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Human Metabolism of Dietary Flavonoids: Identification of Plasma Metabolites of Quercetin

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The position of conjugation of the flavonoid quercetin dramatically affects biological activity *in vitro*, therefore it is important to determine the exact nature of the plasma metabolites. In the present study, we have used various methods (HPLC with diode array detection, LCMS, chemical and enzymic synthesis of authentic conjugates and specific enzymic hydrolysis) to show that quercetin glucosides are not present in plasma of human subjects 1.5 h after consumption of onions (a rich source of flavonoid glucosides). All four individuals had similar qualitative profiles of metabolites. The major circulating compounds in the plasma after 1.5 h are identified as quercetin-3-glucuronide, 3′-methyl-quercetin-3-glucuronide and quercetin-3′-sulfate. The existence of substitutions in the B and/or C ring of plasma quercetin metabolites suggests that these conjugates will each have very different biological activities.

Keywords: Flavonol glucoside; Metabolism; Conjugation; Glucuronidation; Sulfation; Human

Abbreviations: LPH, lactase phlorizin hydrolase; SGLT1, sodium-dependent glucose transporter

INTRODUCTION

Flavonoids are a group of polyphenolic compounds formed as secondary metabolites in higher plants.[1] Quercetin is the major flavonol (a sub-class of the flavonoids) in the plant kingdom and is found ubiquitously in the diet. Quercetin may be a powerful bioactive constituent of the human diet, both as a free radical scavenging agent[2,3] and through interaction with various endogenous proteins (e.g. as an inhibitor of enzyme activity).[4,5] However, the majority of evidence for the activity of quercetin

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is derived from *in vitro* experimentation using the aglycone as substrate, with very little information on the relative activity of conjugated forms. It has been shown that the nature of the conjugate and the position of attachment to the flavonol does affect the biological activity of the polyphenol *in vitro*[6–8] and hence it is vital to determine the form(s) of the circulating flavonol(s) in human plasma if the contribution to human health is to be fully understood.

Quercetin is found in plant food conjugated to various sugars. Cooking has no effect on the glycosides,[9,10] but lactase phlorizin hydrolase (LPH)[11] and the colonic bacterial β-glycosidases[12] will hydrolyse the glycosidic link, releasing the aglycone. Quercetin has not been detected in plasma using sensitive techniques[13–15] and so absorbed quercetin must be rapidly conjugated to glucuronide or sulfate groups.[14–17] An alternative hypothesis for absorption of flavonoid glycosides was postulated by Hollman *et al.*[18,19] The authors suggested that the sodium-dependent glucose transporter (SGLT1) may be responsible for the absorption of some flavonoid glucosides directly from the small intestine. Transport of flavonoid glucosides across the small intestine has not been shown conclusively, although an interaction of some quercetin glucosides with the transporters has been demonstrated.[20] Furthermore, quercetin-4′-glucoside was a substrate for SGLT1 in Caco-2 cells and when expressed in Chinese hamster ovary cells.[21] Although quercetin-4′-glucoside is a substrate for the broad-specificity cytosolic β-glucosidase present in human small intestine and liver, quercetin-3-glucoside is not[22,23] and, therefore, if quercetin-3-glucoside is transported across the intestine, it will circulate in plasma.

Some reports have suggested that flavonol glucosides circulate in plasma after consumption of flavonol-rich foods.[24–26] The evidence was based on comparison of retention times with commercially available standards, or flavonol glucoside standards obtained from the food source. In contrast, other research using similar methods suggests that flavonol glucosides are not present in plasma after consumption of flavonol-rich foods.[14,15,17,27] The aim of the present paper was to use more substantive analytical methods than used previously for identification of the conjugated species of quercetin in human plasma after consumption of quercetin glucoside-rich onions. Evidence for position of conjugation was obtained by reference to appropriate metabolic standards produced *in vitro*.[8] The identification of the major quercetin metabolites circulating in human plasma emphasizes the need for more relevant flavonoid conjugates (rather than the aglycone) to be assessed for potential biological activity.

