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Absorption and Excretion of Conjugated Flavonols, Including Quercetin-4′-O-β-Glucoside and Isorhamnetin-4′-O-β-Glucoside by Human Volunteers after the Consumption of Onions

AZLINA A. AZIZ[a,b], CHRISTINE A. EDWARDS[b], MICHAEL E.J. LEAN[c] and ALAN CROZIER[a,*]

[a]*Plant Molecular Science Group, Bower Building, Division of Biochemistry and Molecular Biology, Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow G12 8QQ, UK;* [b]*University of Glasgow Department of Human Nutrition, Yorkhill Hospitals, Glasgow G3 8SJ, UK;* [c]*University of Glasgow Department of Human Nutrition, Queen Elizabeth Building, Royal Infirmary, Glasgow G31 2ER, UK*

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Flavonols are polyphenols found ubiquitously in plants and plant-products. Flavonols, particularly quercetin, are potent antioxidants *in vitro* and their intake has been associated inversely with the incidence of coronary heart disease. The aim of this study was to investigate the accumulation in plasma and excretion in urine of flavonol glucosides following ingestion of lightly fried onions. Five healthy volunteers followed a low-flavonoid diet for 3 days. On day 4, after an overnight fast, subjects were given 300 g of lightly fried yellow onions which contain conjugates of quercetin and isorhamnetin, including quercetin-3,4′-diO-β-glucoside, isorhamnetin-4′-O-β-glucoside and quercetin-4′-O-β-glucoside. Blood collection was carried out at 0 min, 0.5, 1.0, 1.5, 2, 3, 4, 5 and 24 h after the supplement. In addition, subjects collected all their urine for 24 h following the onion supplement. Isorhamnetin-4′-O-β-glucoside and quercetin-4′-O-β-glucoside accumulated in plasma with maximum levels, defined as proportion of intake, of $10.7 \pm 2.6\%$ and $0.13 \pm 0.03\%$ respectively. The time of the quercetin-4′-glucoside peak plasma concentration was 1.3 ± 0.2 h after the ingestion of onions while a value of 1.8 ± 0.7 h was obtained for isorhamnetin-4′-glucoside. Excretion in urine, as a proportion of intake, was $17.4 \pm 8.3\%$ for isorhamnetin-4′-O-β-glucoside and $0.2 \pm 0.1\%$ for quercetin-4′-O-β-glucoside. Possible reasons for the accumulation and excretion of isorhamnetin-4′-glucoside in proportionally much higher amounts than quercetin-4′-glucoside are discussed. It is concluded that flavonols are absorbed into the bloodstream as glucosides and minor structural differences affect markedly both the level of accumulation and the extent to which the conjugates are excreted.

Keywords: Flavonol conjugates, quercetin-4′-O-β-glucoside, isorhamnetin-4′-O-β-glucoside, accumulation in plasma, excretion in urine

INTRODUCTION

Flavonoids are polyphenols with widespread occurrence in plants and plant-derived foods.

* Corresponding author. Tel.: (44) 141-330-4613. Fax: (44) 141-330-4447. E-mail: a.crozier@bio.gla.ac.uk.

They have been linked with various biological effects in humans including maintenance of capillary wall integrity and capillary resistance, besides having several clinically relevant anti-inflammatory and anti-allergic properties.[1] Flavonols, such as quercetin (I, Figure 1), myricetin (II), isorhamnetin (III) and kaempferol (IV), and the flavones apigenin (V) and luteolin (VI), are the most commonly studied dietary flavonoids. They are normally present in fruits,

