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Free phenolic acids in human urine after drinking coffee rich in chlorogenic acids

(Asid fenolik bebas dalam air kencing manusia selepas meminum kopi kaya dengan asid klorogenik)

R. Suri* and A. Crozier**

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Abstract

The pharmacokinetics study of free urinary excretion of phenolic acids in human volunteers (with and without a colon) who drank instant coffee was successfully conducted. The objective of the study was to identify the possible degradation products of coffee hydroxycinnamates *in vivo*. All volunteers displayed a substantial increase in excretion of free 3-(3-hydroxyphenyl)-3-hydroxypropionic acid, dihydroferulic acid, ferulic acid and 3-hydroxyhippuric acid within 8 – 24 h after drinking coffee. In total, mean concentration of urinary free phenolic acids excreted was 134 ± 43 µmole, which was equivalent to 29.4% of chlorogenic acid intake and significantly higher (p <0.05) than phenolic acids excreted when only water was consumed. The presence of free phenolic acids in urine after drinking coffee indicated that coffee maybe able to provide health benefits to humans. This is because free phenolic acids have higher antioxidant activities in comparison to conjugated phenolic acids.

Introduction

Coffee is the prime contributor of dietary antioxidant intake followed by red wine, fruit juice and tea (Nardini et al. 2006). Chlorogenic acids, which are mainly found in coffee, are also found in a wide range of fruits and vegetables and are formed by esterification of hydroxycinnamic acids, such as caffeic, ferulic and *p*-coumaric to quinic acid (Clifford 1999). These compounds comprise a number of subgroups such as caffeoylquinic acids, feruloylquinic acid and *p*-coumaroylquinic acid, with at least three isomers per group (Clifford et al. 2003). Among them, 5-*O*-caffeoylquinic acid (5-CQA) is the major component in coffee (*Figure 1*) (Stalmach et al. 2006). Like other polyphenols, chlorogenic acids, especially caffeoylquinic acid and feruloylquinic acid, possess strong antioxidant activity *in vitro* (Stalmach et al. 2006) and *in vivo* by increasing the resistance of LDL to lipid peroxidation (Nardini et al. 1995; Abu-Amsha Caccetta et al. 1996).

The biological effects of drinking coffee rely on metabolism and catabolism of chlorogenic acids, but little is known on the catabolism of free phenolic acids in human urine after drinking coffee and this topic has been poorly studied. Stalmach et al. (2009) reported 21 metabolites which corresponded to 29.1% intake of chlorogenic acids following the ingestion of 200 ml of instant coffee. Most of the metabolites were

*Food Technology Research Centre, MARDI Headquarters, Serdang, P.O. Box 12301, 50774 Kuala Lumpur, Malaysia
**Wolfson Medical School Building, University Avenue, Glasgow, G12 8QQ, Scotland
Authors' full names: Suri Roowi and Alan Crozier
E-mail: suri@mardi.gov.my

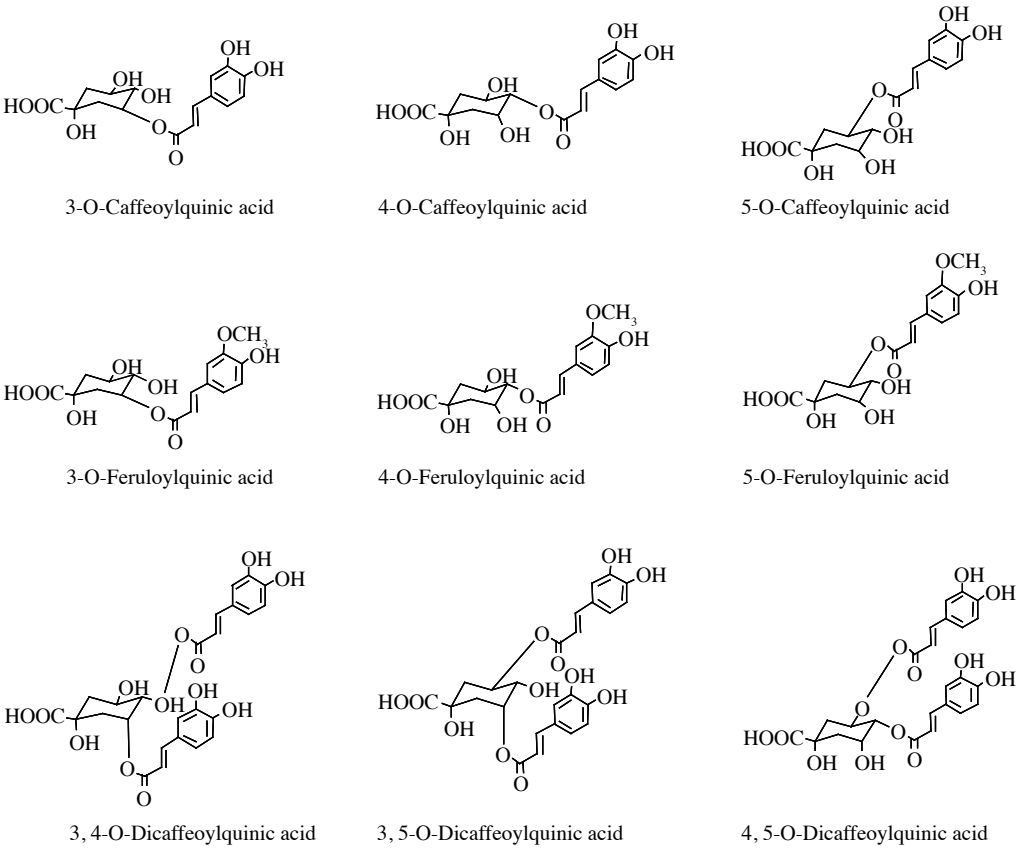

Figure 1. Structure of phenolic acids in coffee (Stalmach et al. 2006)

found either in the form of glucuronide or sulfate. Glucuronidation and sulfatation are two ways of detoxification of phenolic compounds to increase their solubility and promote their excretion in urine and bile. However, these processes may modify the biological activity of the parent molecules resulting in a partial loss of antioxidant activity (Manach et al. 1998)