MATERIALS AND METHODS

Materials

Quercetin, quercetin-3-glucoside, quercetin-4′-glucoside, 3′-and 4′-methylquercetin, quercetin-3-sulfate and apigenin were purchased from Extrasynthese (Genay, France). Quercetin-3,4′-diglucoside was purified from onion bulb tissue[20] and quercetin-3-glucuronide was purified from green bean tissue.[28] The identity of these compounds has previously been confirmed by MS and NMR. Qurecetin-7-glucuronide, quercetin-4′-glucuronide, quercetin-3′-glucuronide, 3′-methylquercetin-3-glucuronide, 3′-methylquercetin-7-glucuronide and 3′methylquercetin-4′-glucuronide were produced enzymically using human liver cell-free extracts and UDP-glucuronic acid, as described previously.[8] Quercetin di-glucuronide was produced enzymically using pig liver cell-free extract.[29] Quercetin-7-sulfate was synthesised chemically as described previously.[30] All flavonols were checked for purity by HPLC prior to use and were found to be >98% pure. Arylsulfatase from *Aerobacter aerogenes* was purchased from Fluka Chemicals (Gillingham, Dorset, UK; <0.1% β-glucuronidase activity)

and β-glucuronidase from *E. Coli* was purchased from Roche (Mannheim, Germany; <0.1% sulphatase activity). All other reagents were purchased from Sigma (Poole, UK) and were of analytical reagent grade or HPLC grade where applicable. Water was purified *via* a Millex Q-plus system (Millipore, Watford, UK).

Quercetin-3′-sulfate

Quercetin (1 g; 3 mmol) and sulphamic acid (0.58 g; 6 mmol) were heated in pyridine (5 ml; 80°C; 1 h) with continuous stirring. After cooling the reaction medium was diluted with 5% aqueous potassium acetate (100 ml; pH 7.2) and extracted with ethyl acetate. The precipitate from the ethyl acetate extract was isolated by filtration and purified by gel filtration on Sephadex® G-10 using a gradient of methanol in water as solvent, yielding 162 mg of quercetin-3′-sulfate. ^{1}H NMR (DMSO-d_6): 8.05 (1H, brs, H-2′), 7.79 (1H, brd, J = 8.5 Hz, H-6′), 6.04 (1H, d, J = 8.5 Hz, H-5′), 6.12 (brs, H-8) and 5.95 (1H, brs, H-6). ^{13}C NMR: see [31]. LSIMS (glycerol): 419.0 (M+K−H) and 381.0 (M^-).

Quantification of Flavonol Glycosides in Onions

Locally grown yellow onions (*Allium cepa*: cultivar-renate) were donated within 3 months of harvesting. The flavonol glycoside composition was assessed according to the method of Rhodes and Price;[32] briefly, onions (500 g) were sliced and immediately frozen in liquid nitrogen. The onion was then freeze dried, ground to a powder using a domestic food processor and stored at −20°C until analysed. Onion powder (2 g) was extracted three times with 70% methanol (50 ml) at 1200 rpm for 1 min (Pro400 homogeniser, Connecticut, USA) and filtered under reduced pressure through filter paper (Whatman number 541, Maidstone, UK). The filtrate was dried to approximately 20 ml under vacuum by rotary evaporation (30°C) and made up to 25 ml with methanol. An aliquot (1 ml) was diluted with 1 ml methanol, filtered (0.22 μm PTFE filter units; Chromos express, Macclesfield, UK) and subjected to HPLC analysis.

Subjects

Ethical approval for the study was obtained from the Norwich District Research Ethics Committee. Volunteers (2 male and 2 female) were recruited through local advertisement and all gave written informed consent to the study. Subjects were aged between 30 and 44 years and had a mean body mass index of 22.1 kg/m^2 (range: 18.8–24). Fasting blood samples were screened for biochemical parameters (e.g. glucose, leucocyte, platelet, erythrocyte counts) and all were considered normal. Subjects were not taking medication.

Study Design

Subjects were required to avoid flavonol-rich foods (i.e. most fruits and vegetables—except root vegetables; especially onion, broccoli, spinach, green beans, lettuce and tomatoes, and beverages such as red wine and tea) for two days prior to the study. On the third morning, after an overnight fast, a baseline level blood sample (20 ml) was taken from each subject. Ten medium sized onions (180–220 g) were fried in 150 g butter until soft, on the day of the study. Each subject was served a breakfast consisting of fried onions (200 g cooked weight), bread and coffee. The remaining onion was immediately frozen and stored at −20°C. A further blood sample (60 ml) was taken at 1.5 h post-ingestion of the onion breakfast.

Plasma was immediately prepared from all samples by centrifugation (800 g, 10 min, room temperature) in heparin-coated tubes (Sarstedt Ltd., Leicester, UK). The plasma was separated from the red blood cells and after addition of ascorbic acid (final concentration 1 mM) samples

were frozen on dry ice. Samples were stored at −20°C until analysed.