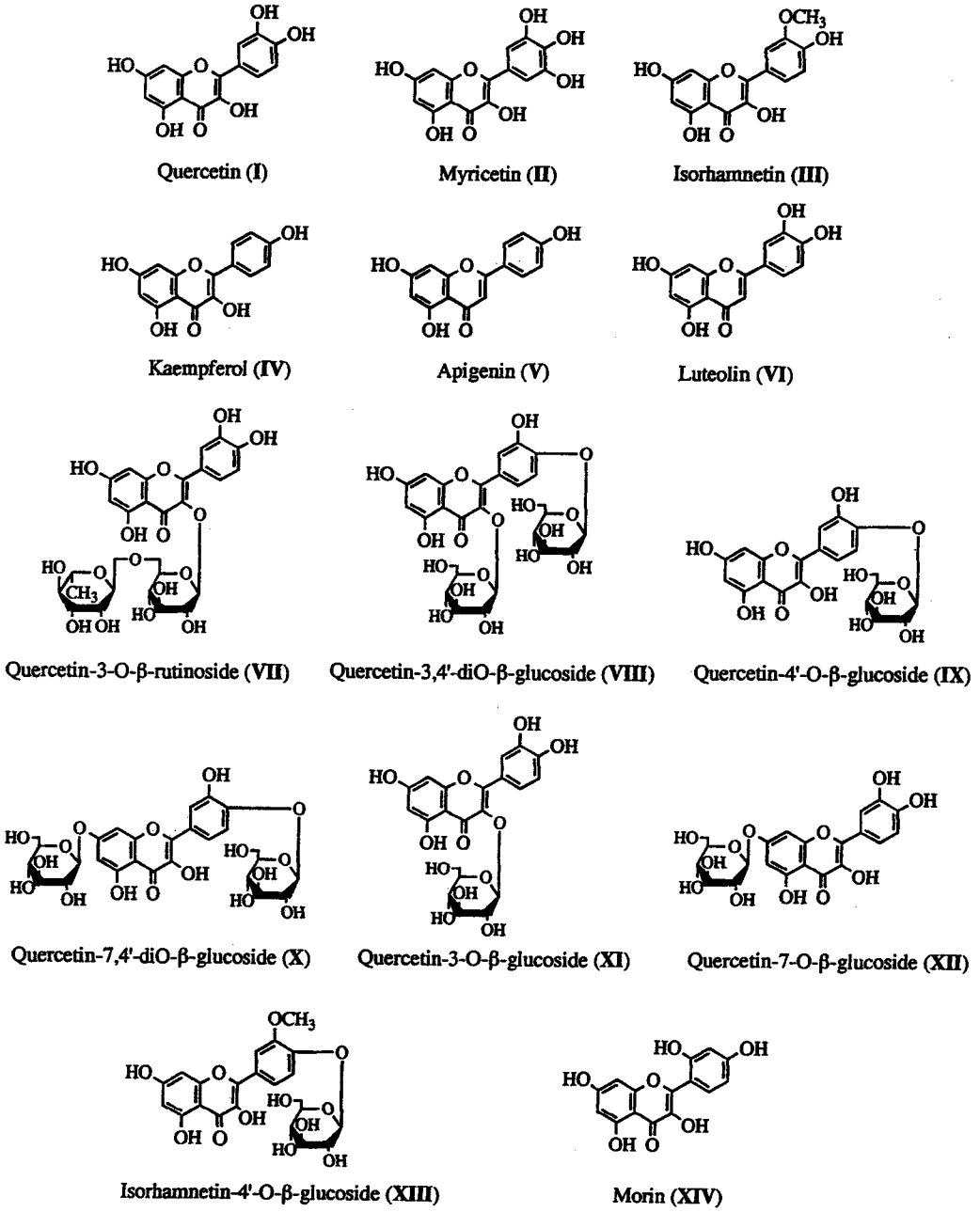

FIGURE 1 Structures of flavonols, flavones and flavonol conjugates.

vegetables and teas as sugar conjugates although many red wines can contain significant amounts of free as well as conjugated quercetin and myricetin.[2–5]

Recent evidence has strongly supported their antioxidative role and their protection against LDL oxidation,[6,7] a process in the pathogenesis of atherosclerosis. Quercetin, which in conjugated forms is commonly present in fruits and vegetables in high concentrations,[2–4] possesses strong antioxidative properties.[8,9] Several epidemiological studies have shown an association between increased intakes of flavonols and lower incidence of coronary heart disease.[10,11] Based on estimates from diet questionnaires and the composition of key foods, the average intake of all flavonols has been estimated at 23 mg/d in the Netherlands diet with tea and onions being the major sources at 48% and 29% of total intake respectively.[11]

The absorption and metabolism of individual flavonols in man is however still poorly understood. Attempts to investigate their absorption have shown conflicting results. Previously, it was speculated that only free flavonols were absorbed and not the glycosides due to their conjugation to sugar molecules.[12] However, recent research has detected the presence of rutin (quercetin-3-O-β-rutinoside, **VII**) in human plasma[13] while data obtained with ileostomy patients has been interpreted as indicating that conjugated forms of quercetin may be better absorbed than the aglycone.[14]

Especially high concentrations of flavonols are found in onions[3] in the form of quercetin-3,4′-diO-β-glucoside (quercetin-3,4′-diglucoside) (**VIII**) and quercetin-4′-O-β-glucoside (quercetin-4′-glucoside) (**IX**), as well as smaller amounts of quercetin-7,4′-diO-β-glucoside (**X**), quercetin-3-O-β-glucoside (quercetin-3-glucoside) (**XI**), quercetin-7-O-β-glucoside (**XII**) and isorhamnetin-4′-O-β-glucoside (isorhamnetin-4′-glucoside) (**XIII**).[15] Onions, therefore, represent useful material for absorption studies as recently developed HPLC techniques[16] with post-column derivatization[17] can be used to quantify the overall levels of free and conjugated quercetin and isorhamnetin. This sensitive and selective method of analysis can also be used to monitor trace levels of quercetin-4′-glucoside and isorhamnetin-4′-glucoside in body fluids and, as such, facilitates more detailed studies on the absorption of flavonol conjugates than has previously been achieved. These developments raise interesting possibilities as the identification of flavonol conjugates that are readily absorbed would open up the opportunity of providing advice not only on flavonol-rich fruits and vegetables but on foods that contain antioxidant flavonol conjugates, which when consumed will accumulate in the blood stream in elevated concentrations.

The aim of this present study was to investigate the extent of accumulation of flavonols in plasma and their excretion in urine after a meal of lightly fried onions and to establish whether any of the different flavonol conjugates present in the dietary supplement were absorbed without undergoing structural modifications.