Other *in vivo* studies have shown that chlorogenic acid is hydrolysed to caffeic acid and quinic acid and further metabolised to ferulic acid, isoferulic acid, 3-hydroxyhippuric acid, 4-hydroxy-3-methoxyphenylpropionic acid, dihydroferulic acid, 3-hydroxybenzoic acid and hippuric acid (Rechner et al. 2001; Gonthier et al. 2003; Olthof et al. 2003; Hodgson et al. 2004; Mennen et al. 2006). However, in many studies involving coffee and 5-CQA, sample preparation involves the use of β-glucuronidase from *Helix pomatia* to hydrolyse conjugated phenolic acids in urine. This enzyme also contains esterase that is able to convert 5-CQA to caffeic acid (Manach et al. 2005) that will make the data less accurate.

At present, only two possible catabolised products are reported as free forms, namely, dihydrocaffeic acid and dihydroferulic acid (Stalmach et al. 2009). However, the identification was made using high performance liquid chromatography mass spectrometry (HPLC-MS-MS), which may be less sensitive to detect a whole range of free phenolic acids in human urine after drinking coffee. According to Mullen et al. (2006), phenolic acids have a low extinction

coefficient and a λ_{max} below 250 nm, so they are not readily detected using photo diode array detector (PDA) and as a further complication, they are not readily ionized when an electrospray interface is used. Therefore, the present study was conducted to identify the possible degradation products of coffee hydroxycinnamates *in vivo*.

Materials and Methods

Materials

Standards of hippuric acid, 4-hydroxybenzoic acid, 3-methoxy-4-hydroxyphenylacetic acid, 2,4,5-trimethoxycinnamic acid, hydrocaffeic acid and 4-hydroxyphenylacetic acid were obtained from Sigma-Aldrich (Poole, Dorset, UK). Dihydroferulic acid was purchased from Alfa Aesar (Heysham, Lancs., UK). Ferulic acid was obtained from AASC Chemicals, Southampton, UK). Derivatisation reagent (*NO*-BSTFA + 1% TMCS (trimethylchlorosilane) was purchased from Sigma-Aldrich (Poole, Dorset, UK). Methanol and ethyl acetate were obtained from Rathburn Chemicals (Walkerburn, Scotland, UK). All other chemicals and reagents were obtained from Sigma-Aldrich (Poole, Dorset, UK) unless otherwise stated.

Study design

Eleven volunteers, six healthy and five with an ileostomy (no colon), took part in the study. The study protocol was approved by the University of Glasgow Royal Infirmary Ethics Committee. Subjects were non-smokers, not pregnant and were not under any medication. Ileal volunteers had an ileostomy for 16.6 ± 9.8 years, due to ulcerative colitis (n = 4) and Crohn's disease (n = 1). The participants were otherwise healthy. Subjects were required to follow a diet low in flavonoids for 2 days prior to the study, avoiding fruits, vegetables, high fibre products and beverages such as tea, coffee, fruit juice and wine. Two feeds (control and coffee study) were conducted for the different healthy volunteers. Volunteers were on a low flavonoid diet for 2 days prior to the study before they consumed 200 ml of brewed coffee or 200 ml of water. Blank study (volunteers consumed 200 ml water) was done one week before the coffee study. Brewed coffee was made from 3.4 g of instant Nescafe Gold Blend coffee dissolved in 200 ml of boiled hot water. Urine, collected prior to supplementation and at 0 – 2, 2 – 5, 5 – 8 and 8 – 24 h periods thereafter, was stored at –80 °C prior to analysis.

Analysis of free phenolic acids in human urine

Urine was prepared according to Olthof et al. (2003) and Borges et. al. (2007). After thawing, 1 ml of urine sample was added to 4 ml of 0.2 M HCl containing 100 µg of 2,4,5-trimethoxycinnamic acid as an internal standard. A styrene divinyl benzene solid phase extraction cartridge (Phenomenex, Macclesfield, UK) was used for sample purification. Before loading with the acidified samples, the cartridge was pre-conditioned with 5 ml of ethyl acetate, followed by 5 ml of methanol and finally 5 ml of 0.1 M HCl. After the urine was added, the cartridge was washed with 5 ml of 0.1 M HCl and eluted in 3 ml of ethyl acetate. The upper ethyl acetate phase was separated from the lower aqueous phase and dried using an activated molecular sieve prior to being reduced to dryness *in vacuo*. The extracts were then derivatised using NO-bistrimethylsilyl acetamide (NO-BSTFA) in preparation for analysis by GC-MS.

GC-MS conditions

The GC-MS conditions were described as in Borges et al. (2007). Phenolic acids in silylated extracts of urine were analysed by GC-MS (Trace DSQ, Thermo Finnigan) using a ZB-5MS 30 m x 0.25 i.d. x 0.25 µm capillary column (Phenomenex, Cheshire, UK) with helium as the carrier gas (1.0 ml/litre). The GC-MS conditions were as follows: injection volume is one µl,

initial temperature at 80 °C for 5 min then increased to 160 °C at 10 °C/min for 10 min followed by an increase to 235 °C at 5 °C/min for 10 min, injector temperature at 280 °C, MS transfer line at 290 °C, ion source at 200 °C and a split ratio of 1:100. Mass spectra were scanned at 50 – 650 m/z at 0.82 scans/s. Electron impact energy was 70 eV. Phenolic compounds were identified according to their retention time, mass spectra of authentic standards and the National Institute of Standards and Technology (NIST 98) library screening. Quantifications were based on a standard curve of 2,4,5-trimethoxycinnamic acid (internal standard) with typical recoveries of 80%. All standards and samples were analysed in triplicate.

