DOI 10.1002/mnfr.201200222 *Mol. Nutr. Food Res.* 2012, *56*, 1488–1500

RESEARCH ARTICLE

Dose-dependent absorption of chlorogenic acids in the small intestine assessed by coffee consumption in ileostomists

Thomas Erk[1], Gary Williamson[2], Mathieu Renouf[3], Cynthia Marmet[3], Heike Steiling[3], Fabiola Dionisi[3], Denis Barron[3], Ralf Melcher[4] and Elke Richling[1]

[1] Food Chemistry and Toxicology, Molecular Nutrition, University of Kaiserslautern, Germany
[2] School of Food Science and Nutrition, University of Leeds, Leeds, UK
[3] Nestlé Research Center, Vers-Chez-les-Blanc, Lausanne, Switzerland
[4] Department of Medicine II, Gastroenterology, University of Würzburg, Germany

Received: April 19, 2012
Revised: July 6, 2012
Accepted: July 26, 2012

Scope: Until now, the question of how the ingested doses of chlorogenic acids (CGA) from coffee influence their absorption and metabolism remains unresolved. To assess absorption in the small intestine, we performed a dose-response study with a randomized, double-blinded, crossover design with ileostomist subjects.

Methods and results: After a polyphenol-free diet, the volunteers consumed, on three separate occasions, coffee with different total CGA contents (high 4525 μmol; medium 2219 μmol; low 1053 μmol). CGA concentrations in plasma, ileal effluent, and urine were subsequently determined by HPLC-DAD-ESI-MS and -ESI-MS/MS. The results show that the consumption of higher CGA concentrations leads to a faster ileal excretion. This corresponds to a renal excretion of 8.0 ± 4.9% (high), 12.1 ± 6.7% (medium), and 14.6 ± 6.8% (low) of total CGA and metabolites. Glucuronidation of CGA became slightly greater with increasing dose. After enzyme treatment, the area under the curve $(AUC)_{0-8h}$ for CGA metabolites in plasma was 4412 ± 751 nM × $h_{0-8}{}^{-1}$ (high), 2394 ± 637 nM × $h_{0-8}{}^{-1}$ (medium), 1782 ± 731 nM × $h_{0-8}{}^{-1}$ (low), respectively. Additionally, we were able to identify new metabolites of CGA in urine and ileal fluid.

Conclusion: We conclude that the consumption of high CGA concentrations via coffee might influence the gastrointestinal transit time and consequently affect CGA absorption and metabolism.

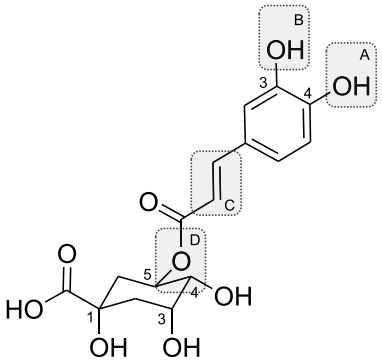
Keywords:
Chlorogenic acids / Coffee / Dose response / Ileostomy / Quinic acid

1 Introduction

Chlorogenic acids (CGA) are esters of hydroxycinnamic acids with D-(–)-quinic acid (QA, 1L-1(OH),3,4,5–tetrahydroxycyclohexane carboxylic acid, according to IUPAC numbering [1]), microconstituents of plants and therefore a part of our diet [2, 3]. The daily lifelong exposure of these secondary plant metabolites and their potentially beneficial effects on human health generate a growing interest in them, including their antioxidant activity in vitro and in vivo [4–9]. CGA intake varies from 25 up to 1000 mg a day. Coffee beans are one of the richest dietary sources of CGA with about 400 μmol CGA per 200-mL cup [10, 11]. From the ileostomy model, it has been shown that high amounts of CGA can reach the colon in concentrations that may inhibit the proliferation of cancer cells [12–15]. However,

Correspondence: Dr. Elke Richling, Department of Chemistry, Division of Food Chemistry and Toxicology, University of Kaiserslautern, Erwin-Schroedinger-Str. 52, 67663 Kaiserslautern, Germany
E-mail: richling@chemie.uni-kl.de
Fax: +49-631-205-4398

Abbreviations: **AUC**, area under the curve; **CA**, caffeic acid; **CGA**, chlorogenic acids; **CQA**, caffeoylquinic acid; **CQL**, caffeoylquinides; **Di-CQA**, dicaffeoylquinic acid; **DHCA**, dihydrocaffeic acid; **DHFA**, dihydroferulic acid; **Di-MeCA**, dimethylcaffeic acid; **Di-MeDHCA**, dimethyldihydrocaffeic acid; **FA**, ferulic acid; **FQA**, feruloylquinic acid; **GIT-TT**, gastrointestinal transit time; **GlucA**, acid-glucuronide; **IFQA**, isoferuloylquinic; **IFA**, isoferulic acid; **IS**, internal standard; **QA**, D-(–)-quinic acid



metabolic action	enzyme	action site
sulfation	sulfuryl-*O*-transferase	A, B
glucuronidation	UDP-glucuronyltransferase	A, B
hydrolysis	esterase	D
reduction	reductase	C
methylation	catechol-*O*-methyltransferase	A, B
conjugation (glycine)*	co-enzyme A-dependent	D

*after hydrolysis

Figure 1. Possible sites of metabolism on 5-*O*-caffeoylquinic acid (see Stalmach et al. [11], Wong et al. [21]).

the colonic availability varies from 26% after cloudy apple juice consumption [16] up to more than 70% after coffee or apple smoothie consumption [15]. The bioavailability of CGA in healthy subjects is somewhat controversial. Some studies found only minor concentration of intact CGAs in plasma [17, 18], whereas a few studies suggest a much higher systemic circulation [19].

For renal excretion, studies with several food sources containing different amounts of CGAs detected a urinary recovery of up to 5.5% of the ingested dose [18, 20]. However, Stalmach [11] showed an extensive metabolism of CGA in the human body after coffee consumption with a renal recovery of 29 ± 4% in healthy subjects and 8 ± 1% in subjects without a colon. Furthermore, extensive phase II metabolism [11, 21] (Fig. 1) highlights the importance of conjugation and metabolism in the overall understanding of polyphenols/CGAs bioavailability. Conjugation of CGA predominantly takes place at the free hydroxyl groups of the hydroxycinnamic acid moiety, which influence biological activities [16, 21, 22].

By taking into account not only high interindividual differences in the daily CGA intake [10] but also the controversial and somewhat inconsistent data in bioavailability, we pose the question of whether the bioavailability of CGA is affected by consumption of different amounts consumed. Furthermore, it would be important to know if different intakes of CGA affect their patterns of metabolism. Therefore, we chose three different nutritional doses of CGA ingested as soluble coffee and performed an ileostomy study as previously described [23]. The aim of this study was to monitor dose-response effects on the absorption, metabolic pattern and colonic availability of CGA and free D-(–)-quinic acid (QA) after the consumption of three different amounts of CGA in a randomized, double-blinded, crossover study with five ileostomists.

2 Materials and methods

2.1 Chemicals

QA and formic acid were from Sigma-Aldrich (Steinheim, Germany). Ethanol (EtOH) p.a. was from Roth (Karlsruhe, Germany). HPLC solvents ACN and methanol were from J.T. Baker (Deventer, The Netherlands). Water was purified by double distillation. Caffeic acid (CA), 5-caffeoylquinic acid (5-CQA) and 3,4,5-trimethoxyphenylacetic acid, *o*-coumaric acid, picric acid, and H_3PO_4 were from Sigma (Steinheim, Germany), and 1-CQA was synthesized [24, 25], 3-CQA (99% purity), and 4-CQA (95% purity) were obtained by interesterification of 5-CQA [26].

2.2 Subjects

Criteria for the five female volunteers were to be in good health with no required medication, nonsmoker, and having a stoma of the terminal ileum. Anthropometric data: average age of 41 ± 3.6 years, BMI of 27.4 ± 2.1 kg/m^2, body fat content of 33.9 ± 2.6% (measured by BF-906, Maltron, Gauting, Germany). The reason for colectomy was a Morbus crohn disease or ulcerative colitis. The study protocol was approved by the Ethics Committee of the Medical Faculty, University of Wuerzburg (No. 124/04 and 32/10).

2.3 Study design

Subjects consumed a single dose of decaffeinated coffee with different amounts of CGA (high, medium, and low) on three separate days. For 2 days before the start and during the study, volunteers consumed a polyphenol-free diet. The administered coffee volume was without sugar or milk. Volunteers remained fasted for 5 h after breakfast and then consumed a light meal. Water consumption was allowed ad libitum. Ileal fluid (−12 to 0, 0, 0.5, 1, 2, 3, 4, 6, 8 h), urine (−24 to 0, 0, 0.5, 1, 2, 3, 4, 6, 8, 8 to 24, and 24 to 48 h) and blood samples (0, 0.5, 1, 2, 4, 6, and 8 h) were collected post consumption. Accordingly, on a separate day, volunteers consumed an anthocyanin-rich blueberry beverage to determine the gastrointestinal transit time (GIT-TT).

2.4 Determination of CGA in coffee and ileal fluid samples

Coffee samples were centrifuged (5 min, 5000 × *g*), membrane filtered (0.45 μm PFDV), and diluted (100-fold) with solution I (ethanol/water/formic acid; 29.9/70/0.1 (v/v/v)).