MATERIALS AND METHODS

Study Design

Five healthy volunteers (4 females, 1 male), mean age 29.4 y (range: 23–37 y), who were not on any medication and were non-smokers, participated in this study and gave their written consent. The subjects followed a low-flavonol diet for 3 days prior to each experiment and fasted overnight before being fed 300 g of lightly fried onions. Venous blood samples were collected immediately prior to the consumption of the onions and at 0.5, 1, 1.5, 2, 3, 4, 5 and 24 h after completing the meal. A 10 ml blood sample was collected at each time point into heparinised tubes which were centrifuged immediately at 3000 × g at 0°C for 10 min after which plasma was separated and stored at −80°C prior to analysis. In addition, urine samples were collected between 0–6, 6–12 and 12–24 h after the meal. The urine samples

were stored in a cooler on ice in plastic bottles from which aliquots were taken and stored at −80°C prior to analysis. This study protocol was approved by the University of Glasgow Human Ethics Committee for Non-Clinical Research.

Preparation of Onions

After the removal of the dry outer scales, yellow onions (Safeway plc., 373 Byres Road, Glasgow G12, UK) were chopped into slices, lightly fried in olive oil before 300 g samples were eaten by volunteers. An earlier study showed that much more extensive frying of onions resulted in only a 21% loss of flavonols.[2] Triplicate samples of fried onions were taken from each feeding experiments for quantitative analysis of their flavonol content.

Optimization of acidic conditions for the hydrolysis of flavonol conjugates in a range of plant tissues has been described by Hertog *et al.*[18] following an earlier detailed study by Harborne[19] on the release of free flavonols by acid and enzymic hydrolyses. It has been reported that this procedure cleaves not only flavonol glucosides but also quercetin glucuronide and sulphate conjugates.[20,21] In the present investigation, preliminary screening was carried out to ascertain the most effective acid hydrolysis conditions for the samples under study. As a result, samples of plasma (600 µl), urine (750 µl) and fried onions (20 mg lyophilised tissue) were hydrolysed at 90°C for either 2 h (urine and fried onions) or 3 h (plasma) in a 3-ml glass V-vial containing 2 ml 1.2 M HCl in 50% aqueous methanol and 20 mM sodium diethyldithiocarbamate as an antioxidant. With onion samples, 5µg of morin (**XIV**) was also added as an internal standard. The presence of a peak with a retention time very close to that of morin in some urine and plasma samples precluded the use of morin as an internal standard with these fluids. A teflon-coated magnetic stirrer was placed in the vial which was sealed tightly with a PTFE-faced septum prior to heating in a Reacti-Therm Heating/Stirring Module (Pierce, Rockford, IL, USA). Extract aliquots of 100 µl, taken both before and after acid hydrolysis, were made up to 250 µl with distilled water adjusted to pH 2.5 with trifluoroacetic acid and filtered through a 0.2 µm Anopore filter (Whatman, Maidstone, Kent, UK), prior to the analysis of 100 µl volumes (1/50th aliquot of total sample) by gradient elution reversed phase HPLC. Because of the presence of precipitated proteins, prior to being filtered, plasma samples were centrifuged for 5 min at $5000 \times g$ at 4°C, after which 1/50th aliquots were analysed by HPLC.

High Performance Liquid Chromatography and Post-Column Derivatization

Samples were analysed using a Shimadzu (Kyoto, Japan) LC-10A series automated liquid chromatograph comprising a SCL-10A system controller, two LC-10A pumps, a SIL-10A auto injector with sample cooler, a CTO-10A column oven and SPD-10A UV-VIS detector and an RF-10A fluorimeter linked to Reeve Analytical (Glasgow, UK) 2700 data handling system. Reversed phase separations were carried out at 40°C using a 150 × 3.0 mm i.d., 4 µm Genesis C_{18} cartridge column fitted with a 10 × 4.0 mm i.d. C_{18} Genesis guard cartridge in an integrated holder (Jones Chromatography, Mid-Glamorgan, UK). The mobile phase was a 25 min, 15–40% gradient of acetonitrile in water adjusted to pH 2.5 with trifluoroacetic acid, eluted at a flow rate of 0.5 ml/min. Column eluent was first directed to the SPD-10A absorbance monitor operating at 365 nm,[16] after which post-column derivatization was achieved by the addition of methanolic, aluminium nitrate containing 7.5% glacial acetic acid, as described by Hollman *et al.*[17] and pumped at a flow rate of 0.5 ml/min by a pulse-free Model 9802 precision mixer/splitter (Reeve Analytical). The mixture was directed to a RF-10A fluorimeter and fluorescent flavonol complexes detected at excitation 420 nm and

emission 485 nm. The limit of detection at $A_{365\,nm}$ was < 5 ng and linear 5–250 ng calibration curves were obtained for morin, myricetin, quercetin, kaempferol, isorhamnetin, quercetin-3,4′-diglucoside, quercetin-4′-glucoside and isorhamnetin-4′-glucoside. The fluorescence intensity of the individual flavonoid derivatives varied, however, 0.1–100 ng linear calibration curves were obtained for morin, myricetin, quercetin, kaempferol, isorhamnetin, quercetin-4′-glucoside and isorhamnetin-4′-glucoside.

Estimates of Free and Conjugated Flavonol Levels

Free flavonols were detected in the unhydrolysed samples while the hydrolysed samples contained free flavonols as well as aglycones released by cleavage of conjugated flavonols. Conjugated flavonol levels were estimated by subtracting the amounts found in the unhydrolysed samples from that detected after acid hydrolysis. With each analysis the flavonol content of hydrolysed onion samples was corrected for sample handling/hydrolysis losses on the basis of the recovery of the morin internal standard, which typically was ca. > 90%. This provided estimates of overall levels of free and conjugated quercetin and isorhamnetin. In addition, the availability of standards of quercetin-3,4′-diglucoside, quercetin-4′-glucoside and isorhamnetin-4′-glucoside, enabled the presence of these compounds to be investigated in unhydrolysed samples of onion, plasma and urine.