Statistical analysis

Each sample was analysed in triplicate and data were presented as mean values ± standard error (n = 3). Where appropriate, data were subjected to statistical analysis using paired and unpaired t-test with Minitab software, version 13 (Minitab Inc., Addison-Wesley Publishing, Reading, MA, U.S.A).

Results and discussion

Free phenolic acids in urine of healthy and ileostomy volunteers

Eight urinary phenolic acids were detected in healthy volunteers (*Table 1*). Six phenolic acids were detected using standards, whereas two compounds were detected using NIST library and had similarity indexes of more than 95%. GC-MS analysis showed that five phenolic acids, namely, hippuric acid, 3-(3-hydroxyphenyl)-3-hydroxypropionic acid, dihydroferulic acid, ferulic acid and 3-hydroxyhippuric acid, increased in the urine of healthy volunteers after coffee ingestion, most probably as the result of colonic degradation of chlorogenic acids (*Figure 2* and *Tables 2, 3*).

In contrast to the volunteers with colons, only 4-hydroxyphenylacetic acid and hippuric acid were detected in ileostomy volunteers (*Table 3*). No 3-(3-hydroxyphenyl)-3-hydroxypropionic acid, dihydroferulic acid, ferulic acid and 3-hydroxyhippuric acid were detected in all ileostomy volunteers. Since no phenolic acids are likely to be produced from breakdown of CQA in urine from ileostomy volunteers, the CQA hydrolysis is unlikely to have taken place in the stomach and small intestine (Olthof et al. 2003). Takenaka et al. (2000) also reported that CQA was stable in the stomach at pH 2.

Jacobson et al. (1983) reported that caffeoylquinic acids are not cleaved in the stomach under acidic conditions in the gastric lumen. Human colon contains ~10^{12} microorganisms that are able to produce various enzymes involved in hydrolysis, dehydroxylation, demethylation, ring cleavage, decarboxylation and deconjugation of various polyphenols in humans (Scheline 1999). These microorganisms cannot be found in the small intestine epithelium, liver or plasma (Plumb et al. 1999). Various colonic microflora such as *Pseudomonas fluorescens* (Andreason et al. 2001), *Escherichia coli*, *Bifidobacterium lactis* and *Lactobacillus gasseri* (Donaghy et al. 1998; Williamson et al. 1998; Couteau et al. 2001) are able to produce esterases in the human colon.

In this study, 3-(3-hydroxyphenyl)-3-hydroxypropionic acid is the novel free phenolic acid biomarker for chlorogenic acid catabolism in humans (*Table 2*). This compound is reported here for the first time in human urine after drinking coffee. The only phenylpropionic acids reported in human urine were 3-(3,4-dihydroxyphenyl) propionic acid (dihydrocaffeic acid) (Olthof et al. 2003) and 3-(3-hydroxyphenyl) propionic acid (Booth et al. 1957). Although commercial standards for these compounds were available, they were not detected in this study.

Dihydroferulic acid, thought to be a biomarker for chlorogenic acid catabolism in humans, was also found in this study. This compound is most probably derived

Table 1. GC-MS identification of catabolites detected in human urine with and without colons before and after drinking coffee*

Peak	Catabolites	^{t}R (min)	Base ion (m/z)	Qualifier ions (m/z)	Identification
1	4-Hydroxybenzoic acid	16.8	267	223; 193	Standard, NIST, Olthof et al. (2003)
2	4-Hydroxyphenylacetic acid	17.2	296	281; 252	Standard, NIST, Olthof et al. (2003)
3	3-Methoxy-4-hydroxyphenylacetic acid	22.9	326	209; 179	Standard, Olthof et al. (2003); Jenner et al. (2005)
4	Hippuric acid	25.3	105	206; 236	Standard, NIST, Olthof et al. (2003)
5	3-(3-Hydroxyphenyl)-3-hydroxypropionic acid	25.9	267	207; 147	NIST
6	Dihydroferulic acid	27.3	340	209; 192	Standard, NIST
7	Ferulic acid	32.8	338	249; 323	Standard, NIST
8	3-Hydroxyhippuric acid	34.0	294	193; 73	NIST

*GC-MS traces in *Figure 2*; t_R-retention time

from ferulic acid by the colonic microflora, which reduces the aliphatic carbon-carbon double bond (Rechner et al. 2001). In the present study, dihydroferulic acid showed a weak relationship to the chlorogenic acid catabolism in humans as it was found in relatively small amounts in the urine of two out of the six healthy volunteers.

According to Andreoni et al. (1995), dihydroferulic acid is formed from ferulic acid catabolism by the action of *Pseudomonas fluorescens* in the large intestine. Ferulic acid, which is also a biomarker for dietary caffeic acid derivatives (Booth et al. 1957), was present in 4 out of 6 volunteers, but in trace quantities. This compound was mainly conjugated with glucuronide and or sulphate (Stalmach et al. 2009) and it was also detected in other studies where samples were treated with β-glucuronidase (Rechner et al. 2001; Hodgson et al. 2004).

In this study, dihydroferulic acid was present in urine in higher amounts than ferulic acid (*Table 2*). Rechner et al. (2001) obtained similar data and speculated that this was due to dihydroferulic acid being excreted in the free form or it may also be due to moderate uptake of ferulic acid in the human liver cell (Mateos et al. 2006). The formation of dihydroferulic acid and ferulic acid in healthy volunteers is probably due to methylation of chlorogenic acids (Mateos et al. 2006). Isoferulic acid, reported to be a specific biomarker for chlorogenic acid catabolism (Rechner et al. 2001; Hodgson et al. 2004), was not found in this study. However, Stalmach et al. (2009) reported that this compound occurs as glucuronide and sulphate conjugates.