After freeze-drying of the ileal fluid (Christ Alpha 1–4 apparatus, Osterode, Germany), samples were carefully homogenized. Aliquots ($n = 3$) of 20 mg were extracted (1 mL solution I), subsequently centrifuged (5 min., 4°C, 10 000 × *g*) (Centrifuge 5417 R, Eppendorf) and supernatant was collected. The pellet was again re-extracted twice. The received supernatants were filtered (0.45 μm PVDF), diluted (20-fold, solution I), and analyzed by HPLC-DAD. Extraction efficiency was between 86 and 90% for 5-CQA, CA, and ferulic acid (FA).

2.5 HPLC-DAD analysis

Prior to analysis, the internal standard (IS) 3.4.5-trimethoxyphenylacetic acid ($\lambda_{max} = 270$ nm, 30 μL, 1 mM) was added to the samples (300 μL) and 20 μL were injected (Agilent Technologies 1200 Series). CGAs were detected at 320 nm. The mobile phase used was water containing 0.1% formic acid and ACN. CQA, feruloylquinic acid (FQA), and CA were analyzed with a Synergi polar-RP 250 × 4.6 mm, 4 μm, 80 Å column (Phenomenex, Aschaffenburg, Germany) (flow rate: 0.5 mL × min^{-1}, gradient: 0 min 5% ACN; 21 min 17.5% ACN, 27 min 24% ACN, 30 min 28% ACN, 60 min 30% ACN). Caffeoylquinides (CQL), FA, and *di*CQA were analyzed with an Atlantis RP 18, 4.6 × 150 mm, 3 μm column (Waters, Eschborn, Germany), (flow rate: 1.1 mL min^{-1}, gradient: 0 min 7% ACN, 80 min 27% ACN).

Calibration conditions: $R^2 > 0.99$; LOD 0.20 to 1.62 *n*g; and LOQ 0.68 to 5.42 *n*g. CQA and CQL were quantified as 5-CQA equivalents. FQA was quantified as 5-FQA equivalents and di-CQA as 3,4-diCQA equivalents. Data acquisition and evaluation were performed with Agilent Chemstation software (Jasco, Groß-Umstadt, Germany).

2.6 Determination of metabolites in ileal fluid

The ileal extraction solution (1.0 mL, as described in section 2.4) was dried in a vacuum centrifuge (UniEquip, Martinsried, Germany) and subsequently redissolved with water to 1.0 mL (pH 1.5, acidified with H_3PO_4). All solutions were acidified with HCl (0.01%, v/v). The SPE cartridges were preconditioned (2 mL MeOH and 2 mL H_2O) (Supelco, Bellefonte, US). After sample application, the SPE was washed (1 mL H_2O), dried carefully, and analytes were eluted (1.8 mL MeOH) and concentrated.

Residues were re-dissolved (200 μL solution I). To 100 μL of this sample, 10 μL of the IS (*o*-coumaric acid, 61 μM) was added. Extraction efficiencies were determined greater than 90%. Calibration conditions: concentration range (6.2 mg × L^{-1} to 1.3 μg × L^{-1}); $R^2 = 0.99$; LOQ 0.71 to 6.22 ng and LOD 0.21 to 1.86 ng.

Determination was semiquantitative, since compounds were quantified based on calibration curves of the available standards: CQA, CQL and their corresponding metabolites, as 5-CQA equivalents; FQA and metabolites as 5-FQA equivalents; FA and feruloylglycine (FA-Gly) as FA equivalents; FA-acid-glucuronide (GlucA) derivatives as isoferulic acid (IFA)-GlucA equivalents; sulfated derivatives as FA-Sulf equivalents. CA- and dihydrocaffeic acid (DHCA) metabolites were quantified using the 3′ conjugated metabolites, respectively.

2.7 Determination of CGA and metabolites in urine

Aliquots were defrosted, warmed up to 37°C, and 3 mL were acidified with H_3PO_4 to a pH of 1.5. Supernatant of samples (centrifuge, 4°C, 5 min, 5000 × *g*) was used for SPE as described above. In urine, LOQ was from 0.55 to 43.4 ng and LOD was from 0.16 to 13.0 ng.

2.8 HPLC-MS analysis of ileal fluids and urine samples

Chromatographic separations were achieved with the same conditions as described above on a Synergi Polar-RP 250 × 4.6 mm, 4 μm, 80 Å column (Phenomenex). Aliquots (40 μL) were injected. For each analyte, the corresponding deprotonated molecular ions $[M\text{-}H]^-$ was used as quantifier in an ESI multimethod (Fig. 2) (scan duration: 1.0 s, dwell time: 70 ms) (Jasco HPLC system (Jasco) coupled to SCIEX API 3200 MS/MS (Applied Biosystems, Darmstadt, Germany)). ESI settings were as follows: spray capillary voltage, 4.5 kV; curtain gas, nitrogen (370°C at 30 psi); GS1, 70 psi; GS2, 40 psi; electron multiplier voltage, 2.1 kV. The main characteristic fragmentation ion from $[M\text{–}H]^-$ of each analyte was used as a qualifier in a multiple reaction mode.

2.9 Determination of CGA in plasma

Blood samples were centrifuged (3500 × *g*, 10 min, 4°C). To 0.5 mL supernatant (plasma), 20 μL of storage solution (0.4 M NaH_2PO_4 buffer pH 3.6, containing 20% ascorbic acid and 0.1% EDTA) was added. Duplicate samples of 100 μL plasma were spiked with an IS (5 μL; 1 μM labeled d13-C2-caffeic acid; Orphachem s.a., Clermont-Ferrand, France) and protein was precipitated (500 μL ethanol) by centrifugation (5 min, 4°C, 17 500 × *g*). The residue was further twice re-extracted and the supernatants dried under N_2. After evaporation, the residue was incubated at 37°C for 60 min with β-glucuronidase (1000 units) and sulfatase (60 units) (pH 5.5, 0.1 M sodium acetate). After adding 10 μL perchloric acid (6 M), CGA was extracted twice (30 min at 4°C) with ACN. The supernatants were adjusted to pH 7 (0.75 M potassium carbonate; 40 μL), pooled, dried, and re-suspended (water

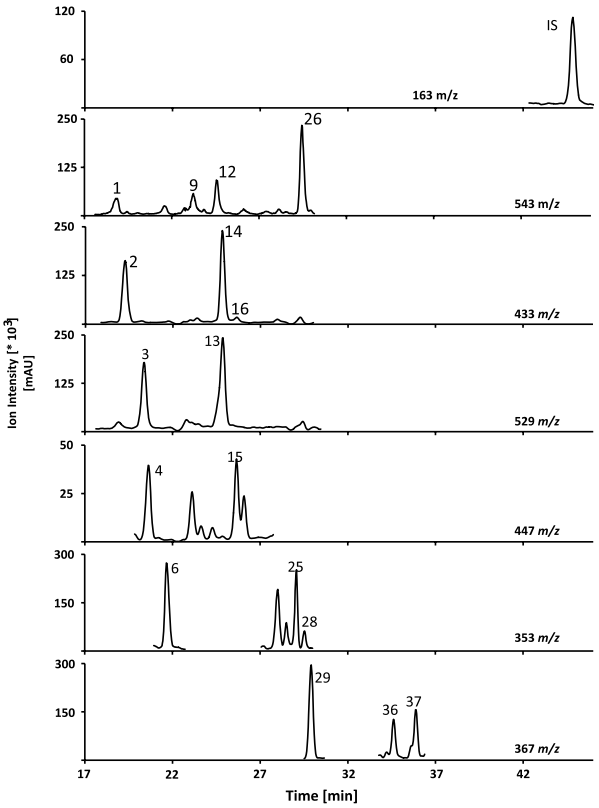


Figure 2. Typical HPLC-MS molecular ion $[M\text{-}H]^-$ chromatogram of CQAs and FQAs and corresponding metabolites detected in urine of an ileostomist 2 h after coffee consumption. IS: *o*–coumaric acid. For identification of peaks and peak numbers, see Table 1.

with 1% acetic acid, 5% ACN), and then samples (50 μL) were analyzed by HPLC-MS.

2.10 HPLC-MS analysis of plasma samples

An HPLC system (Thermo Scientific, Dreieich, Germany) coupled to a TSQ Vantage MS was used. Chromatographic separations were achieved: water containing 1% acetic acid (A) and ACN 1% acetic acid (B); on an UPLC Acquity BEH C18 1.8 μm, 2.1 × 150 mm column (Waters), flow rate 0.2 mL × min^{-1}, gradient: 1 min hold 3% B; 16 min 20% B and 26 min 30% B. The MS was used with ESI operating in the SRM negative ionization mode with the following parameters: collision gas pressure 1.5 mTorr; cycle time 1 s; DCV 2 V; capillary temperature + 270°C; sheath gas pressure 40; aux valve flow 20; spray voltage −2500 V. Calibration curves were generated for each volunteer in duplicate by spiking 100 μL blank plasma (0-h collection point) with 5 μL of labeled CA as IS and a stock solution including the following compounds (SRM transition from $[M–H]^-$ to product ion): CA 179 *m/z* → 135 *m/z*, FA 193 *m/z* → 134 *m/z*, IFA 193 *m/z* → 178 *m/z*, DHCA 181 *m/z* → 137 *m/z*, DHFA 195 *m/z* → 136 *m/z*, 3-CQA 353 *m/z* → 191 *m/z*, 4-CQA 353 *m/z* → 173 *m/z*, 5-CQA 353 *m/z* → 191 *m/z*, 3-FQA 367 *m/z* → 193 *m/z*, 4-FQA 367 *m/z* → 173 *m/z*, 5-FQA 367 *m/z* → 191 *m/z*, 1,3-*di*CQA 515 *m/z* → 353 *m/z*, 1,5–*di*CQA 515 *m/z* → 191 *m/z*, 3,4-*di*CQA 515 *m/z* → 179 *m/z*, 3,5-*di*CQA 515 *m/z* → 191 *m/z*, 4,5-*di*CQA 515 *m/z* → 173 *m/z*, dimethylcaffeic acid (*di*-MeCA) 207 *m/z* → 103 *m/z*, and dimethyldihydrocaffeic acid (*di*-MeDHCA) 209 *m/z* → 150 *m/z*. The LOD was set to approximately 10 nM for all the compounds, except for *di*-CQA where LOQ was set to 30 nM.