Extraction of Flavonols from Plasma

Baseline and 1.5 h plasma samples were treated in the same way. Apigenin (100 µl, 60 µM) was added to plasma (10 ml) as an internal standard. For all samples, plasma was acidified to pH 5 with acetic acid (0.65 mM; 0.1 vol.). Ascorbic acid was added to help stabilize the samples during processing (final concentration, 1 mM). Acetonitrile (2.5 vol.) was used to precipitate plasma proteins and extract flavonol metabolites for all plasma samples. The samples were vortexed for 30 s every 2 min over a 10 min period, before centrifuging the precipitate (13,600*g*, 4°C, 10 min). The supernatants were then taken to approximately 100 µl by rotary evaporation under vacuum (50°C). Samples were made up to 500 µl with water followed by a further 500 µl methanol. Samples were centrifuged (13,600*g*, 4°C, 2 min), and filtered directly into HPLC vials. Aliquots (30 µl) were injected on to the HPLC column for analysis. External standards of quercetin and quercetin-3-glucoside were analysed approximately every five runs.

Indirect Determination of Conjugated Metabolites

Combined plasma samples were taken to dryness by rotary evaporation and redissolved in buffer (1 ml, 0.1 M potassium phosphate, pH 7). A portion (200 µl) was incubated (37°C, 30 min) with (a) β-glucuronidase (5 U, 25 µl) and buffer (25 µl), (b) sulfatase (0.4 U, 25 µl) and buffer (25 µl), (c) β-glucuronidase and sulfatase (25 µl of each), or (d) buffer (50 µl). For β-glucuronidase one unit is 1 µmol of 4-nitrophenol-released from 4-nitrophenyl-β-D-glucuronide per min at pH 7, 37°C. For sulfatase one unit is 1 µmol of 4-nitrophenyl-sulfate hydrolyzed per min at pH 7.1, 37°C. Reactions were stopped by addition of methanol (200 µl) containing ascorbic acid (1 mM). Samples were centrifuged (13,600*g*, 4°C, 5 min), and then filtered for analysis by HPLC.

HPLC Analysis

A modified version of the analytical HPLC method from Price *et al.*[9] was used. Solvents A (water: tetrahydrofuran: trifluoroacetic acid, 98:2:0.1, v:v:v) and B (acetonitrile), were run at a flow rate of 1 ml/min, using a gradient of 17% B (2 min), increasing to 25% B (5 min), 35% B (8 min), 50% B (5 min) and then to 100% B (5 min). A column clean-up stage maintained B at 100% (5 min) followed by a re-equilibration at 17% B (15 min). The column was packed with Prodigy 5 µm ODS3 reversed-phase silica, 250 mm by 4.6 mm id (Phenomenex, Macclesfield, UK). Diode array detection was at 270 and 370 nm.

Liquid Chromatography–Mass Spectrometry (LC–MS)

Positive ion electrospray LC–MS measurements were performed using a Micromass Quattro II (Manchester, UK), equipped with a Z-spray source. Samples were introduced using a Hewlett Packard 1050 HPLC equipped with a diode array detector. Solvent gradient conditions were the same as those above. Eluent flow (1 ml/min) was split between the diode array detector and the mass spectrometer ion source in the approximate ratio 8:1. The Electrospray capillary voltage was set to 3.5 kV and the cone voltage to 28 V. Source block temperature was 140°C and desolvation temperature 350°C. Nitrogen was used as the drying and nebulizing gas at flows of 400 and 20 L/h respectively.

Selected ion monitoring was conducted on mass channels 303, 317, 383, 397, 465, 479, 493, 573 and 655 (maximum of four mass channels monitored per injection) with a scan window of 0.2, dwell times, of 0.1 s/channel and an interchannel delay of cycle time of 0.03 s.

Concurrent diode array spectra were scanned from 190 and 450 nm, with an interval of 2 nm. Instrument control, data acquisition and processing were performed using Micromass MassLynx™ version 3.4 data system and software.

RESULTS

The flavonol glucoside composition of the onion extract is shown in Fig. 1. Based on a 200 g cooked portion of onion, the subjects consumed: 37.1 mg quercetin-3,4′-diglucoside, 0.7 mg quercetin-3-glucoside, 39.5 mg quercetin-4′-glucoside, 1.8 mg 3′-methylquercetin-4′-glucoside and 0.1 mg quercetin aglycone, providing a total of 79.2 mg flavonol glucoside (expressed as quercetin aglycone equivalent).