Although hippuric acid was present in urine in substantial amounts after drinking coffee, the amount of total hippuric acid excreted was well in excess of the 433 μmoles of CQAs present in the coffee. A portion of the hippuric acid maybe produced from benzoic, quinic and amino acids. In this study, the hippuric acid increased in healthy volunteers who drank coffee probably due in part to degradation of chlorogenic acid yielding quinic acid and caffeic acid with the latter being converted to hippuric acid after conjugation with glycine (Gonthier et al. 2003).

Similarly, Olthof et al. (2003) reported that 5.6 mmol/day phenolic acids were

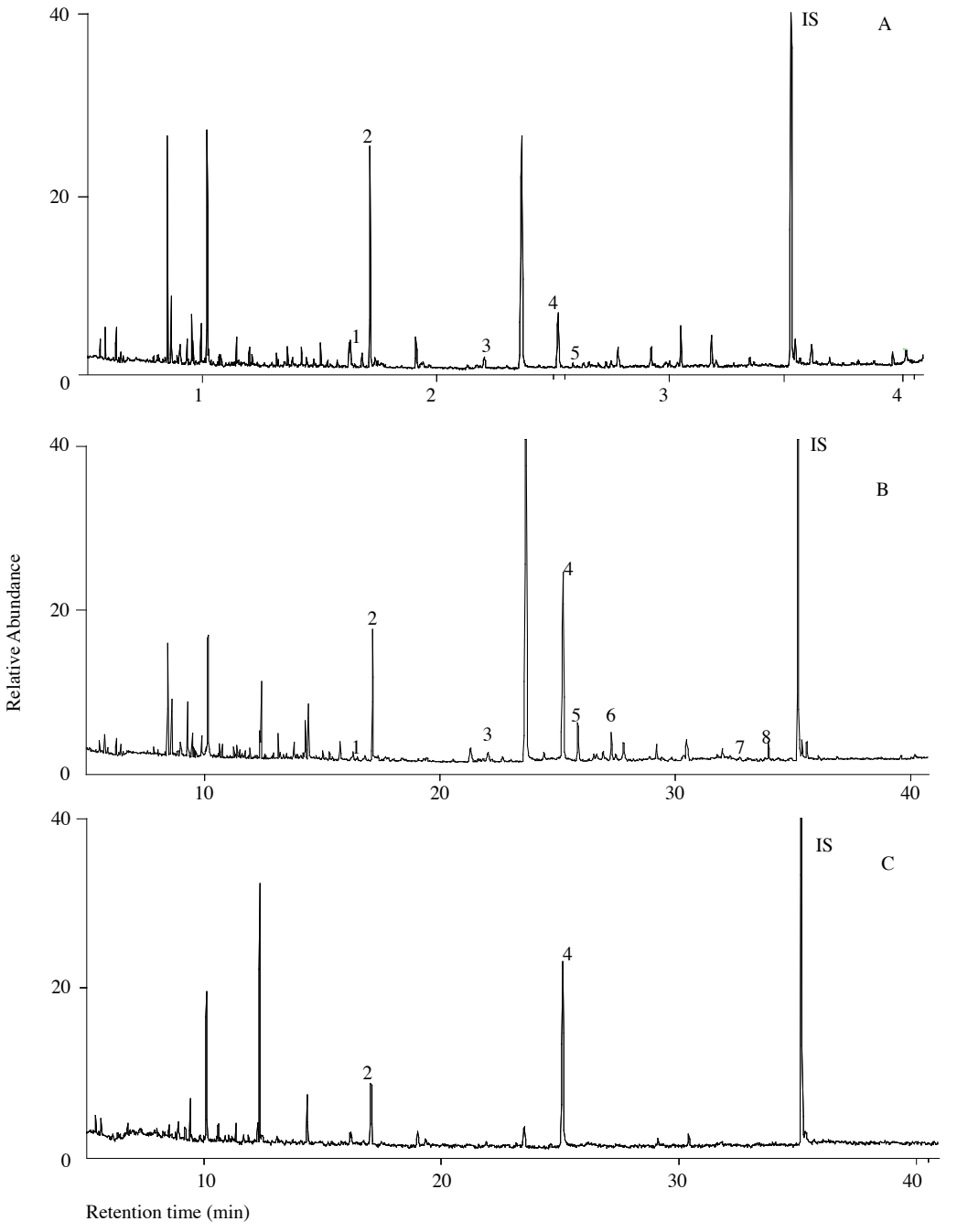

Figure 2. GC-MS traces of phenolic acids in urine of healthy and ileostomy volunteers 8 – 24 h after drinking coffee or water. (A) Healthy volunteer after drinking plain water, (B) Healthy volunteer after drinking coffee and (C) ileostomy volunteer after drinking coffee. For identification of peaks, refer to Table 1. The coffee contained 433 µmoles chlorogenic acid

Table 2. Mean concentration of phenolic acids in the urine of five healthy human subjects after drinking coffee containing 433 µmoles chlorogenic acids or plain water[a]