2.11 Analysis of QA

Coffee samples and ileal extraction solutions were diluted and QA content was assessed according to the previously published method by LC-MS [27].

For the measurement of QA in plasma, 120 μL was mixed with 30 μL of U-^{13}C-D-(–)-QA, (5.1 μM; in solution I) and deproteinized with 50-μL acidified ethanol. Samples were vortexed (1 min, 1600 min^{-1}) and kept at 4°C for 30 min. After this, samples were vortexed briefly and centrifuged (4°C, 30 min, 14 000 × *g*). The QA content of supernatants was assessed as previously published [27], using 70 μL as injection volume and a multiple reaction mode transition from the deprotonated molecular ion $[M–H]^-$ *m/z* 191 to *m/z* 93 as quantifier with a calculated LOQ of 273 ng and LOD of 91 ng.

2.12 Creatinine determination

For this, aliquots of diluted urine were added to picric acid solution (9.0 g NaCl, 1.31 g picric acid in 1 L H_2O) and sodium hydroxide solution (1 M). Absorption was measured according to [28].

2.13 Synthesis of caffeoylquinides

A mixture of 3-CQA, 4-CQA, and 5-CQA was generated [26] and then quinides formed [29]. Isolation was carried out by HPLC-DAD at 320 nm with an Atlantis RP 18 4.6 × 150 mm, 3 μm column (12.0/87.9/0.1; ACN/H_2O/formic acid, v/v/v; 0.8 mL × min^{-1}). Identification of the obtained pure compounds was by comparison of the fragmentation pattern with literature data and NMR spectroscopy data (Bruker AMX–600 apparatus with WIN–NMR v6.0 software) [30].

2.14 Statistics

Experimental results are expressed as mean of at least three independent extractions and analyses ± SD. Excel 2007 (Microsoft, Unterschleißheim, Germany) was used for statistical evaluations (two-sample *t*-test) of significance and differences were considered significant with $p \leq 0.05$ or $p \leq 0.01$.

Table 1. Product ion scan of CGA and metabolites detected in urine and ileal fluid after coffee consumption

Number	Compound	t_R [Min]	CE eV	(*m/z*)		Location
				[M-H]$^-$	Product ion	
1	3-*O*-FQA-*O*-GlucA	18.7	−40	543	367; 193; 113; 175; 149; 85	Ileal fluid, urine
2	3-*O*-CQA-3′-*O*-Sulf	19.0	−40	433	353; 191; 179; 135	Ileal fluid, urine
3	3-*O*-CQA-*O*-GlucA	20.0	−40	529	353; 179; 191; 135	Ileal fluid, urine
4	3-*O*-FQA-*O*-Sulf	20.1	−40	447	367; 193; 191; 134; 173	Ileal fluid, urine
5	1-*O*-CQA	20.9	−50	353	191; 85	Ileal fluid
6	3-*O*-CQA	21.8	−50	353	135; 191; 93	Ileal fluid, urine
7	5-*O*-FQA-*O*-GlucA	22.2	−40	543	367; 191; 113; 85; 173	Ileal fluid[a)], urine[a)]
8	DHCA-4′-*O*-Sulf	22.9	−50	261	59; 88	Urine
9	4-*O*-FQA-*O*-GlucA	23.0	−40	543	367; 173; 193; 113; 85	Ileal fluid, urine
10	DHCA-3′-*O*-Sulf	23.6	−50	261	59; 80; 121; 135; 181	Ileal fluid, urine
11	CA-4′-*O*-GlucA	24.0	−40	355	135; 179	Urine
12	3-*O*-IFQA-*O*-GlucA	24.3	−40	543	367; 193; 113; 173; 85	Ileal fluid, urine
13	4-*O*-CQA-*O*-GlucA	24.5	−40	529	353; 173; 179.0; 191; 135	Ileal fluid, urine
14	4-*O*-CQA-3′-*O*-Sulf	24.5	−40	433	353; 173; 179; 191; 135	Ileal fluid, urine
15	4-*O*-FQA-*O*-Sulf	25.2	−40	447	367; 173; 191; 193; 134	Ileal fluid, urine
16	5-*O*-CQA-3′-*O*-Sulf	25.3	−40	433	353; 191	Ileal fluid
17	CA-4′-*O*-Sulf	25.7	−50	259	135; 107; 80; 179; 97	Ileal fluid, urine
18	DHFA-4′-*O*-Sulf	25.7	−40	275	59; 80; 135; 195	Ileal fluid, urine
19	DHCA-3′-*O*-GlucA	26.0	−40	357	181; 85; 113; 59; 138	Urine[a)]
20	CA-3′-*O*-Sulf	27.4	−50	259	135;107; 80; 97; 179	Ileal fluid, urine
21	DHFA -4′-*O*-GlucA	27.4	−40	371	85; 59; 195	Urine
22	CA-3′-*O*-GlucA	27.7	−40	355	135; 179; 85	Ileal fluid, urine
23	FA-4′-*O*-GlucA	28.0	−40	369	134; 178; 193; 85; 59; 149	Urine
24	DHCA	28.5	−50	181	59; 41; 109; 93; 121; 138	Ileal fluid, urine
25	4-*O*-CQA	29.0	−50	353	135; 191; 93; 173	Ileal fluid, urine
26	4-*O*-IFQA-*O*-GlucA	29.0	−40	543	367; 173; 113; 193	Ileal fluid, urine
27	FA-4′-*O*-Sulf	29.1	−40	273	134; 178; 149; 193; 121; 97; 80	Ileal fluid, urine
28	5-*O*-CQA	29.8	−50	353	191; 85; 93; 127	Ileal fluid, urine
29	3-*O*-FQA	30.0	−50	367	134; 117; 193	Ileal fluid, urine
30	IFA-3′-*O*-Sulf	31.0	−40	273	134; 178; 193; 137; 149; 80	Ileal fluid, urine
31	IFA-3′-*O*-GlucA	32.0	−40	369	178; 193; 134; 85; 59	Urine
32	CQL-*O*-GlucA	33.0	−40	511	335; 161; 135; 179	Ileal fluid, urine
33	CA	33.1	−50	179	134	Ileal fluid, urine
34	CQL-*O*-Sulf	34.0	−50	415	335; 161; 135; 173; 179	Ileal fluid, urine
35	FA-glycine	34.3	−30	250	206; 191; 177; 149; 163; 134; 100	Ileal fluid, urine
36	4-*O*-FQA	34.6	−50	367	134; 93; 173	Ileal fluid, urine
37	5-*O*-FQA	36.0	−50	367	93; 191; 134	Ileal fluid, urine
38	3-*O*-CQL	36.7	−50	335	161; 133	Ileal fluid, urine
39	4-*O*-CQL	37.8	−50	335	161; 133	Ileal fluid, urine
40	FA	41.5	−30	193	134; 178	Ileal fluid, urine
41	3,4-*O*-*di*CQA	55.3	−50	515.5	173; 179; 135; 191	Ileal fluid
42	3,5-*O*-*di*CQA	57.5	−50	515.5	191; 179; 135	Ileal fluid
43	4,5-*O*-*di*CQA	63.2	−50	515.5	173; 179; 135; 191	Ileal fluid

a) Trace levels.

3 Results

Identification of CGA and corresponding metabolites (Table 1) in coffee and biological fluids was achieved by co-chromatography and matching MS^2 spectra with available reference compounds. CGA compounds without available references were assigned using literature data [11, 31] and similar MS^2 spectra of corresponding precursor compounds. Glucuronides were additionally confirmed by neutral loss scan mode (176 amu).

Sulfates of CQA (conjugated at position 3′) were detected as precursor ion of *m/z* 433 and their characteristic product ions, especially the *m/z* 353 ion indicating a loss of 80 amu. Fragmentation pattern of 3-*O*-CQA-sulfate shows the *m/z* 135 and *m/z* 191 product ion ratio similar to 3-*O*-CQA. The 4-*O*-CQA-sulfate showed a characteristic product ion *m/z* 173 similar to 4-*O*-CQA and 5-*O*-CQA-sulfate showed the dominating *m/z* 191 ion similar to 5-*O*-CQA. Two CQA-glucuronides were detected in the product ion scan mode at *m/z* 529 by the product ion *m/z* 353 indicating the loss of

a glucuronide unit (176 amu). A 3-*O*-CQA-glucuronide was identified by the product ion ratio *m/z* 135 and *m/z* 191 and 4-*O*-CQA-glucuronide by the characteristic *m/z* 173 product ion.

CQL-sulfates showed an $[M-H]^-$ of *m/z* 415. The product ion spectrum is dominated by *m/z* 161, typically for CQL and *m/z* 335 is generated by a specific loss of 80 amu, according to the work of Stalmach [11]. CQL-glucuronides have an $[M-H]^-$ of *m/z* 511 and the characteristic *m/z* 161. A *m/z* 335 is generated by a specific loss of 176 amu.