Flavonols were not detected in any of the plasma samples taken before ingestion of onions. Figure 2 shows HPLC UV-chromatograms of extracted plasma from four individuals 1.5 h after onion consumption. The recovery of the internal standard, apigenin, was >90%. The plasma samples showed several new peaks (denoted P1–P12) that could correspond to quercetin metabolites. The qualitative profile of metabolites was similar for all subjects. Quercetin-3,4′-diglucoside, quercetin-3-glucoside, quercetin-4′-glucoside and quercetin were not detected in any of the plasma samples as indicated by arrows in Fig. 2 (limit of detection: 22, 19, 17 and 8 nM in plasma respectively).

Concentrated plasma samples were combined and treated with β-glucuronidase, aryl-sulfatase or a combination of both enzymes as shown in Fig. 3. After treatment with β-glucuronidase, peaks P1–P10 and P12 disappeared, peak P11 increased in area by 40%, and quercetin and 3′-methylquercetin were formed. After sulfatase treatment, peak P11 disappeared, with concomitant appearance of quercetin, 3′-methylquercetin was not observed, and there did not appear to be any significant changes in area of P1–P10 or P12. After treatment of the plasma sample with both enzymes all peaks disappeared with formation of quercetin and 3′-methylquercetin.

Table I shows the amount of quercetin or 3′-methylquercetin released after treatment of the combined plasma sample with each deconjugating enzyme. All the sulfate conjugates are present as unmethylated quercetin, whereas approximately 30% of the glucuronide conjugates are methylated. Only a further 11% quercetin was released after treatment with both deconjugating enzymes, which represents the mixed conjugate fraction within the plasma.

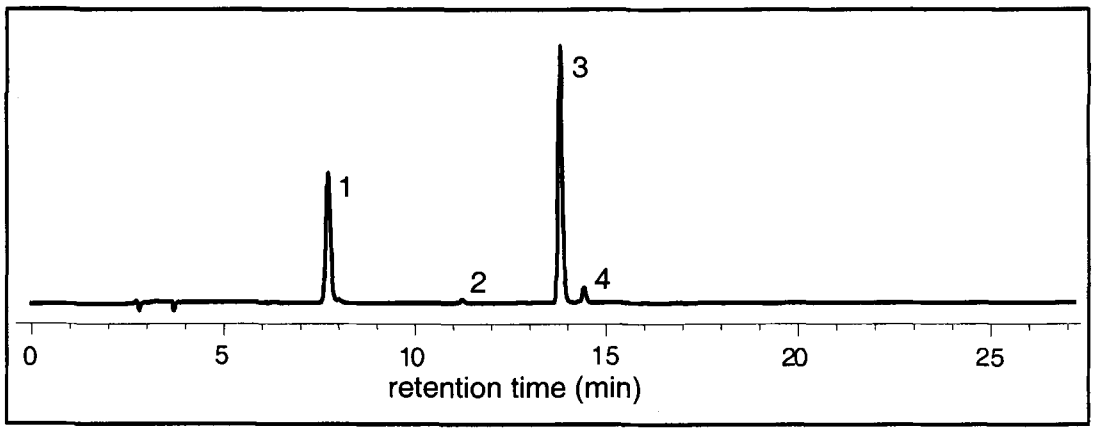

FIGURE 1 Chromatogram of quercetin glucosides from onion. 1, quercetin-3,4′-glucoside; 2, quercetin-3-glucoside; 3, quercetin-4′-glucoside; 4, 3′-methylquercetin-4′-glucoside. Absorbance was measured at 370 nm.