Reference Compounds

Apigenin, kaempferol, morin, myricetin and quercetin were purchased from Sigma Chemicals (Poole, Dorset, UK). Isorhamnetin and quercetin-3-glucoside were obtained from Apin Chemicals (Abingdon, Oxford, UK). Quercetin-3,4′-diglucoside, quercetin-4′-glucoside and isorhamnetin-4′-glucoside were generously provided by Dr T. Tsushida, National Food Research Institute, Ibaraki, Japan.

RESULTS

Flavonols in Fried Onions

HPLC provided an effective separation of the flavonol standards with quercetin-3,4′-diglucoside, quercetin-3-glucoside, quercetin-4′-glucoside, isorhamnetin-4′-glucoside, morin, quercetin, kaempferol and isorhamnetin being detected with an absorbance monitor operating at 365 nm (Figure 2A). The subsequent formation of fluorescent complexes by a postcolumn reaction with aluminium nitrate facilitated the detection of trace levels of all these compounds with the exception of quercetin-3-glucoside and quercetin-3,4′-diglucoside (Figure 2B). Typical traces obtained when these procedures were used to analyse unhydrolysed fried onion extracts are illustrated in Figure 2C and D. Peaks that co-chromatographed with quercetin-3,4′-diglucoside, quercetin-4′-glucoside, the morin internal standard and trace levels of isorhamnetin-4′-glucoside were detected at $A_{365\,nm}$ (Figure 2C) while fluorescent postcolumn derivatization traces contained peaks corresponding to the 4′-glucosides of quercetin and isorhamnetin together with small amounts of quercetin and kaempferol (Figure 2D).

Quantitative estimates of quercetin-3,4′-diglucoside, quercetin-4′glucoside, isorhamnetin-4′-glucoside, quercetin and kaempferol in the five lightly fried onion meals fed to human volunteers are presented in Table I. The variation in flavonol levels between the different samples of onions, which were purchased over a six-month period, is in keeping with previous observations.[2] In all instances, however, quercetin-3,4′-diglucoside and quercetin-4′-glucoside were the major flavonols present together with much lower levels of isorhamnetin-4′-glucoside, quercetin and kaempferol.

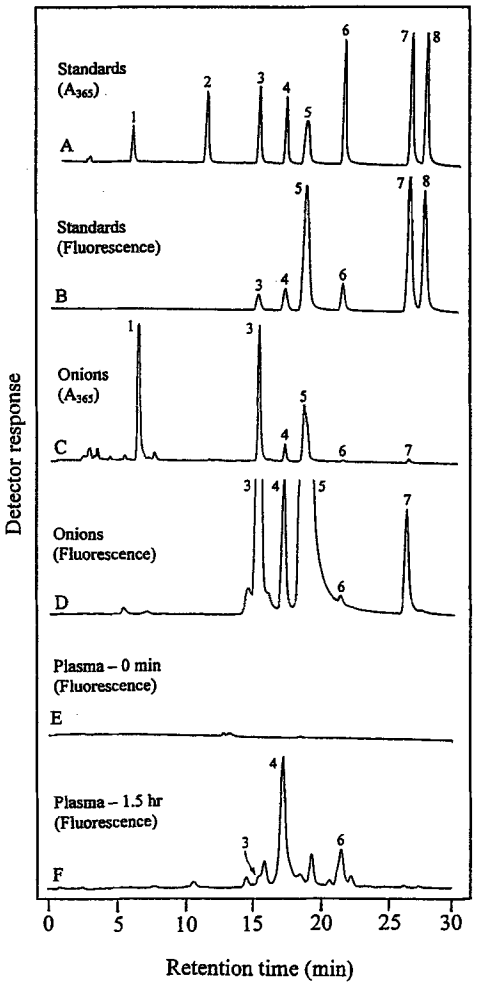

FIGURE 2 Gradient reverse phase HPLC analysis of flavonols. Column: 150 × 3.0 mm i.d. 4-μm Genesis C_{18} cartridge column with a 10 × 4.0 mm 4-μm Genesis C_{18} guard cartridge. Mobile phase: 25 min gradient of 15–40% acetonitrile in water containing 0.1% trifluoroacetic acid. Flow rate: 0.5 ml/min. Detector: absorbance monitor operating at 365 nm and, after on-line post-column reaction with methanolic aluminium nitrate, a fluorimeter operating at excitation 420 nm and emission 485 nm. Samples: (A) 150 ng of (1) quercetin-3,4′-diglucoside, (2) quercetin-3-glucoside, (3) quercetin-4′-glucoside, (4) isorhamnetin-4′-glucoside, (5) morin, (6) quercetin, (7) kaempferol and (8) isorhamnetin with detection at $A_{365\,nm}$; (B) as A but with post-column derivatization and fluorescence detection; (C) aliquot of an unhydrolysed extract of lightly fried onions, with detection at $A_{365\,nm}$; (D) as C but with post-column derivatization and fluorescence detection; (E) unhydrolysed 12 μl aliquot of plasma collected immediately prior to the consumption of 300 g of lightly fried onions, with post-column derivatization and fluorescence detection; (F) as E but plasma collected 1.5 h after eating fried onions. Numbers indicate peaks that co-chromatograph with standards listed for sample A.