Peak	^{t}R(min)	Coffee	Phenolic compounds	0 – 2 h	2 – 5 h	5 – 8 h	8 – 24 h	Total
1	16.8	W	4-hydroxybenzoic acid	2.9 ± 1.6	3.2 ± 0.9	3.8 ± 1.9	3.5 ± 1.7	13.5 ± 1.9
		CF		0.2 ± 0.2	0.4 ± 0.3	2.3 ± 0.8	5.6 ± 2.3	8.4 ± 3.5
2	17.2	W	4-hydroxyphenylacetic acid	79.5 ± 47.5	69.9 ± 25.8	57.1 ± 23.4	64.4 ± 13.1	271 ± 106
		CF		6.5 ± 1.9	7.3 ± 1.6	35.7 ± 14.7	91.1 ± 27.3	141 ± 42.2
3	22.1	W	3-methoxy-4-	4.3 ± 1.9	4.6 ± 2.0	3.6 ± 1.6	5.6 ± 1.8	18.1 ± 7.0
		CF	hydroxyphenylacetic acid	0.3 ± 0.3	0.3 ± 0.2	1.3 ± 0.9	7.3 ± 4.1	9.2 ± 5.6
4	25.3	W	Hippuric acid	26.5 ± 9.6	29.4 ± 10.5	25.6 ± 8.6	56 ± 20.7	137 ± 42.6
		CF		12.7 ± 3.1	22.3 ± 6.3	114 ± 47.8	400 ± 142	549 ± 170
5	25.9	W	3-(3-hydroxyphenyl)-3-	1.8 ± 0.7	1.1 ± 0.5	1.2 ± 0.5	2.7 ± 0.8	6.7 ± 1.8
		CF	hydroxypropionic acid	0.4 ± 0.4	2.8 ± 1.0	24.6 ± 9.3	58.7 ± 16.0	86.5 ± 24.0**
6	27.2	W	Dihydroferulic acid	0	0	0	0	0
		CF		0.1 ± 0.1	0.1 ± 0.1	10.1 ± 8.1	11.1 ± 8.8	21.3 ± 14.8*
7	32.8	W	Ferulic acid	0	0	0	0	0
		CF		0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1*
8	34.0	W	3-hydroxyhippuric acid	0	0	0	0	0
		CF		0.5 ± 0.3	0.3 ± 0.2	5.3 ± 3.0	20.1 ± 7.4	26.1 ± 10.6*

[a]Mean values expressed as µmoles ± standard error [n = 6 (CF) and n = 5 (W)]
For GC-MS data and identification of peaks, refer to *Table 1* and *Figure 2*
^{t}R = Retention time; CF = Healthy volunteer after drinking coffee; W = Healthy volunteer after drinking water
Statistical significant for peaks 5 – 8 from w/o were based on paired t-test
*Significantly different from w/o, p <0.001. **Significantly different from W, p <0.05

Table 3. Quantity of phenolic acids in urine of five ileostomy volunteers 0 – 24 h after drinking coffee containing 401 μmoles chlorogenic acids*

Peak	Phenolic compounds	0 – 2 h	2 – 5 h	5 – 8 h	8 – 24 h	Total
2	4-hydroxyphenylacetic acid	3.0 ± 2.0	2.2 ± 1.2	6.8 ± 2.9	9.6 ± 3.3	21.6 ± 7.9
4	Hippuric acid	12.7 ± 10.5	8.6 ± 6.4	25.8 ± 9.2	15.6 ± 8.0	62.8 ± 22.9

*Data for individual subjects expressed as μmoles ± standard error (n = 3). Mean values expressed as μmoles ± standard error (n = 5)
For GC-MS data and identification of peaks, refer to *Table 1* and *Figure 2*
n.d. = Not detected

excreted by healthy humans after drinking 5.5 mmol/day chlorogenic acid – a more than 100% excretion. Rechner et al. (2001) also reported that volunteers showed an increase in hippuric acid excretion after coffee ingestion of between 11% and 228% relative to control.

Thus, it is very difficult to equate chlorogenic acid catabolism to the amount of hippuric acid excreted in urine, especially as both compounds can be produced by alternative routes. These compounds are probably nonspecific metabolites of chlorogenic acids. The only appropriate marker for CQA catabolism would appear to be 3-hydroxyhippuric acid (*Table 2*). This compound has an hydroxyl group at the 3-position that maybe derived from caffeic acids. Booth et al. (1957) also suggested that the 3-hydroxyl group of hydroxyhippuric acid could be derived from caffeic acid.

Calculation based on selected free phenolic acids contributed by peaks 5 – 8 showed mean concentration of phenolic acids in urine of healthy volunteers 0 – 24 h after drinking coffee reached statistical significance at $p < 0.05$. This value represents excretion equivalent to 29.4% of total phenolics in coffee (*Table 4*). Excretion of 4-hydroxybenzoic acid, 4-hydroxyphenylacetic acid and 3-methoxy-4- hydroxyphenylacetic acid were lower in subjects who drank coffee than in volunteers who drank only water (*Table 2*) indicating that these phenolic acids were not derived from the breakdown of CQAs. Although hippuric acid was the main urinary phenolic acid, it is unlikely to be derived exclusively from CQAs

Table 4. Excretion of phenolic acids 0 – 24 h after ingestion of coffee or water in humans

	Type of sample	Healthy	Ileostomy
I	Coffee	433 ± 11	401 ± 4
II	Urine (after coffee)[a]	134 ± 43	n.d.
III	Urine (blank) [a]	6.7 ± 2	n.t.

[a]Quantification based on peaks 5 – 8 in *Table 2*.
Data expressed as μmoles
n.d. = Not detected; n.t. = not tested
Data expressed as mean values ± SE
(n = 6 for I and n = 5 for III)
Statistical significance between II and III for healthy volunteers based on unpaired t-test where $p < 0.05$

catabolism and it is known to be produced independently from benzoic acid, quinic acid, tryptophan, tyrosine and phenylalanine (Roowi 2008).

The relatively low amounts of total phenolic acids excreted in urine of ileostomy volunteers as compared to healthy volunteers (*Table 4*) may arguably be a reflection of the low amount of chlorogenic acid reaching the colon after absorption in the upper part of the gasterointestinal tract (Azuma et al. 2000; Nardini et al. 2002; Stalmach et al. 2009). However, there is only one report of unexpectedly large amounts of CQAs being detected in plasma after coffee consumption (Monteiro et al. 2007).