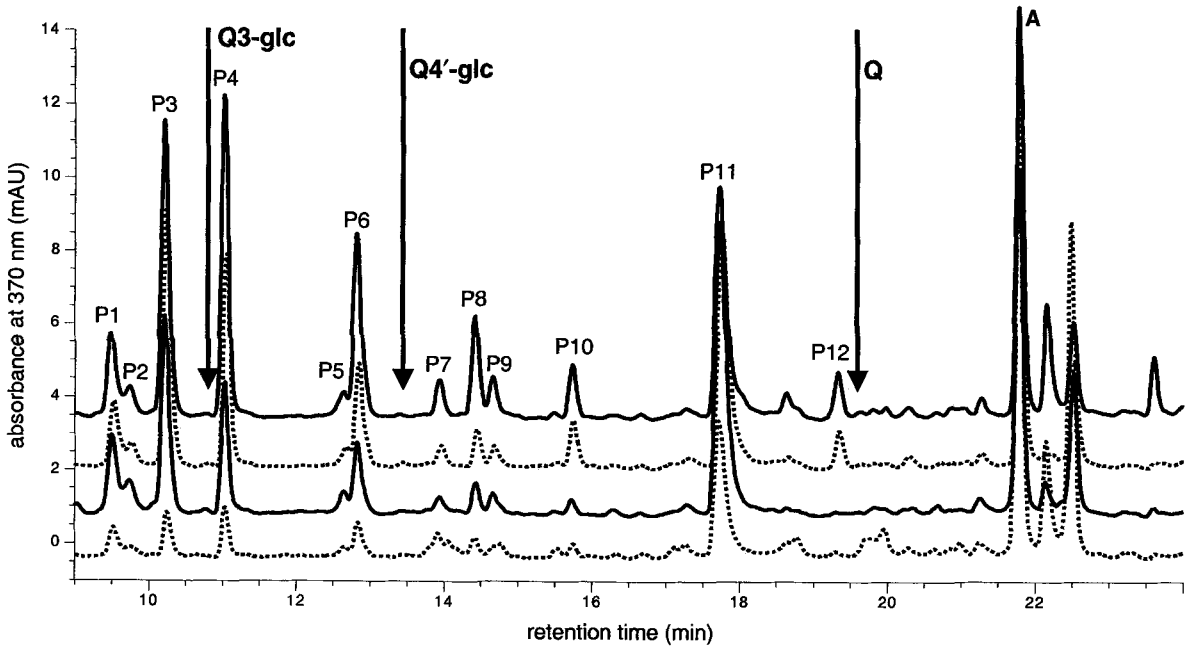

FIGURE 2 Plasma profiles of four subjects at 1.5 h after consumption of onion. P1–P12 are possible quercetin metabolites. A is apigenin as internal standard. Arrows indicate quercetin standards; Q is quercetin, Q3-glc is quercetin-3-glucoside, Q4′-glc is quercetin-4′-glucoside.

Overall a fifth of the absorbed quercetin was methylated and a third of all quercetin was present as sulfate conjugates.

P4 (Fig. 2) has a UV-absorption spectrum showing a Band I maximum at 356 nm, indicating conjugating of the flavonol at the 3-position (Fig. 4). Comparison of P4 with the external standards quercetin-3-glucoside and quercetin-3-glucuronide, showed that P4 eluted at the same retention time as quercetin-3-glucuronide. LC–MS analysis of P4 provided further confirmation of the glucuronide conjugate as the molecular ion $[M+H^+]$ of m/z 479 and fragment ion of the quercetin aglycone (m/z 303) were identified at the corresponding retention time. Selected ion monitoring for a molecular ion $[M+H^+]$ of m/z 465 confirmed the absence of quercetin-3-glucoside and quercetin-4′-glucoside in the plasma sample. P6 eluted under the HPLC conditions at the same retention time as 3′-methylquercetin-3-glucuronide. LC–MS analysis of P6 showed a molecular ion $[M+H^+]$ of m/z 493 and fragment ion of the 3′methylquercetin aglycone (m/z 317). Another major peak on the chromatogram P11, which responded to sulfatase treatment, had a molecular ion $[M+H^+]$ of m/z 383 and fragment ion of the quercetin aglycone (m/z 303) at the corresponding retention time. The UV-absorption spectrum showed a Band I maximum at 369 nm, and the peak co-eluted with quercetin-3′-sulfate standard.

Analysis by LC–MS indicated a group of peaks eluting at positions P1–P3 with $[M+H^+]$

TABLE I Release of quercetin and methylquercetin by deconjugating enzymes

Enzyme	Quercetin (μmol/l) in plasma*	Methylquercetin
Buffer	0	0
β-glucuronidase	0.26	0.11
Sulfatase	0.16	0
β-glucuronidase+sulfatase	0.45	0.12

*Plasma from four subjects combined to give an average concentration.