Analysis of Flavonols in Plasma

Typical HPLC postcolumn derivatization traces of plasma collected at 0 and 1.5 h after the ingestion of onions are illustrated in Figure 2E and F. The 0 h sample of plasma contained only very minor fluorescent components. Plasma collected at 1.5 h after eating 300 g of onions contained isorhamnetin-4′-glucoside in far larger amounts than quercetin-4′-glucoside which was present in only trace quantities as a shoulder on an impurity (Figure 2F) despite being found in onions in much larger amounts than the isorhamnetin conjugate (Figure 2C and D, Table I). In some plasma samples quercetin (Figure 2F), kaempferol and isorhamnetin were also found but in trace amounts very close to the limit of detection. The major onion flavonol, quercetin-3,4′-diglucoside, does not fluoresce following the postcolumn derivatization (see Figure 2C and D) and it was not detected with an absorbance monitor operating at 365 nm in any of the plasma samples that were analysed (data not shown). This does not necessarily mean it was not present in levels broadly comparable to quercetin-4′-glucoside because the limit of detection at $A_{365\,nm}$ for the quercetin-3,4′-diglucoside was 500 ng/ml while postcolumn derivatization enabled quercetin-4′-glucoside to be monitored at concentrations as low as 10 ng/ml.

In all instances, identifications of quercetin-4′-glucoside, isorhamnetin-4′-glucoside, quercetin, kaempferol and isorhamnetin were confirmed by co-chromatography with standards. The slight band broadening that is apparent in the 1.5 h plasma sample illustrated in Figure 2F, compared to the standards (Figure 2B) and onion extract (Figure 2D), was observed frequently when unhydrolysed plasma was analysed. This probably is a consequence of the presence of residual proteins in the sample.

Accumulation of Flavonols in Plasma

The onions were well accepted and tolerated by the human volunteers and no adverse effects

TABLE I Flavonol content ± standard error ($n = 3$) of 300 g samples of lightly fried onions eaten by five subjects

Flavonol	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Mean
Quercetin	0.41 ± 0.03 mg (1.21 ± 0.1 μmoles)	0.46 ± 0.08 mg (1.4 ± 0.2 μmoles)	0.94 ± 0.07 mg (2.8 ± 0.2 μmoles)	7.4 ± 0.2 mg (21.9 ± 0.6 μmoles)	2.6 ± 0.1 mg (7.8 ± 0.4 μmoles)	2.8 ± 1.3 mg (7.0 ± 3.9 μmoles)
Quercetin-3,4′-diglucoside	228 ± 2 mg (326 ± 3 μmoles)	133 ± 9 mg (190 ± 12 μmoles)	361 ± 2 mg (517 ± 2 μmoles)	374 ± 1 mg (535 ± 2 μmoles)	340 ± 2 mg (487 ± 3 μmoles)	287 ± 46 mg (411 ± 67 μmoles)
Quercetin-4′-glucoside	65 ± 2 mg (126 ± 4 μmoles)	37 ± 4 mg (70 ± 7 μmoles)	111 ± 3 mg (215 ± 5 μmoles)	147 ± 6 mg (283 ± 11 μmoles)	150 ± 1 mg (290 ± 3 μmoles)	102 ± 22 mg (197 ± 43 μmoles)
Isorhamnetin	n.d. —	n.d. —	0.06 ± 0.01 mg (0.11 ± 0.01 μmoles)	0.33 ± 0.01 mg (0.66 ± 0.02 μmoles)	0.13 ± 0.02 mg (0.26 ± 0.04 μmoles)	0.10 ± 0.06 mg (0.21 ± 0.12 μmoles)
Isorharmnetin-4′-glucoside	10.0 ± 0.4 mg (20.2 ± 0.8 μmoles)	5.5 ± 0.2 mg (11.1 ± 0.4 μmoles)	10.9 ± 0.1 mg (22.1 ± 0.3 μmoles)	14.3 ± 0.4 mg (28.8 ± 0.8 μmoles)	12.0 ± 0.1 mg (24.1 ± 0.2 μmoles)	10.5 ± 1.5 mg (21.3 ± 2.9 μmoles)
Kaempferol	0.29 ± 0.06 mg (1.01 ± 0.21 μmoles)	0.52 ± 0.02 mg (1.82 ± 0.07 μmoles)	0.17 ± 0.02 mg (0.59 ± 0.07 μmoles)	0.28 ± 0.001 mg (0.98 ± 0.03 μmoles)	0.21 ± 0.01 mg (0.73 ± 0.02 μmoles)	0.29 ± 0.06 mg (1.03 ± 0.21 μmoles)
Conjugated kaempferol	0.77 ± 0.09 mg (2.69 ± 0.31 μmoles)	0.26 ± 0.02 mg (0.91 ± 0.07 μmoles)	0.52 ± 0.02 mg (1.82 ± 0.07 μmoles)	0.98 ± 0.06 mg (3.42 ± 0.21 μmoles)	0.51 ± 0.06 mg (1.78 ± 0.06 μmoles)	0.61 ± 0.12 mg (2.12 ± 0.43 μmoles)