Sulfates of FQA were detected by the precursor ion of *m/z* 447 and characteristic product ions of the FQA, especially the *m/z* 367 indicating a loss of 80 amu. A 3-*O*-FQA-4′-*O*-sulfate has similar product ions (*m/z* 193 and *m/z* 134) as 3-*O*-FQA. A 4-*O*-FQA-sulfate has the characteristic *m/z* 173 product ion similar to 4-*O*-FQA [32]. Three FQA-glucuronides were detected by an $[M-H]^-$ of *m/z* 543. The product ion of *m/z* 367 indicates the loss of a glucuronic acid unit (176 amu). A 3-*O*-FQA-4′-*O*-glucuronide was identified by the product ion *m/z* 193 similar to 3-*O*-FQA, 4-*O*-FQA-4′-*O*-glucuronide by the characteristic product ion *m/z* 173 typical for 4-FQA and 5-*O*-FQA-4′-*O*-glucuronide by the dominating *m/z* 191 product ion typical for 5-FQA [32].

The identification of isoferuloylquinic acid-glucuronide (IFQA-GlucA) was based on the data obtained from FQA-GlucA. IFQA-GlucA has a later retention time but we assumed the same characteristics as feruloylquinic acid glucuronides. We identified 3-*O*-IFQA-3′-*O*-glucuronide and 4-*O*-IFQA-3′-*O*-glucuronide corresponding to the MS^2 fragmentation pattern. Feruloylglycine was identified by comparison of the MS^2 fragmentation pattern reported by Stalmach et al. [11].

3.1 Coffee CGA content

The main CGA subclass found in the three different coffees under study were caffeoylquinic acids (68.8%) (Table 2). Free CA was only detectable in low amounts. Other CGAs, such as FQA, *di*-CQA, and CQL, were also detected in minor amounts. The total consumed CGA content in coffee was 4525 μmol (1642 mg), 2218 μmol (805 mg), and 1053 μmol (382 mg) for the high, medium and low doses, respectively or in $\mu mol \times kg^{-1} \times BW^{-1}$ 59.7 ± 2.0, 29.1 ± 1.5 and 13.8 ± 0.5. The consumed free QA content is 2547, 1373, and 695 μmol (high; medium; low), respectively.

3.2 Ileal excretion

The time of passage of consumed coffee through the upper gastrointestinal tract varied between the subjects. In particular, the GIT-TT of one subject (number 5) showed a significant difference (Fig. 3, $p < 0.03$) (Table 4). We examined the ileal excretion T_{max} of 5-CQA. Independently of the coffee concentration, subject 5 had a time shift of several hours in $T_{max(5\text{-}CQA)}$ as shown in Fig. 3. This finding was confirmed by the separate GIT-TT determination with an anthocyanin-rich (colored) beverage.

Eight hours after coffee consumption, only small amounts of CGA and metabolites were detectable in the ileal fluid. The total colonic availability is shown in Table 3. All compounds from coffee were observed in the excreted ileal fluid to a certain extent. Between 68.8 ± 9.0% (high) and 77.4 ± 4.3% (low) of the ingested compounds were excreted via the ileal fluid. A part (6.7 ± 2.1% (high), 7.7 ± 1.2% (medium), and

Table 2. Ingested coffee polyphenols per trial in μmol (data expressed as mean values ± SD; $n = 5$)

Compound	High	Medium	Low
1-*O*-Caffeoylquinic acid	29 ± 3	14 ± 2	8 ± 1
3-*O*-Caffeoylquinic acid	878 ± 73	452 ± 59	231 ± 21
4-*O*-Caffeoylquinic acid	869 ± 73	431 ± 53	209 ± 17
5-*O*-Caffeoylquinic acid	1262 ± 106	621 ± 75	298 ± 24
Total Caffeoylquinic acids	3037 ± 148	1519 ± 110	746 ± 36
3-*O*-Caffeolyquinide	481 ± 32	218 ± 16	84 ± 12
4-*O*-Caffeoylquinide	249 ± 21	110 ± 9	41 ± 8
Total Caffeoylquinides	729 ± 38	328 ± 18	125 ± 15
3-*O*-Feruloylquinic acid	122 ± 11	63 ± 8	34 ± 4
4-*O*-Feruloylquinic acid	122 ± 10	60 ± 8	30 ± 3
5-*O*-Feruloylquinic acid	193 ± 16	91 ± 11	46 ± 4
Total Feruloylquinic acids	436 ± 21	214 ± 16	109 ± 6
3,4-*O*-Dicaffeoylquinic acid	112 ± 7	56 ± 5	26 ± 2
3,5-*O*-Dicaffeoylquinic acid	77 ± 6	38 ± 4	19 ± 1
4,5-*O*-Dicaffeoylquinic acid	105 ± 6	51 ± 4	22 ± 2
Total dicaffeoylquinic acids	294 ± 11	145 ± 8	67 ± 3
Caffeic acid	28 ± 2	13 ± 1	7 ± 1
Total chlorogenic acids	4525 ± 155	2219 ± 113	1053 ± 40
D-(–)-Quinic acid	2547 ± 276	1373 ± 129	695 ± 63

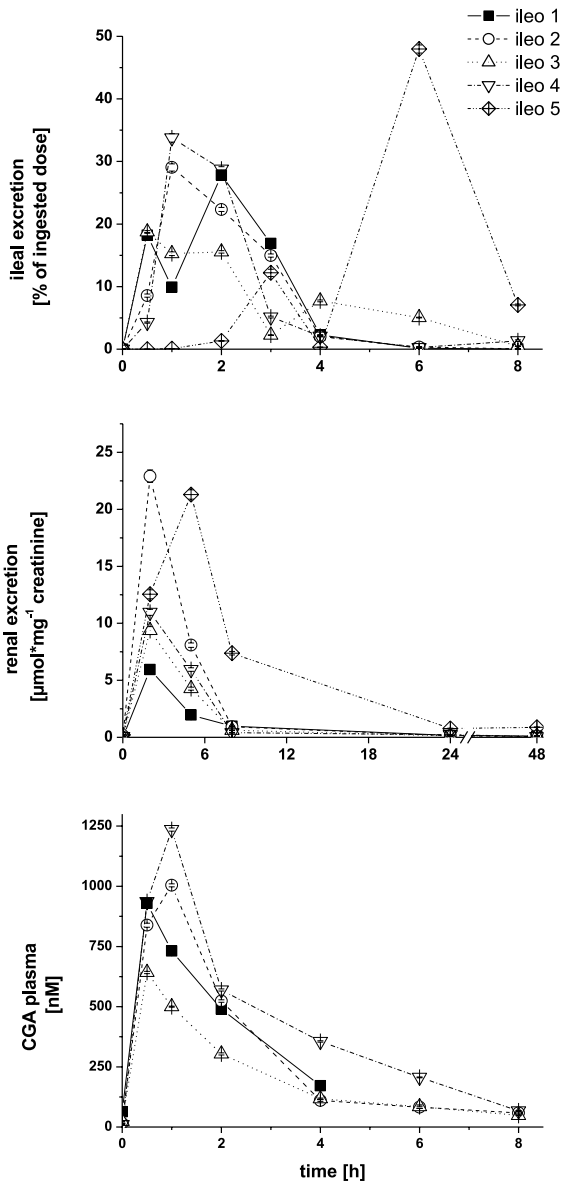


Figure 3. Summarized time periods of the coffee CGA and corresponding metabolite concentrations detected in the biological samples of volunteers after consumption of the medium dose of coffee. Ileal excretion (percentage of ingested dose) ($n = 5$), renal excretion in (μmol $\times$ mg^{-1} creatinine) ($n = 5$), and plasma concentration after enzymatic hydrolysis (nM) ($n = 4$).

8.9 $\pm$ 1.3% (low)) of the ingested CGA dose was metabolized or hydrolyzed during the GIT passage. Therefore, sulfation was the dominating conjugation, with a sulfation:glucuronidation ratio of 8.2:1 (high), 10.4:1 (medium) and 13.4:1 (low) (Table 3). The recovery rate of QA showed high interindividual variation, and was 86.5 $\pm$ 14.5% (high) to 77.6 $\pm$ 17.5% (low) for subjects 1 to 4. For subject number 5, QA ileal recovery was much lower (Table 4).

3.3 Renal excretion

The total renal excretion (see Table 5) of CGA differed considerably among the ileostomists; in particular, subject number 5 showed a longer and significantly higher renal excretion compared to the other ileostomists ($p < 0.01$; Fig. 3). Between 8.0 $\pm$ 4.9 (high) and 14.6 $\pm$ 6.8% (low) of CGAs and metabolites were detected in urine (Table 5). The rate of metabolized or hydrolyzed CGA was 67.0 $\pm$ 6.6% (high), 69.2 $\pm$ 6.5% (medium), and 69.8 $\pm$ 3.4% (low) with a sulfation:glucuronidation ratio of 0.7:1 (high), 1:1 (medium), and 1.3:1 (low). We found 16.4 $\pm$ 6.0 (high), 14.8 $\pm$ 1.5 (medium), and 13.7 $\pm$ 2.7% (low) of the ingested QA in urine after coffee consumption.

3.4 Plasma concentration

We observed increasing CGA concentrations in plasma correlating to an increase in CGA concentrations of the coffee beverages. The total AUC of all measured CGA were 4412 $\pm$ 751 nM $\times$ $h_{0-8}{}^{-1}$ (high), 2394 $\pm$ 637 nM $\times$ $h_{0-8}{}^{-1}$ (medium) 1782 $\pm$ 731 nM $\times$ $h_{0-8}{}^{-1}$ (low). After 1 h, nearly all CGA were almost at their maximal plasma concentration for each trial (Fig. 3, Table 6). Volunteer number 5 showed a slower elimination of CGA in comparison to the other volunteers and thus AUC was significantly increased ($p < 0.05$). All detected compounds, except for IFA and *di*-MeDHCA, showed a dose-dependent relationship between plasma AUC or C_{max} and increasing coffee dose (Table 6). The QA concentration reached its maximum within about 4.5 h after coffee consumption, much later than CGA (Fig. 4 and Table 6).