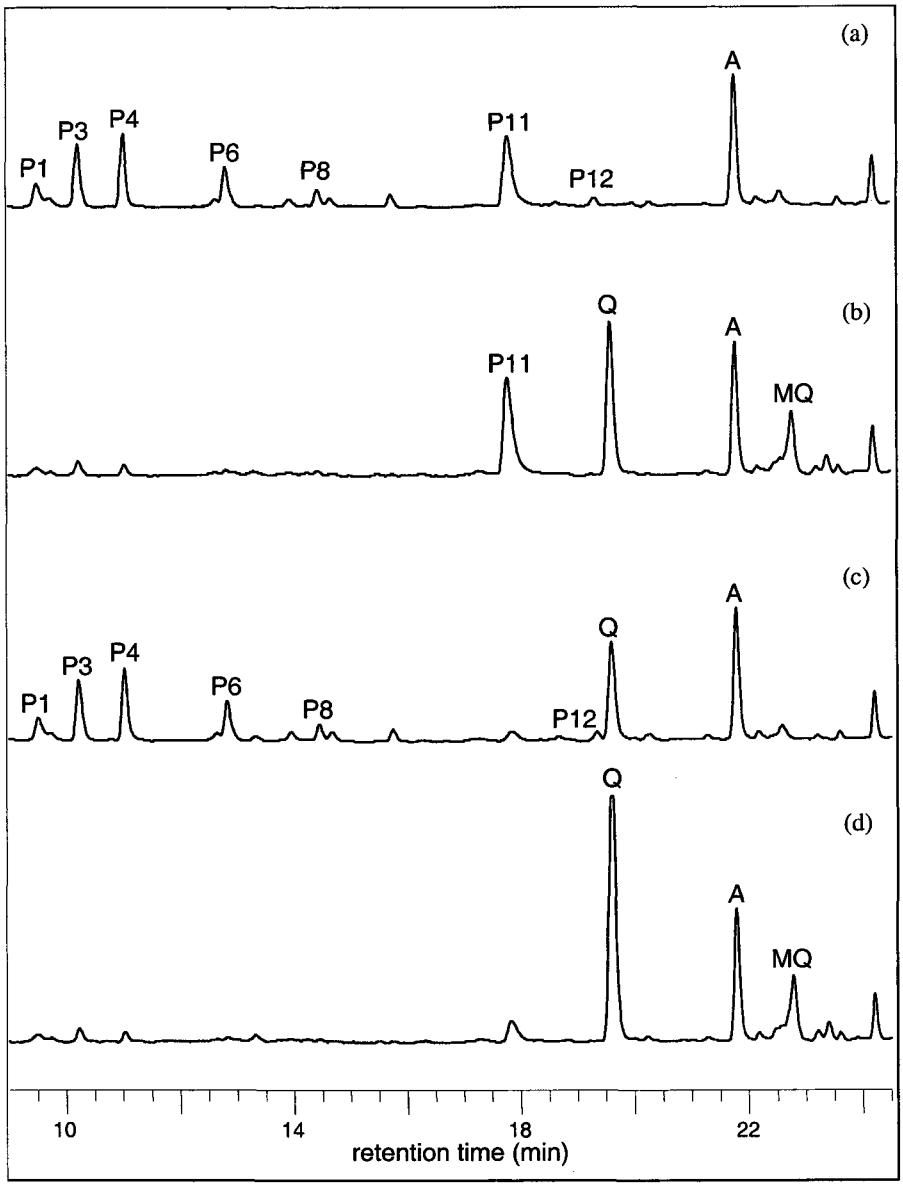

FIGURE 3 Effect of deconjugating enzymes on the quercetin metabolite profile. Combined human plasma samples were incubated for 30 min, 37°C in potassium phosphate buffer (pH 6) with (a) buffer only, (b) β-glucuronidase (5 U), (c) sulfatase (0.25 U) or (d) β-glucuronidase (5 U) and sulfatase (0.25 U). For β-glucuronidase, one unit is defined as 1 μmol of 4-nitrophenol released from 4-nitrophenyl-β-D-glucuronide per min at 37°C, pH 7. For sulfatase, one unit is 1 μmol of *p*-nitophenyl sulfate hydrolyzed per min at pH 7.1 at 37°C.

ions of m/z 655 and 573, which could correspond to quercetin di-glucuronides and mixed sulfo-glucuronide conjugates of quercetin. Quercetin di-glucuronide elutes at the same retention time as P3, but further identification of the positions of conjugation is required. LC–MS data could not be obtained for P5, P7, P8, P9, P10 and P12, however, all responded to β-glucuronidase treatment. Furthermore, P7, P8 and P9 elute at the same retention times as quercetin-4′-

TABLE II Summary of identification parameters for quercetin metabolites (n/a, not available)

Peak*	Abs. max.† (nm)	Reaction to (G) or (S)	LC–MS (SIM-M+H$^+$)	Co-elution with standard
P1–P3	n/a	(G)	weak mixture of 573, 655	(P3) Quercetin di-glucuronide
P4	355	(G)	479, 303	Quercetin-3-glucuronide
P5	n/a	(G)	n/a	–
P6	355	(G)	493, 317	3′-methylquercetin-3-glucuronide
P7	n/a	(G)	n/a	Quercetin-4′-glucuronide and 3′-methylquercetin-4′-glucoside
P8	n/a	(G)	n/a	Quercetin-3′-glucuronide
P9	n/a	(G)	n/a	3′-methylquercetin-4′-glucuronide
P10	n/a	(G)	n/a	–
P11	369	(S)	383, 303	Quercetin-3′-sulfate
P12	n/a	(G)	n/a	–