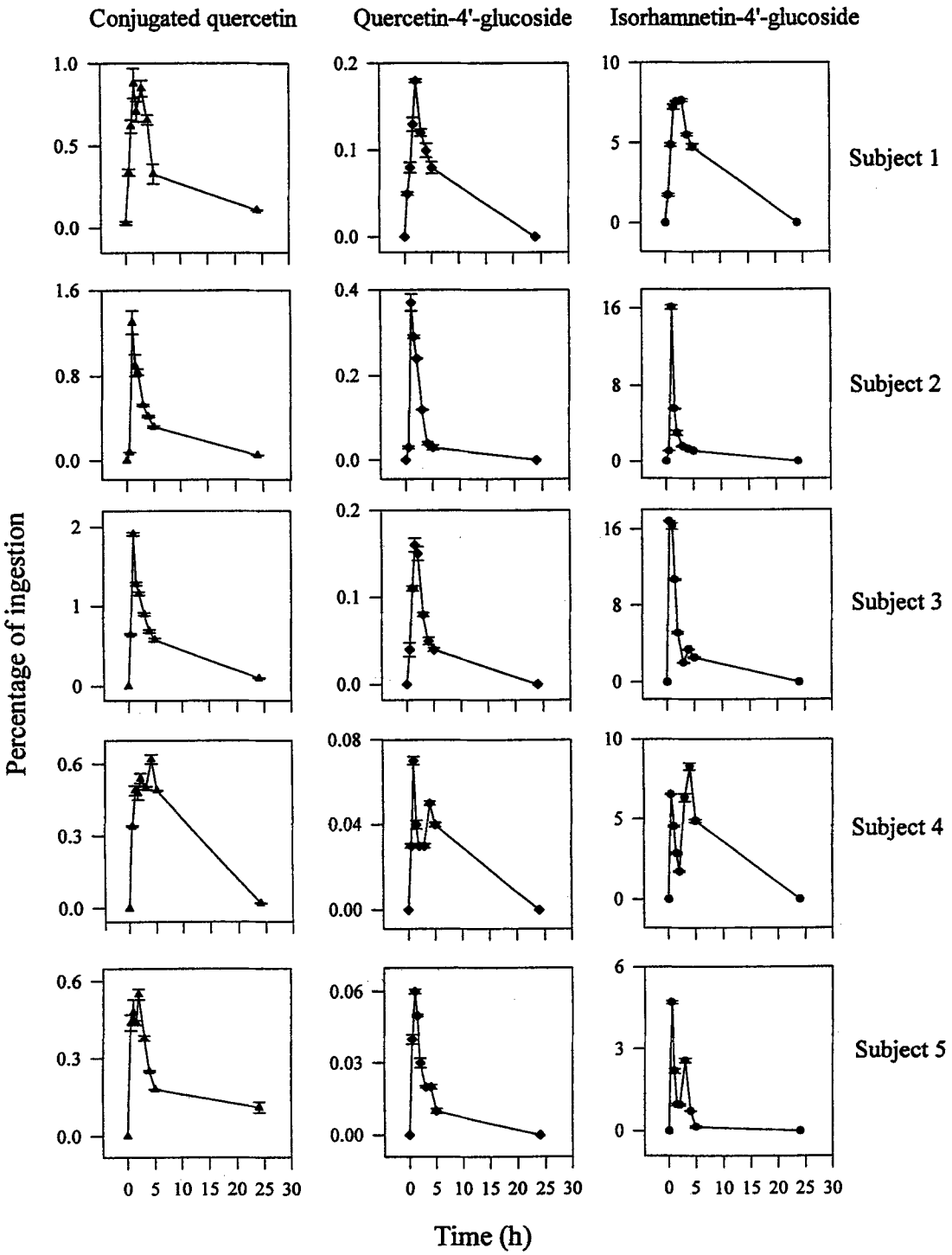

FIGURE 3 Concentration of conjugated quercetin, quercetin-4′-glucoside and isorhamnetin-4′-glucoside in plasma collected from five human volunteers after the ingestion of 300 g of lightly fried onions. Data expressed as percentage of the intake based on flavonol content of onions ± S.E. ($n = 5$) and calculated on the basis of 3000 ml of plasma per person.

were reported. The five subjects followed a low-flavonol diet for three days before being fed fried onions. The time course profiles of the appearance of quercetin-4′-glucoside, conjugated quercetin and isorharmnetin-4′-glucoside in the plasma are presented in Figure 3. As the amounts of flavonols in the onions consumed varied (see Table I), flavonol levels in the plasma are expressed as percent of the amount ingested. For the sake of clarity, data on the intermittent appearance of trace quantities of quercetin, kaempferol, isorhamnetin and conjugated kaempferol are not included.

The plasma flavonol profiles show some variation among the 5 subjects (Figure 3). The 0 h plasma from subject 1 contained 6.8 ng/ml of conjugated quercetin. No flavonols were detected in the other four 0 h plasma samples. Subjects 2 and 3 exhibited similar profiles with a rapid increase of flavonol levels after onion consumption followed by a rapid decline in their concentration. On the other hand, flavonols in subjects 1, 4 and 5 appeared to have a second peak concentration later on in the time course of the experiment and had a slower decline in flavonol content than subjects 2 and 3. Overall, the absorption of flavonols was moderately rapid with peak plasma concentrations being reached in the five subjects at times ranging from 0.5–4.0 h after ingestion of fried onions, although in most instances a figure of 1.0–2.0 h was typical (Table II).

