 14.4 ^a
Hippuric acid	Free	429 ± 67 ^a	398 ± 96 ^a	403 ± 106 ^a	1762 ± 388 ^b	644 ± 108 ^a	5330 ± 1530 ^c	2257 ± 447 ^d
	Total	545 ± 86 ^a	404 ± 95 ^a	409 ± 114 ^a	1768 ± 375 ^b	659 ± 112 ^a	6021 ± 1772 ^c	2842 ± 554 ^b
3-Hydroxyphenylpropionic acid	Free	19.8 ± 7.8 ^a	2.6 ± .04 ^b	3.5 ± 1.1 ^b	155 ± 42 ^c	42.6 ± 14.2 ^d	1311 ± 548 ^e	396 ± 122 ^f
	Total	25.3 ± 9.9 ^a	2.7 ± 0.5 ^b	3.9 ± 1.2 ^b	155 ± 43 ^c	48.2 ± 17.7 ^d	1358 ± 562 ^e	549 ± 174 ^f
Feruloylquinic acid [†] (FQA1)	Free	nd	9.4 ± 2.9 ^a	nd	43.4 ± 6.0 ^b	nd	47.4 ± 13.5 ^b	nd
	Total	nd	11.6 ± 3.3 ^a	nd	54.4 ± 8.9 ^b	nd	57.6 ± 15.7 ^b	nd
Feruloylquinic acid [†] (FQA2)	Free	nd	13.5 ± 4.3 ^a	168 ± 29 ^b	57.5 ± 5.5 ^c	433 ± 63 ^d	64.6 ± 17.5 ^c	849 ± 151 ^e
	Total	nd	15.3 ± 4.8 ^a	182 ± 30 ^b	78.2 ± 9.3 ^c	548 ± 68 ^d	83.5 ± 22.2 ^c	1416 ± 232 ^e
Feruloylquinic acid [†] (FQA3)	Free	nd	11.5 ± 3.4 ^a	117 ± 19 ^b	51.4 ± 6.5 ^c	305 ± 50 ^d	48.8 ± 13.1 ^c	555 ± 93 ^e
	Total	nd	15.6 ± 4.1 ^a	134 ± 22 ^b	84.9 ± 16.5 ^{bd}	471 ± 65 ^c	81.0 ± 21.9 ^d	1207 ± 197 ^e
Sum of excreted compounds	Free	460 ± 75 ^a	528 ± 107 ^a	726 ± 138 ^a	2343 ± 457 ^b	1550 ± 196 ^b	7308 ± 2167 ^c	4355 ± 568 ^c
%recovery of amount ingested (mol/mol)	–	–	0.23 ± 0.04 ^a	0.35 ± 0.06 ^a	1.07 ± 0.21 ^b	0.70 ± 0.09 ^b	3.39 ± 0.99 ^c	2.12 ± 0.27 ^{bc}
	Total	585 ± 98 ^a	691 ± 89 ^a	804 ± 150 ^a	3447 ± 653 ^b	2083 ± 193 ^b	8701 ± 261 ^d	6798 ± 812 ^d
	–	–	0.31 ± 0.04 ^a	0.39 ± 0.07 ^a	1.58 ± 0.30 ^b	0.94 ± 0.08 ^b	4.03 ± 1.19 ^d	3.31 ± 0.41 ^d

Free (not conjugated) and total (free + conjugated) phenolic acids*

Values are mean ± SE ($n = 6$ rats per group in each time interval, each analyzed in triplicate); *nd* not detected; different superscript letters in the same row indicate statistical differences ($p < 0.05$ Kruskal–Wallis and post hoc Dunn)

* Samples incubated with (total) or without (free) β -glucuronidase and sulfatase