4 Discussion

After consumption of coffee by ileostomists, we recovered in ileal fluid about two thirds of the CGA metabolites, implying that about one third is absorbed in the small intestine, comparable to a previous single-dose study on a different group of ileostomist volunteers [12,14]. Different coffee doses did not greatly affect absorption in the small intestine. The high interindividual differences in $T_{max(5\text{-}CQA)}$, especially of ileostomist 5 ($p < 0.03$; Table 4) did not relate to the total amount of CGA reaching the colon. The excreted QA content showed significant interindividual differences, especially in one subject who had the longest transit time (ileo 5 compared to ileo 1–4, $p < 0.01$). High interindividual differences (6.2–12.8 h) have already been observed in transit time [33]. Additionally, we show that a higher coffee dose led to a faster transit time for ileostomists 1–4 ($p < 0.09$, Table 4).

A proportionally lower amount of the ingested CGA dose was metabolized or hydrolyzed with higher ingested dose, which could conceivably be due to enzyme saturation, limitation of substrates, limited transport capacities at the enterocyte (influx and efflux) or an influence of the GIT-TT. In

Table 3. Ileal excretion of CGA and metabolites in ileostomist ($n = 5$) after ingestion of a high, medium, and low dose of coffee (in μmol). (data expressed as mean values ± SD; $n = 5$)

Compound	High	Medium	Low
1-*O*-Caffeoylquinic acid	16 ± 5	10 ± 4	5 ± 2
3-*O*-Caffeoylquinic acid	638 ± 183	342 ± 129	179 ± 69
4-*O*-Caffeoylquinic acid	593 ± 170	312 ± 121	157 ± 62
5-*O*-Caffeoylquinic acid	862 ± 246	455 ± 175	228 ± 89
3-*O*-Caffeoylquinic acid-*O*-GlucA	4 ± 2	2 ± 1	1 ± 1
4-*O*-Caffeoylquinic acid-*O*-GlucA	5 ± 2	3 ± 1	2 ± 1
3-*O*-Caffeoylquinic acid-*O*-Sulf	30 ± 10	19 ± 8	10 ± 4
4-*O*-Caffeoylquinic acid-*O*-Sulf	35 ± 12	22 ± 10	12 ± 5
5-*O*-Caffeoylquinic acid-*O*-Sulf	2 ± 1	1 ± 1	0.8 ± 0.3
3-*O*-Caffeolyquinide	132 ± 63	39 ± 20	16 ± 8
4-*O*-Caffeoylquinide	56 ± 28	22 ± 11	9 ± 5
3-/4-*O*-Caffeolyquinide-*O*-GlucA	16 ± 6	9 ± 4	3 ± 2
3-/4-*O*-Caffeoylquinide-*O*-Sulf	125 ± 36	76 ± 28	33 ± 16
3-*O*-Feruloylquinic acid	86 ± 25	46 ± 17	25 ± 10
4-*O*-Feruloylquinic acid	98 ± 29	43 ± 17	23 ± 9
5-*O*-Feruloylquinic acid	111 ± 32	61 ± 24	32 ± 12
3-*O*-Feruloylquinic acid-*O*-Sulf	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.0
4-*O*-Feruloylquinic acid-*O*-Sulf	0.3 ± 0.1	0.2 ± 0.1	0.1 ± 0.0
3-*O*-Feruloylquinic acid-*O*-GlucA	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.0
4-*O*-Feruloylquinic acid-*O*-GlucA	< LOQ	< LOQ	< LOQ
3-*O*-Isoferuloylquinic acid-*O*-GlucA	0.4 ± 0.1	0.2 ± 0.1	0.1 ± 0.0
4-*O*-Isoferuloylquinic acid-*O*-GlucA	0.9 ± 0.3	0.6 ± 0.2	0.5 ± 0.2
Caffeic acid	27 ± 8	16 ± 6	13 ± 4
Caffeic acid-3′-*O*-GlucA	1 ± 1	1 ± 1	0.2 ± 0.1
Caffeic acid-3′-*O*-Sulf	26 ± 9	17 ± 8	11 ± 5
Caffeic acid-4′-*O*-Sulf	6 ± 2	4 ± 2	3 ± 1
Dihydrocaffeic acid	8 ± 3	4 ± 1	2 ± 1
Dihydrocaffeic acid-3′-*O*-Sulf	1 ± 1	0.9 ± 0.4	0.5 ± 0.2
Ferulic acid	5 ± 2	3 ± 1.0	3 ± 1
Ferulic acid-4′-*O*-Sulf	2 ± 1	2 ± 0	2 ± 1
Feruloylglycine	0.9 ± 0.3	0.3 ± 0.1	0.2 ± 0.1
Isoferulic acid-3′-*O*-Sulf	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
Dihydroferulic acid-4′-*O*-Sulf	4 ± 1	2 ± 1	1 ± 0.0
3,4-*O*-Dicaffeoylquinic acid	76 ± 23	38 ± 15	19 ± 7
3,5-*O*-Dicaffeoylquinic acid	50 ± 15	25 ± 9	12 ± 5
4,5-*O*-Dicaffeoylquinic acid	71 ± 22	37 ± 14	18 ± 7
$\sum$	3093 ± 317	1598 ± 110	816 ± 88
% of ingested amount	68.8 ± 9.0	72.4 ± 4.7	77.4 ± 4.3

nd = not detected (< LOQ).

a similar study on one dose, Stalmach et al. [14] observed that 22% was metabolized or hydrolyzed with a low dose of CGA ingestion (2.7 times smaller than our low dose) with a sulfation:glucuronidation ratio of 10.5:1. In agreement with Stalmach et al, we also identified sulfation as the major phase II conjugation reaction in the upper GIT, but we additionally observed an increasing proportion of glucuronidation with increasing CGA dose. The regional selectivity preference for sulfation and glucuronidation on the 3′-hydroxyl of CA and DHCA was consistent over the three doses in both

Table 4. For ileostomist 1–4 $T_{max(5\text{-}CQA)}$ (ileal excretion) increases with increasing dose ($p < 0.09$). Ileostomist 5 showed a significant difference in $T_{max(5\text{-}CQA)}$ ($p < 0.03$) within all doses compared to the other ileostomists. Further, the total ileal excretion of free QA in percentage is different as well

	Ø Ileostomist no. 1–4		Ileostomist no. 5	
Trial	Ileal excretion (%) D-(–)-QA	$T_{max(5\text{-}CQA)}$ (Hours)	Ileal excretion (%) D-(–)-QA	$T_{max(5\text{-}CQA)}$ (Hours)
High	86.2 ± 14.5	0.5 ± 0.0	38.1 ± 0.9	4
Medium	81.9 ± 8.1	0.8 ± 0.3	23.9 ± 1.0	6
Low	77.6 ± 17.5	1.6 ± 1.0	12.5 ± 0.3	4

Table 5. Renal excretion of polyphenols and metabolites after ingestion of a high, medium, and low dose of coffee (in μmol), ileostomists $n = 5$ (data expressed as mean values ± SD; $n = 5$)