It is notable that compared with the levels present in the ingested onions, isorhamnetin-4′-glucoside accumulated in plasma in amounts ca. 10 times greater than conjugated quercetin and 50-fold more than quercetin-4′-glucoside. This was evident with all five subjects (Figure 3). The level of flavonol accumulation, as a percentage of the amount ingested, varied somewhat between the five subjects. With subjects 2 and 3, the peak isorhamnetin-4′-glucoside level was ca. 16% and that of quercetin-4′-glucoside ca. 0.2–0.4 % while the equivalent figures for subject 5 were ca. 5 % and ca. 0.1% respectively.

The mean values for the key features of flavonol accumulation in plasma is presented in Table II. A peak concentration of 452 ± 100 ng/ml was obtained for the overall level of quercetin conjugates while with quercetin-4′-glucoside it was 45 ± 11 ng/ml, which is less than 10% of the total quercetin conjugate concentration. The mean maximum concentration of isorhamnetin-4′-glucoside was 370 ± 91 ng/ml which when expressed as a proportion of intake from the onions is 10.7 ± 2.6% compared to values of 0.13 ± 0.03% and 0.97 ± 0.21% from quercetin-4′-glucoside and quercetin conjugates, respectively. After 24 h, the levels of all the flavonols in the plasma had declined markedly, and they were either undetectable or present in trace levels (Figure 3).

Urinary Excretion of Flavonols

Isorhamnetin-4′-glucoside showed a higher percentage excretion at 17.4 ± 8.3% as compared to quercetin-4′-glucoside with a percentage

TABLE II Mean values ± standard error of key features of flavonol conjugate accumulation in plasma following the consumption of 300 g of lightly fried onions by five subjects. n.d. – not detected

Flavonol	Intake	Peak plasma concentration	Time of peak plasma concentration	Peak plasma concentration as a proportion of intake*
Conjugated quercetin	139 ± 25 mg (411 ± 74 μmoles)	452 ± 100 ng/ml (1.34 ± 0.30 μM)	1.9 ± 0.6 h	0.97 ± 0.21%
Quercetin-4′-glucoside	102 ± 22 mg (197 ± 43 μmoles)	45 ± 11 ng/ml (0.09 ± 0.02 μM)	1.3 ± 0.2 h	0.13 ± 0.03%
Isorhamnetin-4′-glucoside	10.5 ± 1.5 mg (21 ± 3 μmoles)	370 ± 91 ng/ml (0.75 ± 0.18 μM)	1.8 ± 0.7 h	10.7 ± 2.6%

*Calculated on the basis of 3000 ml plasma/person.

TABLE III Mean values ± standard error for the excretion of flavonol conjugates in urine following the consumption of 300 g of lightly fried onions by five subjects. n.d. – not detected

Flavonol	Intake	Excretion period			Total excreted as a proportion of intake
		0–6 h	6–12 h	12–24 h	
Conjugated quercetin	139 ± 25 mg (411 ± 74 μmoles)	661 ± 281 μg (1.9 ± 0.8 μmoles)	348 ± 184 μg (1.0 ± 0.5 μmoles)	66 ± 29 μg (0.2 ± 0.1 μmoles)	0.8 ± 0.4%
Quercetin-4′-glucoside	102 ± 22 mg (197 ± 43 μmoles)	100 ± 27 μg (0.2 ± 0.05 μmoles)	65 ± 43 μg (0.1 ± 0.08 μmoles)	4.8 ± 2.6 μg (0.01 ± 0.005 μmoles)	0.2 ± 0.1%
Isorhamnetin-4′-glucoside	10.4 ± 1.5 mg (21 ± 3 μmoles)	1175 ± 482 μg (2.4 ± 1.0 μmoles)	620 ± 374 μg (1.2 ± 0.7 μmoles)	23 ± 15 μg (0.05 ± 0.03 μmoles)	17.4 ± 8.3%

excretion of 0.2 ± 0.1% (Table III). Approximately 62% of the cumulative excretion was reached in the first collection period (0–6 h). Urine samples from the last collection period (12–24 h) contained, on average, 3% of the total daily output of the flavonols, indicating that the peak of urinary flavonol excretion lay well within the 12 h period. Quercetin conjugates in the hydrolysed samples showed a percentage of excretion of 0.8% which was four times the percentage excretion of quercetin-4′-glucoside.