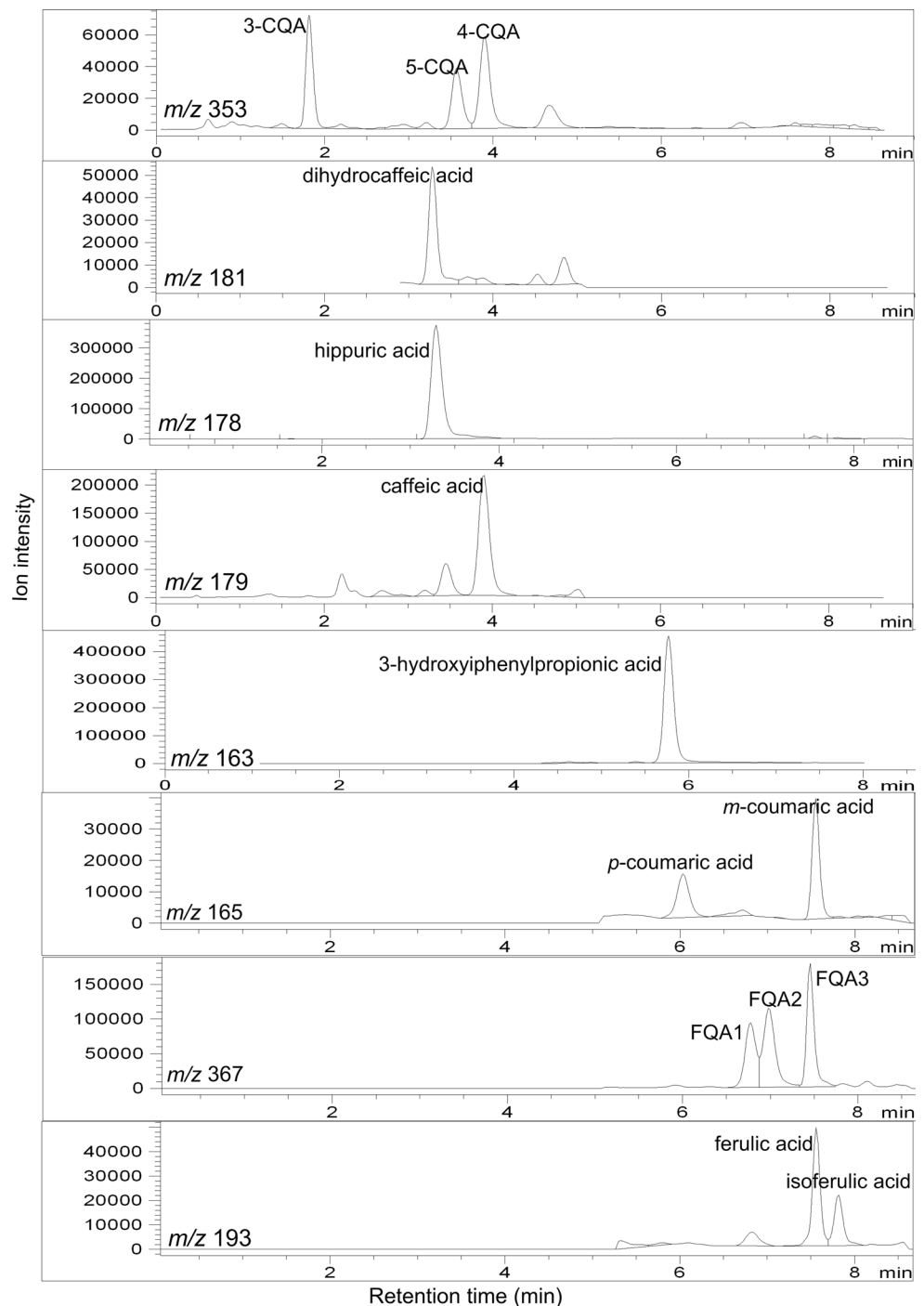
[†] Expressed as 5-CQA equivalents

metabolization of chlorogenic acids by esterases and phase II enzymes (COMT, sulfotransferases and UDP-glucuronosyltransferases) begins in stomach and does not occur only in liver, kidneys, intestinal mucosa or by activity of the colonic microbiota, as hypothesized before [14, 21, 33]. In agreement with our results, the appearance of phase II derivatives of CQAs in vitro has been observed on experiments with gastric mucosa [32]. The presence of COMT

[34, 35] and activity of esterases [12, 13] in gastrointestinal mucosa have already been demonstrated as well.