Compound	High	Medium	Low
3-*O*-Caffeoylquinic acid	26.2 ± 27.8	14.6 ± 0.6	8.2 ± 0.5
4-*O*-Caffeoylquinic acid	14.6 ± 8.0	8.6 ± 0.7	6.7 ± 1.2
5-*O*-Caffeoylquinic acid	9.4 ± 7.9	6.7 ± 0.5	4.4 ± 0.4
3-*O*-Caffeoylquinic acid-*O*-GlucA	8.3 ± 5.6	4.8 ± 0.2	1.6 ± 0.1
4-*O*-Caffeoylquinic acid-*O*-GlucA	23.3 ± 18.2	13.3 ± 0.9	5.5 ± 0.2
3-*O*-Caffeoylquinic acid-*O*-Sulf	5.3 ± 2.1	4.0 ± 0.2	2.2 ± 0.2
4-*O*-Caffeoylquinic acid-*O*-Sulf	5.1 ± 1.8	3.8 ± 0.2	2.7 ± 0.2
3-*O*-Caffeolyquinide	2.8 ± 3.0	0.8 ± 0.1	0.2 ± 0.0
4-*O*-Caffeoylquinide	4.6 ± 4.7	1.5 ± 0.1	0.7 ± 0.1
Caffeolyquinide-*O*-GlucA	4.1 ± 3.0	2.2 ± 0.1	0.6 ± 0.0
Caffeoylquinide-*O*-Sulf	24.1 ± 6.9	23.5 ± 1.6	10.3 ± 0.6
3-*O*-Feruloylquinic acid	33.3 ± 19.5	20.8 ± 1.4	12.3 ± 0.9
4-*O*-Feruloylquinic acid	22.2 ± 17.0	14.9 ± 0.5	7.1 ± 0.8
5-*O*-Feruloylquinic acid	17.0 ± 16.1	12.0 ± 0.6	7.1 ± 0.7
3-*O*-Feruloylquinic acid-*O*-Sulf	0.3 ± 0.3	0.1 ± 0.0	< LOQ
4-*O*-Feruloylquinic acid-*O*-Sulf	0.8 ± 0.7	0.4 ± 0.0	0.1 ± 0.0
3-*O*-Feruloylquinic acid-*O*-GlucA	2.2 ± 1.4	1.8 ± 0.3	1.0 ± 0.1
4-*O*-Feruloylquinic acid-*O*-GlucA	1.3 ± 1.8	0.5 ± 0.1	0.2 ± 0.1
3-*O*-Isoferuloylquinic acid-*O*-GlucA	6.1 ± 3.8	3.9 ± 0.3	1.9 ± 0.1
4-*O*-Isoferuloylquinic acid-*O*-GlucA	19.7 ± 13.8	12.9 ± 0.9	6.2 ± 0.3
Caffeic acid	14.1 ± 17.8	4.6 ± 0.3	3.9 ± 0.7
Caffeic acid-3′-*O*-GlucA	1.6 ± 1.3	0.5 ± 0.1	< LOQ
Caffeic acid-4′-*O*-GlucA	0.6 ± 0.6	n.d.	n.d.
Caffeic acid-3′-*O*-Sulf	12.8 ± 3.6	11.7 ± 0.7	10.9 ± 0.7
Caffeic acid-4′-*O*-Sulf	2.1 ± 0.8	1.8 ± 0.1	1.5 ± 0.2
Dihydrocaffeic acid	5.1 ± 6.2	1.4 ± 0.1	1.1 ± 0.2
Dihydrocaffeic acid-3′-*O*-Sulf	3.4 ± 1.3	5.4 ± 0.2	3.0 ± 0.2
Dihydrocaffeic acid-4′-*O*-Sulf	0.2 ± 0.1	< LOQ	< LOQ
Ferulic acid	17.1 ± 21.3	4.6 ± 0.6	2.8 ± 0.8
Ferulic acid-4′-*O*-GlucA	14.6 ± 9.3	12.3 ± 0.7	9.9 ± 0.5
Ferulic acid-4′-*O*-Sulf	17.5 ± 6.9	23.1 ± 1.2	23.8 ± 1.6
Feruloylglycine	16.5 ± 13.3	19.3 ± 0.6	5.6 ± 0.5
Isoferulic acid-3′-*O*-GlucA	23.7 ± 13.1	15.9 ± 1.2	11.8 ± 0.5
Isoferulic acid-3′-*O*-Sulf	< LOQ	nd	nd
Dihydroferulic acid -4′-*O*-GlucA	2.6 ± 1.4	4.5 ± 0.7	1.1 ± 0.2
Dihydroferulic acid-4′-*O*-Sulf	0.3 ± 0.2	0.2 ± 0.1	0.1 ± 0.1
$\sum$	363.0 ± 223.3	256.7 ± 141.8	154.5 ± 72.1
Percentage of ingested amount	8.0 ± 4.9	12.1 ± 6.7	14.6 ± 6.8

nd = not detected (< LOD)

the ileal effluents as well as in the urine samples [21]. We confirm an extensive metabolism of CGA [11, 14, 21] and additionally the identification of novel metabolites in urine. Furthermore, we observed that the colon is not the only metabolically active compartment for forming DHCA and dihydroferulic acid (DHFA).

We presume a relationship between GIT-TT and CGA bioavailability. More specifically, one volunteer showed a much longer GIT-TT and showed renal excretion twice as high as the other subjects at each trial. Relationship of GIT-TT and renal excretion of each volunteer in Fig. 5 gives a hint that a decelerated passage of CGA in the gastrointestinal tract leads to a higher absorption. Other groups detected a similar total renal excretion for ileostomists after consumption of coffee or pure CGA [11, 14]. We found unmetabolized CGA in urine, as in previous studies [11, 12, 14, 20]. Especially 3-FQA and 3-CQA of these two CGA subgroups dominated in urine independently of the administered dose (Table 5, Fig. 6), whereas in plasma the four acyl compounds dominated after enzyme hydrolysis (Fig. 6). The five acyl compounds (5-FQA, 5-CQA) were detected in both compartments only in minor amounts. Interesterification reactions at physiological pH could be a reason for this. Further, the plasma elimination of CGA and metabolites via urine could be affected by the various phase II conjugation reactions. The SULT enzyme family seems to prefer the 3 and 4 acyl CQA and FQA, similar to the UGT enzyme family, which showed additionally a high affinity for 4-CQA and 4-IFQA (Table 5, Fig. 6). Both enzyme families showed an inhibition of 5-CQA or 5-FQA conjugation.

Despite unmetabolized CGA, we did not identify any *di*-CQA in plasma nor in urine. The higher molecular weight of

Table 6. Maximal plasma concentration of CGA, (after enzyme hydrolysis) and QA (data expressed as mean values ± SD; $n = 4$)

	High			Medium			Low		
Compound	c_{max} (nM)	$AUC_{(nM*0–8h-1)}$	t_{max} (h)	c_{max} (nM)	$AUC_{(nM*0–8h-1)}$	t_{max} (h)	c_{max} (nM)	$AUC_{(nM*0–8h-1)}$	t_{max} (h)
3-CQA	43 ± 10	129 ± 48	0.6 ± 0.2	33 ± 13	89 ± 27	0.8 ± 0.3	14 ± 6	38 ± 19	1.3± 0.4
4-CQA	73 ± 7	222 ± 61	0.8 ± 0.3	57 ± 24	156 ± 68	0.8 ± 0.3	20 ± 7	64 ± 24	0.9 ± 0.2
5-CQA	44 ± 7	125 ± 38	0.8 ± 0.3	30 ± 12	85 ± 32	0.9 ± 0.2	14 ± 5	43 ± 13	0.6 ± 0.2
3-FQA	96 ± 39	251 ± 85	0.9 ± 0.2	56 ± 26	131 ± 53	0.9 ± 0.2	23 ± 10	61 ± 24	1.0 ± 0.0
4-FQA	117 ± 36	317 ± 85	1.0 ± 0.0	62 ± 24	167 ± 62	0.9 ± 0.2	32 ± 10	94 ± 24	0.9± 0.2
5-FQA	45 ± 8	160 ± 51	1.1 ± 0.5	41 ± 23	119 ± 66	0.9 ± 0.2	16 ± 5	45 ± 6	0.8 ± 0.3
CA	214 ± 22	605 ± 176	0.6 ± 0.2	162 ± 52	445 ± 172	0.9 ± 0.2	77 ± 12	243 ± 71	0.5 ± 0.0
DHCA	63 ± 18	153 ± 65	0.5 ± 0.0	35 ± 11	77 ± 30	0.5 ± 0.0	53 ± 13	51 ± 70	0.8 ± 0.3
FA	518 ± 76	937 ± 157	0.5 ± 0.0	214 ± 39	453 ± 93	0.5 ± 0.0	147 ± 27	377 ± 84	0.5 ± 0.0
IFA	262 ± 91	559 ± 249	0.5 ± 0.0	110 ± 6	190 ± 43	0.5 ± 0.0	76 ± 27	344 ± 315	0.7 ± 0.2
DHFA	85 ± 12	173 ± 27	0.6 ± 0.2	34 ± 12	78 ± 50	0.9 ± 0.2	22 ± 16	64 ± 55	0.5 ± 0.0
DiMeCA	305 ± 63	824 ± 154	0.6 ± 0.2	129 ± 55	357 ± 117	0.8 ± 0.2	58 ± 21	88 ± 48	0.6 ± 0.2
DiMeDHCA	67 ± 55	208 ± 185	0.3 ± 0.2	28 ± 22	42 ± 25	0.8 ± 0.2	59 ± 15	213 ± 186	0.8 ± 0.3
	c_{max} (μM)	$AUC_{(μM*0–8h-1)}$	t_{max} (h)	c_{max} (μM)	$AUC_{(μM \times 0–8h-1)}$	t_{max} (h)	c_{max} (μM)	$AUC_{(μM \times 0–8h-1)}$	t_{max} (h)
D-(–)-QA	4.2 ± 0.4	24.4 ± 5.6	5.0 ± 1.0	2.5 ± 0.6	14.8 ± 5.0	4.5 ± 0.9	1.2 ± 0.1	8.0 ± 0.5	5.2 ± 1.0

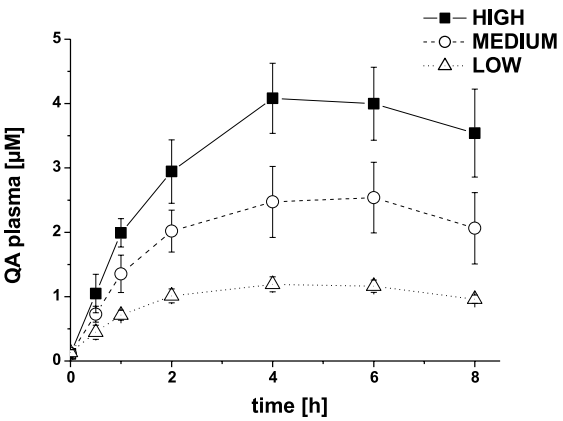


Figure 4. Plasma concentration of free QA after the consumption of a high, medium, and low dose of coffee (data expressed as mean values ± SD; $n = 4$).