In the present study, chlorogenic acid was not detected in urine of healthy volunteers after ingestion of coffee, although Ito et al. (2005), Mennen et al. (2006) and Stalmach et al. (2009) reported the presence of 5-CQA in human urine who drank coffee. It is more likely that GC-MS

is less sensitive than HPLC-MS-MS to detect chlorogenic acids in urine. It is also possible that chlorogenic acid is metabolised in the large intestine due to its low recovery in human and rat urine (Olthof et al. 2001; Gonthier et al. 2003).

Several studies in humans and rats have also been unable to detect chlorogenic acids in plasma or urine after feeding either with pure chlorogenic acid or chlorogenic acid containing foods (Booth et al. 1957; Bourne and Rice-Evans 1998; Choudhury et al. 1999; Azuma et al. 2000; Rechner et al. 2001; Nardini et al. 2002).

Caffeic acid, which is known as a useful urinary biomarker of chlorogenic acid catabolism (Lafay et al. 2006; Mennen et al. 2006), was also not detected in the present study. Caffeic acid may be conjugated with glucuronide and/or sulphate as indicated by Lafay et al. (2006) when sample was treated with β-glucuronidase/sulfatase. In keeping with this possibility, Nardini et al. (2006) also reported that caffeic acid is found mainly conjugated with glucuronides and/or sulphates.

Although coffee is a major source of dietary CQAs, which are strong antioxidants, coffee is frequently consumed with milk. Dupas et al. (2006) reported that when 25% milk is added to coffee, more than 40% of coffee chlorogenic acids bind to proteins. The *in vitro* antioxidant activity of coffee also decreased when milk was added to Espresso coffee (Sánchez-Gonzáles et al. 2005). The interaction of 5-CQA with dietary protein has been investigated by Prigent et al. (2003) and Papadopoulou and Frazier (2004). However, to what degree this affects the absorption and catabolism of CQA's when coffee is consumed with milk remains to be determined.

Proposed scheme for colonic degradation

The possible fate of chlorogenic acids conversion into free phenolic acids in the colon is illustrated in *Figure 3*. Initially, chlorogenic acid probably undergoes

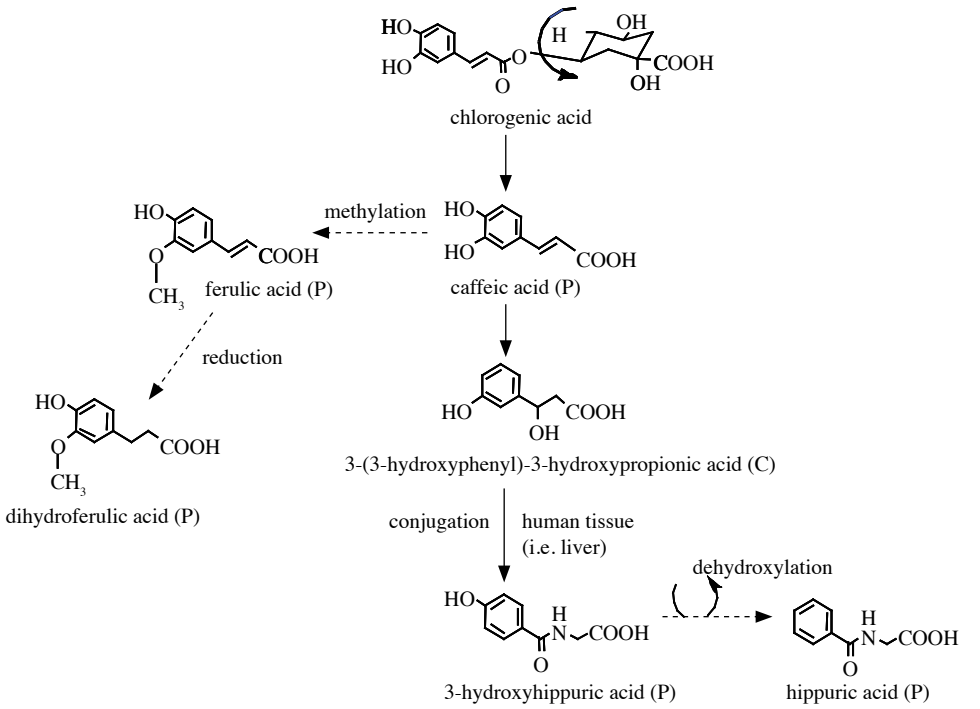

Figure 3. Proposed catabolism of chlorogenic acids in humans. P= proven; C = conjecture

hydrolysis by esterases to form caffeic acid (Plumb et al. 1999). Caffeic acid released from chlorogenic acids is catabolised to form 3-(3-hydroxyphenyl)-3-hydroxypropionic acid, which is converted to 3-hydroxyhippuric acid and hippuric acid. In an alternative route, part of the caffeic acid undergoes reduction to form ferulic acid and is further catabolised to dihydroferulic acid.

Conclusion

This study showed that the colon was mainly involved in chlorogenic acids catabolism in humans. Free phenolic acids were excreted by healthy volunteers mainly at 8 – 24 h after drinking coffee, while none was excreted by ileostomy volunteers or those who drank just plain water. The presence of free phenolic acids in human urine who drank coffee indicated that coffee may provide health benefits in humans. Better knowledge of consumption and catabolism of coffee will be essential in the future to evaluate properly their role in disease prevention.