*Peak as shown in Fig. 2.
†Absorbance maximum of Band I. (G) is β-glucuronidase, (S) is sulfatase.

glucuronide, quercetin-3′-glucuronide and 3′-methylquercetin-4′-glucuronide respectively. Quercetin-4′-glucuronide and 3′-methylquercetin-4′-glucoside, however, have similar elution times and the same molecular ion (m/z 479) therefore more evidence would be required to identify the P7 metabolite. Table II summarises the evidence for identification of the quercetin metabolites in human plasma.

DISCUSSION

Quercetin, derived from quercetin glycosides, is absorbed from food but the nature of the circulating species has been a matter of much debate. Several investigators have shown the presence of glucuronide or sulfate conjugated metabolites,[13–17] with evidence for the conjugating moiety provided chiefly by enzymic hydrolysis. The enzyme preparations used contain additional residual activity and hence and β-glucoside conjugate present in the plasma may be hydrolysed by the β-glucuronidase preparation, particularly if a high concentration of enzyme is incubated for long periods. Erland *et al.*[13] assessed various enzyme preparations and suggested that if glucosides were present in the plasma of individuals after consumption of a flavonol-rich diet, then they would account for less than 17% of the total conjugated forms.

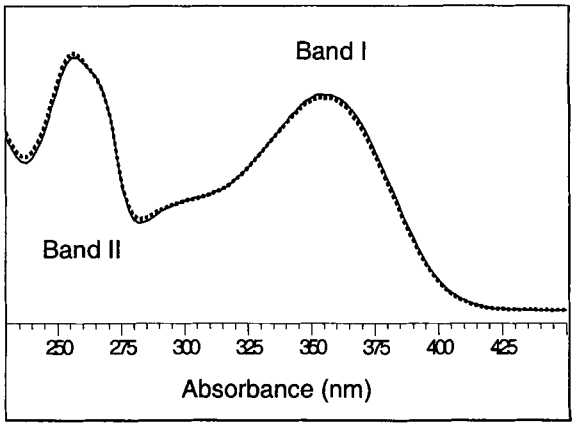

FIGURE 4 UV-absorption spectra of P4 (—) and quercetin-3-glucuronide(- - -).

Some researchers have reported the presence of flavonol glycosides in plasma. Paganga and Rice-Evans[24] identified rutin (quercetin-3-rhamnoglucoside) and phlorizin (phloretin-2′-glucoside) in plasma along with several other unidentified flavonoid glycosides. The evidence was based on UV-absorption spectra and retention times of commercially available standards. Aziz *et al.*[25] identified quercetin-4′-glucoside and 3′-methylquercetin-4′-glucoside in the plasma of individuals after consumption of onions. The evidence was based on co-elution with flavonol glucoside standards, isolated from onion, and detection by fluorescence (which requires the presence of a free 3-hydroxyl group). Boyle *et al.*[26] identified quercetin-3-glucoside

and 3′-methylquercetin-4′-glucoside in plasma again after onion consumption. Identification was based only on the retention time of flavonol glucoside standards.

In contrast, Moon *et al.*[14] could not detect quercetin glucosides in plasma after subjects consumed an onion meal using quercetin glucosides as standards (detection limit <20 nM). Walle *et al.*[27] also could not detect quercetin glucosides in the plasma of ileostomy subjects after onion consumption. Furthermore the authors detected only quercetin aglycone in the ileostomy effluent suggesting hydrolysis of quercetin glucosides prior to absorption across the intestine. Wittig *et al.*[17] could not detect quercetin aglycone or the native quercetin glucosides in plasma after three volunteers consumed a large quantity of stewed onion using highly selective tandom LC–MS/MS analysis. Likewise, Graefe *et al.*[15] did not detect quercetin aglycone or quercetin glucosides in the plasma or urine of 12 subjects consuming stewed onions or quercetin-4′-glucoside as a supplement, using coularray as a sensitive detection method.

The major limitation in many of these studies is the range of standards used. The dietary compounds ingested by individuals should be monitored, but as metabolism will occur other types of conjugates need to be assessed. Glucuronide and glucoside conjugates behave similarly during chromatography at pH values below the pK of the glucoronide carboxyl. Also the nature of the conjugate does not affect the UV-absorption spectrum, although information on position of conjugation can be obtained.[33] Hence chromatographic conditions need to be modified to give adequate separation of both types of conjugate before identification can proceed.