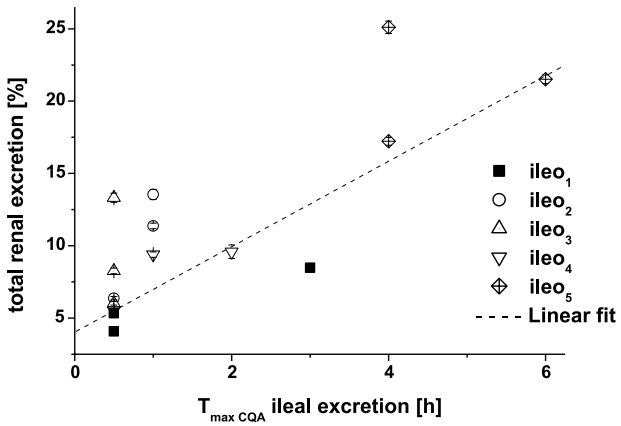


Figure 5. Relationship of total renal excretion of consumed CGA in percentage, with maximum time of ileal excretion ($T_{max\ CQA}$) for each ileostomist for all doses. Different doses are not considered. Linear fit: $R^2 = 0.64$; $y = 2.8x + 6.2$.

di-CQA (516.5 g × mol^{-1}) in comparison to the other CGAs might hinder passage across the GIT barrier. Nevertheless, a lack of *di*-CQA in ileal recovery was observed and may be due to hydrolysis via the GIT passage to CQA, CA, and QA. In contrast to the poorly absorbed *di*-CQA, CQL were highly absorbed, and especially its sulfated metabolites were detected (Table 5). In comparison to CGA, the QA moiety of CQL has an intramolecular ester bridge, which decreases the polarity. Specifically, the calculated Log $D_{(pH6.0)}$ for CQL was 0.4 which is more suitable for passive transcellular absorption than the Log $D_{(pH6.0)}$ for CQA of −2.9 (calculated with MARVIN SKETCH 5.3.1).

The proportion of CGA metabolites (conjugates after or without hydrolysis) in urine were not affected by dose, whereas the ratios of sulfation:glucuronidation in urine showed a dose dependency similar to ileal fluid. In a previous study in ileostomist volunteers [14], the urinary sulfation:glucuronidation ratio was 5.6:1.

The elimination of QA from the circulatory system (Fig. 4) was not complete ($T_{max} \approx 5$ h) even 8 h after coffee consumption. However, maximum plasma concentration of QA was linearly dose dependent.

Within the first 30 min after consumption, plasma concentration of CGA showed a strong ascent and T_{max} was reached dose independently within the first hour of consumption (Table 6, Fig. 3) for all detected plasma CGA, as confirmed by other published data [34, 35]. Hereby, methylation was shown to be a fast phase II conjugation reaction, indicated by the early T_{max} and the high AUC level of methylated hydroxycinnamic acids (Table 6, Fig. 7). Considering a possible

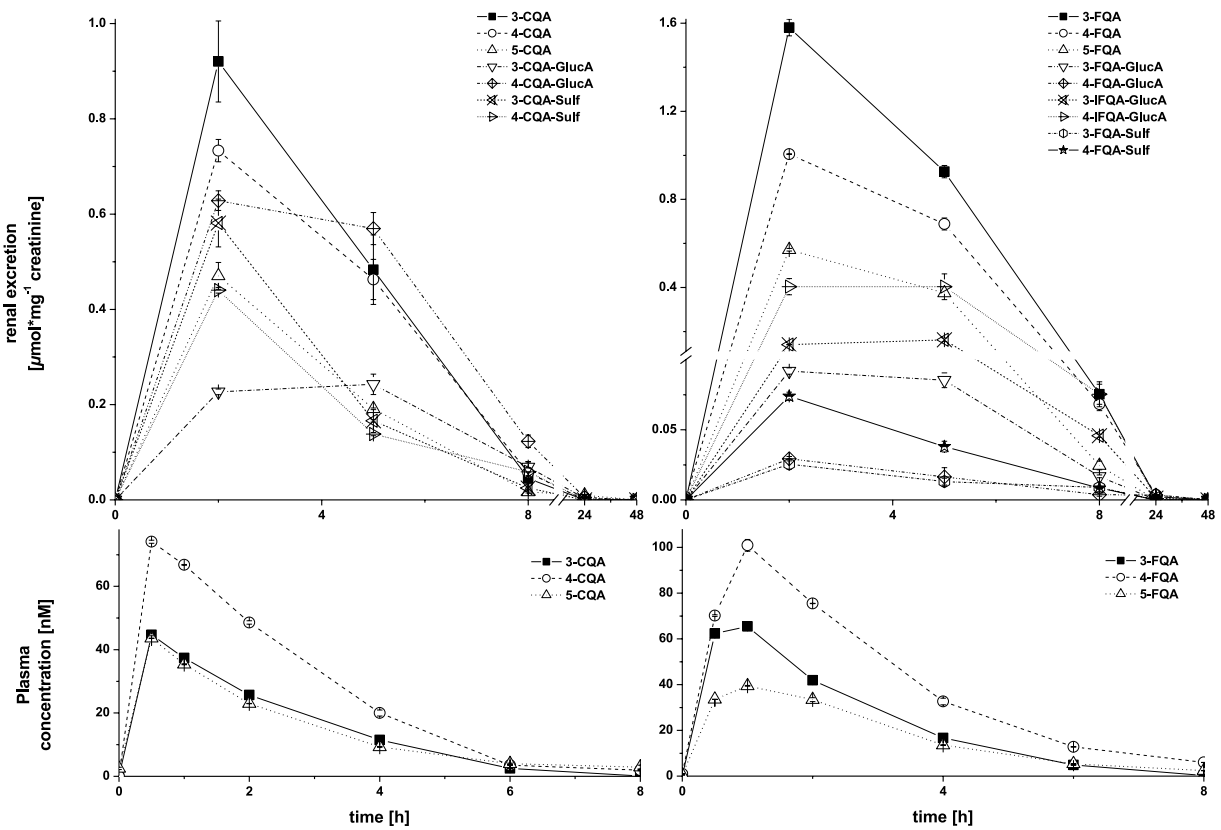


Figure 6. CQAs, FQAs and metabolites in urine (µmol × mg^{-1} creatinine) and plasma (after enzyme hydrolysis, in nM) of ileostomist number 2 after the consumption of coffee (high CGA concentration, 4525 µmol).

gastric residence time from 0.2 up to 3.8 h [33], a CGA uptake and methylation in the stomach is conceivable [36, 37]. Plasma appearance is linearly correlated to the most compounds within the different ingested doses (Fig. 7, Table 6). Deviations could be a reason of the high interindividual differences and maybe interday differences. Plasma concentrations at low coffee dose were confirmed for FA, IFA, with similar coffee dose by (34).

Di-MeDHCA and *di*–MeCA were previously observed in plasma [38, 39]. Their profiles in plasma were similar to the other coffee compounds, although we did not identify any of these molecules in urine nor in ileal fluid. Thus, we conclude that the body might be not able to eliminate these highly lipophilic molecules in their free form via renal or bilary excretion (enterohepatic circulation).

The colonic microflora is thought to be the metabolic key site for reduction of hydroxycinnamic acids to dehydro compounds (DHCA, DHFA) [11, 35]. Our findings of plasma concentrations and T_{max} ($\leq$ 0.9 h) of DHFA, DHCA, and *di*-MeDHCA indicated also a contribution of reduction in the upper parts of the GIT tract (Tables 5 and 6).

Both CQA and FQA were for the first time detected as intact compounds in significant amounts in plasma after

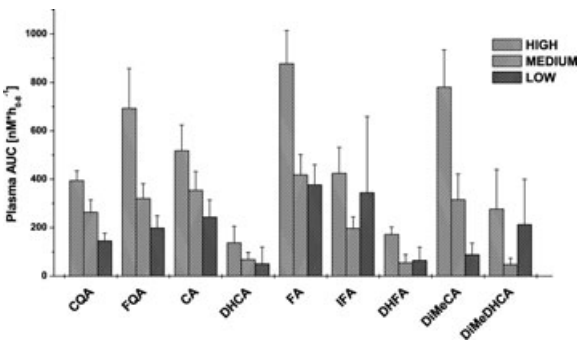


Figure 7. AUC of plasma in nM × $h_{0-8}{}^{-1}$ after the consumption of a high, medium, and low dose of coffee (data expressed as mean values ± SD; $n = 4$).

treatment with β-glucuronidase and sulfatase (Fig. 6) and urine in contrast to others [10, 33, 34]. Plasma C_{max} and AUC correlated with the different ingested dose (Table 6, Fig. 7). AUC as well as the renal excretion seemed to be affected by the different GIT-TT. The CQA and FQA plasma profiles were not similar to the ones observed in the original beverage (Fig. 6). In agreement with our data, minor amounts of

the five acyl compounds of FQA were also observed by others [11] and the dominance of the four acyl compounds in plasma could be explained by a possible facilitated transport component for 4-CQA and 4-FQA [37] or potential isomerization reactions within the CGA subgroups. The contradictory high availability of CQA and *di*-CQA reported from [19, 40] which is discussed in a recent publication [41] could not be explained by our dose response experiment.

Our results show that after coffee consumption with different doses of CGA and QA bioavailability is affected by the GIT-TT. We detected a much more extensive metabolism during the passage through the body for ileostomists than expected from the literature [40]. Also an increasing glucuronidation and an accelerated ileal excretion reduced the systemic bioavailability of nearly all coffee CGA when increasing doses of CGA from soluble coffee were ingested. Approximately 15% of the QA were available for the systemic circulation and about 70% of coffee CGA would be available in the colon independently of the consumed doses.

Due to the somewhat controversial bioavailability data of CGA in the literature after consumption of a single dose, we performed a dose-response study with different CGA doses. Therefore, ileostomists consumed increasing coffee doses in a randomized, double blinded, crossover trial and the observed effects on coffee polyphenol and free D-(–)-QA absorption, colonic availability, and metabolism were documented. Our results show that different doses cannot explain the controversial bioavailability data for intact CGA.