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References

Abu-Amsha Caccetta, A.R., Croft, K.D., Puddey, I.B., Proudfoot, J.M. and Beilin, L.J. (1996). Phenolic content of various beverages determines the extent of inhibition of human serum and low-density lipoprotein oxidation *in vitro*: Identification and mechanism of action of some cinnamic acid derivatives from red wine. *Clin. Sci*. 91: 449 – 458

Andreason, M.F., Kroon, P., Williamson, G. and Garcia-Conesa, M.T. (2001). Esterase activity able to hydrolyze dietary antioxidant hydroxycinnamates is distributed along the intestine of mammals. *J. Agric. Food Chem*. 49: 5679 – 5684

Andreoni, V., Bernasconi, S. and Bestetti, G. (1995). Biotransformation of ferulic acid and related compounds by mutant strain of *Pseudomonas fluorescens*. *Appl. Microbiol. Biotechnol*. 42: 830 – 835

Azuma, K., Ippoushi, K., Nakayama, M., Ito, H., Higashio, H. and Terao, J. (2000). Absorption of chlorogenic acid and caffeic acid in rats after oral administration. *J. Agric. Food Chem*. 48: 5496 – 5500

Booth, A.N., Emerson, O.H., Jones, F.T. and DeEds, F. (1957). Urinary metabolites of caffeic and chlorogenic acids. *J. Biol. Chem*. 229: 51 – 59

Borges, G., Roowi, S., Rouanet, J.M. and Crozier, A. (2007). The bioavailability of raspberry anthocyanins and ellagitanins in rats. *Mol. Nutr. and Food Res*. 51: 714 – 725

Bourne, L.C. and Rice-Evans, C.A. (1998). Urinary detection of hydroxycinnamates and flavonoids in humans after high dietary intake of fruit. *Free Radic. Res*. 4: 429 – 438

Choudhury, R., Srai, S.K., Debnam, E. and Rice-Evans, C.A. (1999). Urinary excretion of hydroxycinnamates and flavonoids after oral and intravenous administration. *Free Radic. Biol. Med*. 27: 278 – 286

Clifford, M.N. (1999). Chlorogenic acids and other cinnamates - nature, occurrence and dietary burden. *J. Sci. Food Agric*. 79: 362 – 372

Clifford, M.N., Johnston, K.L., Knight, S. and Kuhnert, N. (2003). Hierarchical scheme for LC-MS^n identification of chlorogenic acids. *J. Agric. Food Chem*. 51: 2900 – 2911

Couteau, D., McCartney, A.L., Gibson, G.R., Williamson, G. and Faulds, C.B. (2001). Isolation and characterization of human colonic bacteria able to hydrolyse chlorogenic acid. *J. Appl. Microbiol*. 90: 873 – 881

Donaghy, J., Kelly, P.F. and McKay, A.M. (1998). Detection of ferulic acid esterase production by *Bacillus* spp. and *Lactobacilli*. *Appl. Microbiol. Biotechnol*. 50: 257 – 260

Dupas, C.J., Marsset-Baglieri, A.C., Ordonaud, C.S., Ducept, F.M.G. and Millard, M.N. (2006). Coffee antioxidant properties: Effect of milk addition and processing conditions. *J. Food Sc*. 71(3): S253 – S258

Gonthier, M.P., Verny, M.A., Besson, C., Rémésy, C. and Scalbert, A. (2003). Chlorogenic acid bioavailability largely depends on its metabolism by the gut microflora in rats. *J. Nutr.* 133: 1853 – 1859

Hodgson, J.M., Chan, S.Y., Puddey, I.B., Devine A., Wattanapenpaiboon N., Wahlqvist, M.L., Lukito, W., Burke, V., Ward, N.C., Prince R.L. and Croft, K.D. (2004). Phenolic acid

metabolites as biomarkers for tea- and coffee-derived polyphenol exposure in human subjects. *Br. J. Nutr.* 91(2): 301 – 306

Ito, H., Gonthier, M.P., Manach, C., Morand, C., Mennen, L., Remesy, C. and Scalbert, A. (2005). Polyphenol levels in human urine after intake of 6 different polyphenol-rich beverages. *Br. J. Nutr*. 94: 500 – 509

Jacobson, E.A., Newmark, H., Baptista, J. and Bruce, W.R.A. (1983). Preliminary investigation of the metabolism of dietary phenolics in human. *Nutr. Rep. Int*. 28: 1409 – 1417

Jenner, A.M., Rafter, J. and Halliwell, B. (2005). Human fecal water content of phenolics, the extent of colonic exposure to aromatic compounds. *Free Radic. Biol. Med*. 38: 763 – 772

Lafay, S., Gil-Izquierdo, A., Manach, C., Morand, C., Besson, C. and Scalbert, A. (2006). Chlorogenic acid is absorbed in its intact form in the stomach of rats. *J. Nutr.* 136: 1192 – 1197

Manach, C., Morand, C., Crespy, V., Demigne, C., Texier, O., Regerat, F. and Remesy, C. (1998). Quercetin is recovered in human plasma as conjugated derivatives which retain antioxidant properties. *FEBS Lett*. 426: 331 – 336

Manach, C., Williamson, G., Morand, C., Scalbert, A. and Remesy, C. (2005). Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *Am. J. Clin. Nutr*. 81(1): 230S – 242S

Mateos, R., Goya, L. and Bravo, L. (2006). Uptake and metabolism of hydroxycinnamic acids (chlorogenic, caffeic and ferulic acids) by HepG2 cells as a model of the human liver. *J. Agric. Food Chem*. 54: 8724 – 8732

Mennen, L.I., Sapinho, D., Ito, H., Bertrais, S., Galan, P., Hercberg, S. and Scalberg, A. (2006). Urinary flavonoids and phenolic acids as biomarker of intake for polyphenol-rich food. *Br. J. Nutr.* 96: 191– 198

Monteiro, M., Farah, A., Perrone, D., Trugo, L.C. and Donangelo, C. (2007). Chlorogenic acid compounds from coffee are differentially absorbed and metabolized in humans. *J. Nutr.* 137: 2196 – 2201