Three isomers of DiCQAs were found in maté tea and detected in stomach, small and large intestine samples from MT group, but were not present in plasma, urine and other tissues. Two studies reported the presence of DiCQAs in human plasma [17] and urine [18] after coffee ingestion, while other similar experiments did not detect these

Fig. 5 UPLC-MS (*selected ion monitoring*) chromatogram of urine samples collected from 0 to 8 h after oral administration of maté tea (2 g/kg of body weight) to rats



compounds [11, 15, 16, 19]. Due to the size of the molecule, the transport of DiCQAs across the gastrointestinal membrane to the circulation is less likely than the transport of CQAs. Part of the DiCQAs present in the gastrointestinal mucosa may suffer the action of esterases and also phase II enzymes, being transported into the bloodstream in the form of CQAs, caffeic acid or its phase II metabolites, or even return to the gastrointestinal lumen [36] and be further metabolized by the colonic microbiota.

The concentration of intact CGAs and their direct metabolites, formed by endogenous phase I and II metabolism (total CQAs, FQAs, caffeic, ferulic and isoferulic acids), decreased in plasma and liver 2 h after ingestion of maté tea or 5-CQA. These compounds were rapidly excreted in urine, especially on MT group, while their amounts in urine from ST group collected from 0 to 8 h were significantly higher than the quantities in urine collected from 0 to 4 h.