The authors thank all volunteers in the study for participation, K. Ultes, M. Essers, D. Schreiber for preparation of standards. K. H. Merz for NMR interpretation. Lionel Philippe for supervising the study and Ines Holub for her help in performing the intervention studies. The study was funded by the Nestlé Research Centre.

The authors have declared no conflict of interest.

5 References

[1] IUPAC, Nomenclature of cyclitols. *Biochem. J.* 1976, *153*, 23–31.

[2] Manach, C., Scalbert, A., Morand, C., Remesy, C. et al., Polyphenols: food sources and bioavailability. *Am. J. Clin. Nutr.* 2004, *79*, 727–747.

[3] Fukushima, Y., Ohie, T., Yonekawa, Y., Yonemoto, K. et al., Coffee and green tea as a large source of antioxidant polyphenols in the Japanese population. *J. Agric. Food. Chem.* 2009, *57*, 1253–1259.

[4] Bonita, J. S., Mandarano, M., Shuta, D., Vinson, J., Coffee and cardiovascular disease: in vitro, cellular, animal, and human studies. *Pharmacol. Res.* 2007, *55*, 187–198.

[5] Graziani, G., D'Argenio, G., Tuccillo, C., Loguercio, C. et al., Apple polyphenol extracts prevent damage to human gastric epithelial cells in vitro and to rat gastric mucosa in vivo. *Gut* 2005, *54*, 193–200.

[6] Kempf, K., Herder, C., Erlund, I., Kolb, H. et al., Effects of coffee consumption on subclinical inflammation and other risk factors for type 2 diabetes: a clinical trial. *Am. J. Clin. Nutr.* 2010, *91*, 950–957.

[7] Natella, F., Nardini, M., Giannetti, I., Dattilo, C. et al., Coffee drinking influences plasma antioxidant capacity in humans. *J. Agric. Food. Chem.* 2002, *50*, 6211–6216.

[8] Pavlica, S., Gebhardt, R., Protective effects of ellagic and chlorogenic acids against oxidative stress in PC12 cells. *Free Radic. Res.* 2005, *39*, 1377–1390.

[9] Stalmach, A., Mullen, W., Nagai, C., Crozier, A., On-line HPLC analysis of the antioxidant activity of phenolic compounds in brewed, paper-filtered coffee. *Braz. J. Plant Physiol.* 2006, *18*, 253–262.

[10] Clifford, M. N., Chlorogenic acids and other cinnamates–nature, occurrence and dietary burden. *J. Sci. Food Agric.* 1999, *79*, 362–372.

[11] Stalmach, A., Mullen, W., Barron, D., Uchida, K. et al., Metabolite profiling of hydroxycinnamate derivatives in plasma and urine after the ingestion of coffee by humans: identification of biomarkers of coffee consumption. *Drug Metab. Dispos.* 2009, *37*, 1749–1758.

[12] Olthof, M. R., Hollman, P. C., Katan, M. B., Chlorogenic acid and caffeic acid are absorbed in humans. *J. Nutr.* 2001, *131*, 66–71.

[13] Kahle, K., Huemmer, W., Kempf, M., Scheppach, W. et al., Polyphenols are intensively metabolized in the human gastrointestinal tract after apple juice consumption. *J. Agric. Food Chem.* 2007, *55*, 10605–10614.

[14] Stalmach, A., Steiling, H., Williamson, G., Crozier, A., Bioavailability of chlorogenic acids following acute ingestion of coffee by humans with an ileostomy. *Arch. Biochem. Biophys.* 2010, *501*, 98–105.

[15] Hagl, S., Deusser, H., Soyalan, B., Janzowski, C. et al., Colonic availability of polyphenols and D-(−)-quinic acid after apple smoothie consumption. *Mol. Nutr. Food Res.* 2011, *55*, 368–377.

[16] Kahle, K., Kraus, M., Richling, E., Polyphenol profiles of apple juices. *Mol. Nutr. Food Res.* 2005, *49*, 797–806.

[17] Crozier, A., Del Rio, D., Clifford, M. N., Bioavailability of dietary flavonoids and phenolic compounds. *Mol. Aspects Med.* 2010, *31*, 446–467.

[18] Zhao, Z., Moghadasian, M. H., Bioavailability of hydroxycinnamates: a brief review of in vivo and in vitro studies. *Phytochem. Rev.* 2009, *9*, 133–145.

[19] Monteiro, M., Farah, A., Perrone, D., Trugo, L. C. et al., Chlorogenic acid compounds from coffee are differentially absorbed and metabolized in humans. *J. Nutr.* 2007, *137*, 2196–2201.

[20] Kahle, K., Kempf, M., Schreier, P., Scheppach, W. et al., Intestinal transit and systemic metabolism of apple polyphenols. *Eur. J. Nutr.* 2011, *50*, 507–522.

[21] Wong, C. C., Meinl, W., Glatt, H.-R., Barron, D. et al., In vitro and in vivo conjugation of dietary hydroxycinnamic acids by UDP-glucuronosyltransferases and sulfotransferases in humans. *J. Nutr. Biochem.* 2010, *21*, 1060–1068.

[22] Giacomelli, C., Miranda, F. d. S., Gonçalves, N. S., Spinelli, A., Antioxidant activity of phenolic and related compounds: a density functional theory study on the O–H bond dissociation enthalpy. *Redox. Rep.* 2004, *9*, 263–269.

[23] Kahle, K., Kraus, M., Scheppach, W., Richling, E., Colonic availability of apple polyphenols – A study in ileostomy subjects. *Mol. Nutr. Food Res.* 2005, *49*, 1143–1150.

[24] Sefkow, M., First efficient synthesis of chlorogenic acid. *Eur. J. Org. Chem.* 2001, *2001*, 1137–1141.

[25] Sefkow, M., Kelling, A., Schilde, U., First efficient syntheses of 1-, 4-, and 5-Caffeoylquinic Acid. *Eur. J. Org. Chem.* 2001, *2001*, 2735–2742.

[26] Trugo, L. C., Macrae, R., Chlorogenic acid composition of instant coffees. *Analyst* 1984, *109*, 263–266.

[27] Erk, T., Bergmann, H., Richling, E., A novel method for the quantification of quinic acid in food using stable isotope dilution analysis. *J. AOAC Int.* 2009, *92*, 730–733.

[28] Knud, L., Creatinine assay by a reaction-kinetic principle. *Clin. Chim. Acta* 1972, *41*, 209–217.

[29] Rúveda, E. A., Deulofeu, V., Galmarini, O. L., The lactone of neochlorogenic acid. *Chem. Ind.* 1964, *8*, 239–240.

[30] Bennat, C., Engelhardt, U. H., Kiehne, A., Wirries, F. M. et al., HPLC analysis of chlorogenic acid lactones in roasted coffee. *Z. Lebensm. Unters. Forsch. A.* 1994, *199*, 17–21.

[31] Clifford, M. N., Johnston, K. L., Knight, S., Kuhnert, N., Hierarchical scheme for LC-MSn identification of chlorogenic acids. *J. Agric. Food Chem.* 2003, *51*, 2900–2911.

[32] Menozzi-Smarrito, C., Wong, C. C., Meinl, W., Glatt, H. et al., First chemical synthesis and in vitro characterization of the potential human metabolites 5-O-feruloylquinic acid 4′-sulfate and 4′-O-glucuronide. *J. Agric. Food. Chem.* 2011, *59*, 5671–5676.

[33] Fallingborg, J., Christensen, L. A., Ingeman-Nielsen, M., Jacobsen, B. A. et al., Gastrointestinal pH and transit times in healthy subjects with ileostomy. *Aliment Pharmacol. Ther.* 1990, *4*, 247–253.

[34] Nardini, M., Cirillo, E., Natella, F., Scaccini, C., Absorption of phenolic acids in humans after coffee consumption. *J. Agr. Food Chem.* 2002, *50*, 5735–5741.

[35] Renouf, M., Guy, P. A., Marmet, C., Fraering, A.-L. et al., Measurement of caffeic and ferulic acid equivalents in plasma after coffee consumption: small intestine and colon are key sites for coffee metabolism. *Mol. Nutr. Food Res.* 2010, *54*, 760–766.

[36] Lafay, S., Gil-Izquierdo, A., Manach, C., Morand, C. et al., Chlorogenic acid is absorbed in its intact form in the stomach of rats. *J. Nutr.* 2006, *136*, 1192–1197.

[37] Farrell, T. L., Dew, T. P., Poquet, L., Hanson, P. et al., Absorption and metabolism of chlorogenic acids in cultured gastric epithelial monolayers. *Drug Metab. Dispos.* 2011, *39*, 2338–2346.

[38] Nagy, K., Redeuil, K., Williamson, G., Rezzi, S. et al., First identification of dimethoxycinnamic acids in human plasma after coffee intake by liquid chromatography–mass spectrometry. *J. Chromatogr. A.* 2011, *1218*, 491–497.

[39] Redeuil, K., Smarrito-Menozzi, C., Guy, P., Rezzi, S. et al., Identification of novel circulating coffee metabolites in human plasma by liquid chromatography-mass spectrometry. *J. Chromatogr. A.* 2011, *1218*, 4678–4688.

[40] Farah, A., Monteiro, M., Donangelo, C. M., Lafay, S., Chlorogenic acids from green coffee extract are highly bioavailable in humans. *J. Nutr.* 2008, *138*, 2309–2315.

[41] Williamson, G., Dionisi, F., Renouf, M., Flavanols from green tea and phenolic acids from coffee: critical quantitative evaluation of the pharmacokinetic data in humans after consumption of single doses of beverages. *Mol. Nutr. Food Res.* 2011, *55*, 864–873.