Mullen, W., Edwards, C.A. and Crozier, A. (2006). Absorption, excretion and metabolite profiling of methyl-, glucuronyl-, glucosyl- and sulpho-conjugates of quercetin in human plasma and urine after ingestion of onions. *Br. J. Nutr*. 96(1): 107 – 116

Nardini, M., Daquino, M., Tomassi, G., Gentili, V., Difelice, M. and Scaccini, C. (1995). Inhibition of human low-density lipoprotein oxidation by caffeic acid and other hydroxycinnamic acid derivatives. *Free Radic. Biol. Med*. 19: 541 – 552

Nardini, M., Cirillo, E., Natella, F. and Scaccini, C. (2002). Absorption of phenolic acids in humans after coffee consumption. *J. Agric. Food Chem*. 50: 5735 – 5741

Nardini, M., Natella, F., Scaccini, C. and Ghselli, A. (2006). Phenolic acids from beer are absorbed and extensively metabolized in humans. *J. Nutr. Biochem*. 17: 14 –22

Olthof, M.R., Hollman, P.C.H., Bujisman, M.N.C.P., Van Amalsvoort, J.M.M. and Katan, M. (2003). Chlorogenic acid, quercetin-3-ruinoside and black tea phenols are extensively metabolized in humans. *J. Nutr.* 133(6): 1806 – 1814

Olthof, M.R., Hollman, P.C.H. and Katan, M.B. (2001). Chlorogenic acid and caffeic acid are absorbed in humans. *J. Nutr.* 131: 66 – 71
Papadopoulou, A. and Frazier, R.A. (2004). Characterization of protein-polyphenol interactions. *Trends Food Sci. Technol*. 15: 186 – 190

Papadopoulou, A. and Frazier, R.A. (2004). Characterization of protein-polyphenol interactions. *Trends Food Sci. Technol*. 15: 186 – 190

Plumb, G., Garcia-Conesa, M.T., Kroon, P., Rhodes, M., Saxon, R. and Williamson, G. (1999). Metabolism of chlorogenic acid by human plasma, liver, intestine and gut microflora. *J. Sci. Food Agric*. 79: 390 – 392

Prigent, S.V.W., Gruppen, H., Visser, A.J.W.G., van Koningsveld, G.A., de Jong, G.A.H. and Voragen, A.G.J. (2003). Effects of non-covalent interactions with 5-*O*-caffeloylquinic acid (chlorogenic acid) on the heat denaturation and solubility of globular proteins. *J. Agric. Food Chem*. 51(17): 5088 – 5095

Rechner, A.R., Spencer, J.P.E., Kuhnle, G., Hahn, U. and Rice-Evans, C.A. (2001). Novel biomarkers of the metabolism of caffeic acid derivatives *in vivo*. *Free Radic. Biol. Med*. 30: 1213 – 1222

Roowi, S. (2008). Tropical citrus antioxidants and catabolism of phenolics in green tea, coffee, cocoa and orange juice, PhD Thesis, University of Glasgow

Sánchez-Gonzáles, I., Jiménez-Escrig, A. and Saura-Calixto, F. (2005). *In vitro* antioxidant activity of coffees brewed using different procedures (Italian, espresso and filter). *Food Chem*. 90: 133 – 139

Scheline, R.R. (1999). Metabolism of oxygen heterocyclic compounds. In: *Handbook of mammalian metabolism of plant compounds*, p. 243 – 395. Boca Raton: CRC Press, Inc.

Stalmach, A. Mullen, W., Denis, B., Kenichi, U., Takao, Y., Christophe, C., Heike, S., Gary, W. and Crozier, A. (2009). Metabolite profiling of hydroxycinnamate derivatives in plasma and urine after the ingestion of coffee by humans: Identification of biomarkers of coffee consumption. *Drug metabolism and disposition: the biological fate of chemicals* 37(8): 1749 – 1758

Stalmach, A., Mullen, W., Nagai, C. and Crozier, A. (2006). On-line HPLC analysis on the antioxidant activity of phenolic compounds in brewed, paper-filtered coffee. *Braz. J. Plant Physiol*. 18(1): 253 – 262

Takenaka, M., Nagata, T. and Yoshida, M. (2000). Stability and bioavailability of antioxidants in garland (*Chrysanthemum coronarium* L.). *Biosci. Biotechnol. Biochem*. 64: 2689 – 2691

Williamson, G., Kroon, P.A. and Faulds, C.B. (1998). Hairy plant polysaccharides: a close shave with microbial esterases. *Microbiology* 144: 2011 – 2023

Abstrak

Kajian farmakokinetik perkumuhan asid fenolik bebas di dalam air kencing manusia (dengan atau tanpa usus besar) yang minum kopi segera telah berjaya dilakukan. Objektif kajian ini adalah untuk mengenal pasti kemungkinan produk degradasi daripada *hydroxycinnamates* kopi *in vivo*. Semua subjek menunjukkan peningkatan asid 3-(3-hidroksifenil)-3-hidroksipropionik, asid dihidroferulik, asid ferulik dan asid 3-hidroksihippurik dalam masa 8 – 24 jam selepas minum kopi. Keseluruhannya, purata kepekatan asid fenolik bebas yang didapati di dalam air kencing ialah 134 ± 43 μmol dan ini bersamaan dengan 29.4% daripada jumlah pengambilan asid klorogenik. Jumlah ini lebih tinggi (p <0.05) daripada asid fenolik yang dikumuh di dalam air kencing manusia yang hanya minum air kosong. Kehadiran asid fenolik bebas di dalam air kencing manusia selepas minum kopi menunjukkan bahawa kopi mungkin mendatangkan manfaat kesihatan kepada manusia. Ini disebabkan asid fenolik bebas mempunyai aktiviti antioksidan yang lebih tinggi berbanding dengan asid fenolik berkonjugat.

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