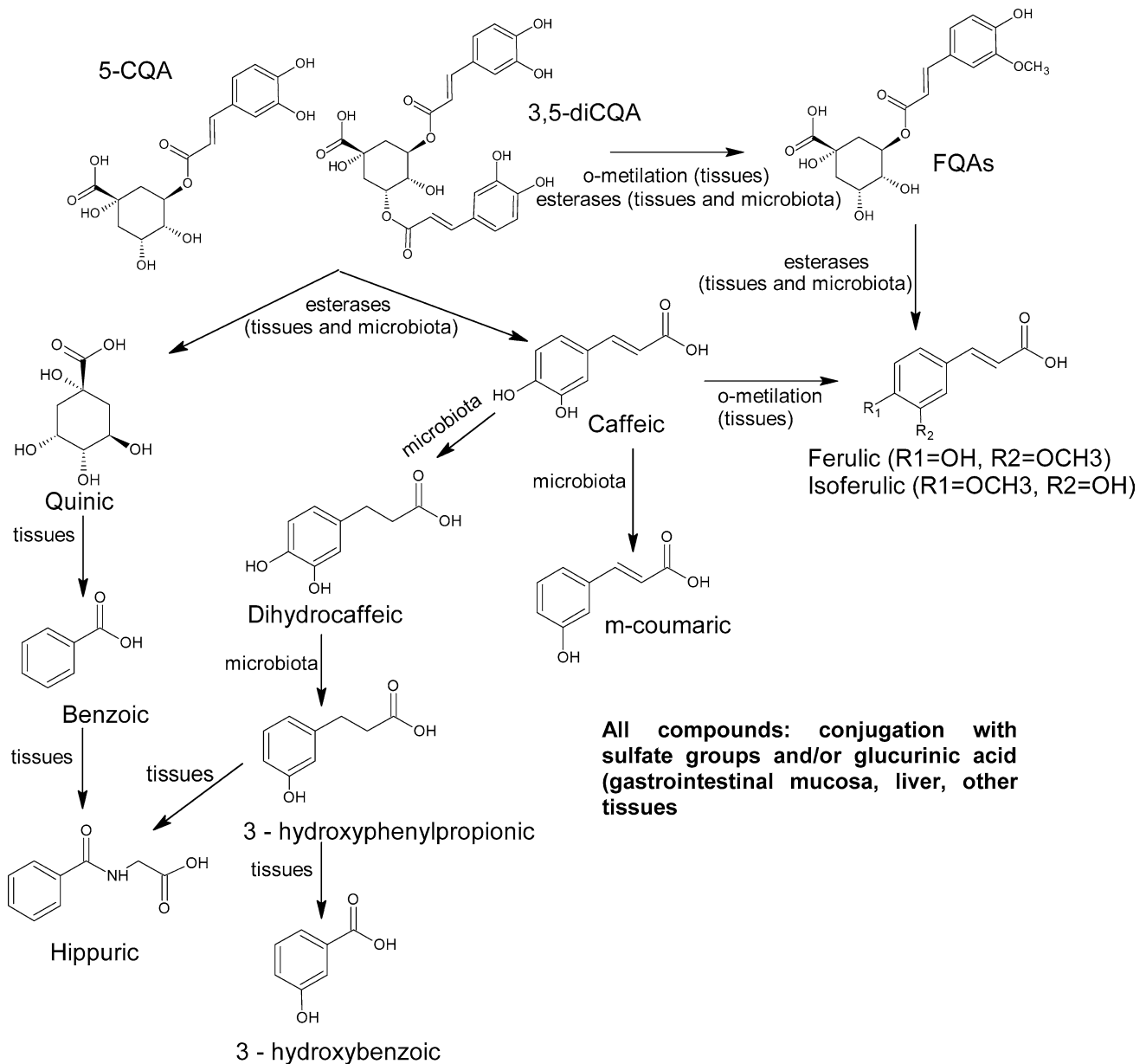


Fig. 6 Schematic representation of the proposed metabolism of chlorogenic acids (CGAs), with 5-caffeoylquinic (5-CQA) and 3, 5-dicaffeoylquinic acids (3, 5-DiCQA) as representatives of CGAs class

According to previous studies, after cleavage of CQAs ester bonds, microbial metabolization of free caffeic acid may originate *m*-coumaric and dihydrocaffeic acids, and this later can be further dehydroxylated to hydroxyphenylpropionic acid and then transformed in hydroxybenzoic or hippuric acid in liver or other tissues, after absorption [7, 14, 20, 31]. Hippuric acid may also originate from quinic acid or from amino acids (L-tryptophan, L-tyrosine and L-phenylalanine), which explains the presence of this compound on tissues (Table 2) and urine (Table 3) of control group.

On animals euthanized 4 h after administration of beverages, plasmatic and hepatic concentrations of substances formed after metabolism of CGAs and free hydroxycinnamates by intestinal microbiota were higher than on previous time points, and these microbial metabolites became the predominant compounds on the last time point evaluated (8 h), as expected, in both groups. For MT group, plasmatic concentrations of dihydrocaffeic, 3-hydroxyphenylpropionic and hippuric acids and hepatic concentration of the latter two were higher than those reached by CQA or by their phase I and/or II metabolites, and also accounted

by the majority of metabolites excreted in urine. Hippuric acid represented 69 % of the total metabolites found in urine collected from 0 to 8 h after maté tea ingestion, followed by 3-hydroxyphenylpropionic acid (~16 %). Both hippuric and 3-hydroxyphenylpropionic acids have been identified as major urinary metabolites of CGAs following ingestion of apple juice [37] and coffee [17] by humans or isolated 5-CQA by humans [38] and rats [20]. In ST group, however, plasmatic and hepatic concentrations of microbial metabolites were lower than those found in MT group. The urinary excretion also differed among the groups, with hippuric acid representing ~42 % of the compounds excreted from 0 to 8 h, followed by FQA2 (~21 %), FQA3 (~18 %) and 3-hydroxyphenylpropionic acid (~8 %), but there were no significant differences between the two groups on the total amount of metabolites excreted in urine (Table 3).

The total metabolites recovered in urine (0–8 h) represented about 4.03 % of the amount of CGAs ingested by MT group (mol/mol) and about 3.31 % of the total 5-CQA ingested by ST group. Although similar absorption rates for CGAs have been found after ingestion of coffee by humans—5.9 % [39] and 5.5 % [31]—more recent studies indicate that the absorption rate may be higher, reaching 29.1 % [15] or even 68 % [18], depending on the amount of CGAs ingested [11]. However, in these studies urine was collected for 24 h, and the majority of phenolics recovered were those formed by colonic microbiota, after 8 h of ingestion of coffee. Therefore, probably a larger amount of metabolites would be absorbed after the last time point evaluated in the present study. Considering only the 5-CQA, free and bound to glucuronic acid and/or sulfate groups, less than 1 % of the amount administered was recovered in the urine in free form in both groups, results similar to those of previous studies in humans after ingestion of coffee [17–19] or pure 5-CQA [38, 40] and rats after oral administration of 5-CQA [20]. FQAs, which are phase II methylated derivatives of CQAs, represent ~39 % of the compounds recovered in urine 0–8 h after ingestion of pure 5-CQA (ST group) and ~25 % of the compounds recovered in urine of MT group in the same period. These results indicate that the rate of absorption of intact CQAs remains very low, although their absorption begins in stomach, and that these compounds suffer extensive metabolization, both by endogenous phase I and II enzymes and by the colonic microbiota, which is in accordance with previous studies [19].