DISCUSSION

Although the literature contains much information on the seemingly low levels of absorption of the aglycone quercetin in a variety of rat test systems, there are few studies on the absorption of flavonol conjugates, the typical constituents of foods, by humans. Hollman and co-workers have investigated the absorption of quercetin glucosides by humans with an ileostomy.[14] The subjects were fed either quercetin glucoside-rich onions, rutin or free quercetin, after which the flavonol content of ileostomy effluent and urine were monitored over a 13 h period. *In vitro* incubations of the three sources of flavonols with gastrointestinal fluids showed minimal degradation and extremely low levels of flavonols were excreted in urine. After corrections for sample handling losses and low level degradation in the ileostomy bag, absorption was estimated by subtracting the flavonol content of the ileostomy effluent from the oral intake. This albeit indirect procedure, indicated surprisingly high levels of absorption, 52% of onion quercetin glycosides, 17% for rutin and 24% for quercetin. Subsequently, the same group, who analysed samples only after acid hydrolysis, which does not allow distinction between free and conjugated quercetin pools, monitored flavonol levels in plasma after the ingestion of onions.[20] The time course profiles obtained with two volunteers were similar to those obtained in the present study (Figure 3), as was the peak quercetin (free plus conjugated) plasma concentration of 196 ng/ml. This is equivalent to ca. 0.9% of the flavonol content of the ingested onion flavonols and comparable to figures in Table II. These low plasma concentrations imply that quercetin/quercetin conjugates, if they are absorbed into the bloodstream in the quantities reported by Hollman *et al.*,[14] are being rapidly metabolised and/or removed from the bloodstream, presumably by the liver.

In the present study, it has been demonstrated for the first time that the onion flavonol glucosides, quercetin-4′-glucoside and isorhamnetin-4′-glucoside accumulate in the bloodstream and are excreted in urine without seemingly undergoing structural modification. The main flavonol in onions, quercetin-3,4′-diglucoside, was not detected in body fluids but this is likely to be a consequence of the relative lack of sensitivity of the HPLC detection systems for this conjugate.

The level of quercetin released from conjugated forms by acid hydrolysis, although low, was invariably several-fold higher than the concentration of quercetin-4′-glucoside in both plasma and urine (Tables II and III). This may be due to the presence of metabolites such as quercetin glucuronide and sulphate conjugates, which release free quercetin when acid hydrolysed,[20, 21] as would trace levels of quercetin-3,4′-diglucoside, which may also have been present. However, when compared to the levels present in the ingested onions, it is evident that isorhamnetin-4′-glucoside accumulated in both plasma and urine in proportionally far higher amounts than quercetin-4′-glucoside and other quercetin conjugates (Tables II and III). Further study is required to determine whether this is due to more effective absorption of the isorhamnetin conjugate or whether it is a consequence of the absorbed quercetin conjugates being removed from the bloodstream more rapidly than isorhamnetin-4′-glucoside. There is, however, an alternative possibility that at least part of the isorhamnetin-4′-glucoside pool is formed by 3′-O-methylation of quercetin-4′-glucoside. Isorhamnetin is one of a number of metabolites that appear in the bile of rats after oral intake of quercetin.[22]

After the consumption of onions, flavonols accumulated rapidly in plasma with peak concentrations being reached within 1–2 h in most instances (Table II, Figure 3). This observation agrees with previous findings by Hollman *et al.*[20] discussed above and implies that absorption of flavonol conjugates occurs primarily from the stomach and/or the small intestine. The variation in the profile of absorption of the subjects (Figure 3) may be due to the differences in their intestinal physiology which influence the extent of flavonol absorption or alternatively they could be a consequence of different rates of metabolism/sequestration of the absorbed conjugates. Interestingly the mean peak plasma concentrations for conjugated quercetin and isorhamnetin-4′-glucoside (Table II) are both in excess of the levels of β-carotene that are typically found in human plasma.[23]

In their reports on the absorption of flavonols derived from onions and other vegetables, the Dutch group fitted data on flavonol levels in plasma and urine into a two-open compartment model using the equation $C(t) = Ae^{-kt} + Be^{-\alpha t} + Ce^{-\beta t}$.[24] The different parameters calculated from the equation were used to estimate the half-lives of the absorption, distribution and elimination phases and the bioavailability of total quercetin was calculated by comparing the areas under the percentage flavonol ingested-time curve.[20,21] Application of this model to the data obtained in the present study produced similar figures for the half-lives of the absorption and distribution phases and a shorter value for the elimination phase (data not shown). The validity of such extrapolations is, however, open to question. Although the figures for isorhamnetin-4′-glucoside were higher, the peak quercetin levels detected in plasma and urine were low, ca. 1% of the amounts in the ingested onions. Homeostasis of plasma flavonol pools is almost certainly in a state of flux because of the combined effects of transport through the gut wall into the bloodstream and removal by sequestration, metabolism and excretion. In the circumstances, figures obtained from the two-open compartment model are likely to be of little value until much more is known about the underlying physiological and metabolic events.

Information regarding the mechanism of absorption of flavonols are still not well understood. It has been postulated that the Na^+–glucose co-transport system may play a role in flavonol absorption.[25] This co-transport system is involved in the transport of glucose across the intestinal wall[26] and since the present study has provided unequivocal evidence for the absorption of the flavonol glucosides, the possible involvement of this method of transport merits investigation.

In conclusion, this study has shown that following the ingestion of lightly fried onions,

there is a proportionally higher accumulation of isorhamnetin-4′-glucoside than quercetin conjugates, including quercetin-3,4′-diglucoside and quercetin-4′-glucoside, in plasma and urine of humans. This is likely to be a consequence of either preferential absorption of isorhamnetin-4′-glucoside or, a post-absorption conversion of quercetin-4′-glucoside to isorhamnetin-4′-glucoside via 3′-O-methylation. Distinguishing between these processes and clarification of the mechanisms involved requires further detailed metabolic studies.

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