It is important to highlight that calibration curves for estimation of phenolic acids concentration in urine were built with standards diluted in mobile phase, not in urine from the control group, in order to compare the amounts of MT and ST groups with control. However, this approach has limitations, especially when analyses are performed using MS detectors, since matrices affects ionization (and

therefore the response) of compounds. In addition, recovery rates of phenolic acids in urine were calculated by comparing data generated by DAD (concentration ingested through yerba maté) and MS detectors (concentration in urine), and different isomers ionize differently under MS conditions. These factors may not be an issue when comparing concentrations of phenolic acids in urine between groups, but may lead to inaccuracies on the calculation of urinary recoveries.

The profile of CGAs and their metabolites differed between MT and ST groups, in all analyzed fluids and tissues. In MT group, a larger number of phenolic acids were quantified and/or identified, not only because of the greater variety of compounds arising from maté tea (3 isomers of CQAs and 3 DiCQAs), but also a largest number of compounds formed from the metabolization of caffeic acid (ferulic, isoferulic, *m*-coumaric, 3-hydroxyphenylpropionic, dihydrocaffeic and hippuric acids) were observed. Quantitatively, caffeic acid and its metabolites were more abundant in plasma, liver and urine of MT group compared to ST group. We hypothesized that these differences are due to the presence of di-caffeoylquinic acids in maté tea, accounting for about 34 % of the phenolic acids ingested by the animals in MT group (Table 1). DiCQAs provide two caffeic acid molecules for each quinic acid, while 5-CQA has one molecule of each substance. Therefore, animals of MT group ingested relatively more caffeic acid than ST group, also generating more compounds derived from this phenolic acid.

To our knowledge, this is the first study that evaluated the bioavailability and biotransformation of phenolic compounds from yerba maté. Other experiments were performed with coffee, also rich in chlorogenic acids, in humans [11, 15–19, 41] or with isolated 5-CQA in humans [38, 40] and animals [20, 28, 42, 43]. However, it is not always possible to compare the different studies, mainly due to the different metabolites evaluated in each. Also, the profile of chlorogenic acids in coffee differs from maté tea: While coffee has a higher content of moncaffeoylquinic acids (65–72 %) and feruloylquinic acids (12–16 %) and small amounts of di-caffeoylquinic acids (3–10 %) [11, 15–19, 29, 31, 32, 41], maté tea contains mainly moncaffeoylquinic (62 %) and di-caffeoylquinic acids (34 %). As demonstrated in our results, differences in the profile of CGAs ingested may result in qualitative and quantitative differences in the metabolites formed in the body.

This study confirmed that not only absorption of CGAs begins in stomach, but also their metabolization by phase I and II enzymes. Moncaffeoylquinic acids, which are the main CGAs found in maté tea, are absorbed intact and can be transported to organs, but are rapidly excreted in urine either in their native form or as phase II metabolites (glucuronides, sulfates and/or FQAs). Ingestion of maté tea resulted in a more diverse profile of metabolites than

ingestion of isolated 5-CQA, especially derivatives of caffeic acid. Greater emphasis should be given to metabolites in studies that assess biological properties and mechanisms of action of phenolic compounds from yerba maté and other food sources, but attention can also be drawn to intact CGAs when assessing properties related to the gastrointestinal tract, as most of the compounds ingested remain unabsorbed and may have a local effect on the gastrointestinal mucosa.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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