We have shown by using complementary methods of analysis that the major quercetin glucosides present in onion are not present in plasma 1.5 h after consumption of an onion meal. Furthermore we have provided evidence, based on co-elution of metabolite standards, enzyme hydrolysis and selected ion monitoring LC–MS, for the identity of several of the major metabolites present in human plasma: quercetin-3-glucuronide, 3′-methylquercetin-3-glucuronide, and quercetin-3′-sulfate. In the study of Aziz *et al.*,[25] quercetin-3-glucuronide and 3′-methylquercetin-3-glucuronide would not have been observed since detection was by fluorescence, which involves chelating the 3-hydroxyl group with aluminium.[34] Boyle *et al.*[26] did not confirm the presence of quercetin-3-glucoside or 3′-methylquercetin-4′-glucoside in their onion meal. These flavonol glucosides are minor components of onion and their contribution in the plant tissue can vary.[35] Furthermore, quercetin-3-glucuronide elutes only 0.3 min after quercetin-3-glucoside under the HPLC conditions used in our study and has an identical UV-absorption spectrum. Hence it is probable that Boyle *et al.*[26] observed the glucuronide conjugate rather than the glucoside in their study.

We were not able to distinguish between quercetin-4′-glucuronide and 3′-methylquercetin-4′-glucoside and so the identity of P7 cannot be confirmed. However, 3′-methylquercetin-4′-glucoside is likely to be a substrate for both LPH and the cytosolic β-glucosidase based on ready hydrolysis of quercetin-4′-glucoside by these enzymes.[11,22] Quercetin-4′-glucuronide is also readily formed by UDP-glucuronosyltransferase activity of cell-free liver extracts.[8]

The four individuals involved in our study showed remarkable qualitative consistency in their metabolic profiles. The average concentration in the plasma (combined sample) was 0.5 μM for quercetin and 0.1 μM for 3′-methylquercetin. These values are comparable to published quercetin concentrations after similar onion consumption (0.6 μM;[36] 0.74 μM[37]) or a flavonol-rich meal (0.6 μM).[16] Graefe *et al.*[15] also found identical metabolic profiles in subjects after consumption of four different quercetin glucoside sources (onion, buckwheat tea, quer-

cetin-4′-glucoside and quercetin-3-rhamnoglucoside supplements), suggesting that the same metabolic pathway was followed regardless of the administered form of quercetin glucosides. Interestingly, quercetin-7-glucuronide was not observed in the human plasma samples of our study. This conjugate was a major metabolite after passage of quercetin-3-glucoside or quercetin across the rat small intestine,[38] but quercetin-3-glucuronide has recently been identified in rat plasma.[39] The apparent difference between intestinal metabolism and metabolites circulating in plasma may be due to further metabolism in the liver (e.g. glucuronidase activity) after intestinal absorption.[40]

Previously we have shown that quercetin-3-glucuronide does not retain the ability of quercetin to inhibit xanthine oxidase or lipoxygenase *in vitro*.[8] We have now shown by analysis of human plasma 1.5 h after consumption of fried onions that quercetin-3-glucuronide and 3′-methylquercetin-3-glucuronide are two of the predominant circulating metabolites. Hence it would be unlikely for these compounds to exert such biological activity *in vivo*. However, quercetin-3-glucuronide retains free radical scavenging activity.[39] Quercetin-3′-sulfate was also a major metabolite, accounting for an average of a third of all quercetin in the plasma. Although the biological activity of quercetin-3′-sulfate has not been assessed, it might behave in a similar way to quercetin-3′-glucuronide, i.e. a potent inhibitor of xanthine oxidase, at a concentration that can be achieved after flavonol-rich foods are consumed.

In conclusion, the major circulating compounds identified in human plasma after consumption of onions were quercetin-3-glucuronide, 3′-methylquercetin-3-glucuronide and quercetin-3′-sulfate. Quercetin di-glucuronides and mixed sulfoglucuronide conjugates were also present along with other possible quercetin glucuronide conjugates. Some of these compounds are likely to retain the biological activity of the aglycone. Quercetin-3,4′-diglucoside, quercetin-3-glucoside and quercetin-4′-glucoside were not present in human plasma. Undoubtedly quercetin circulates in plasma predominantly as conjugated metabolites and it is these more complex compounds that need to be the focus of future studies on the biological activity of flavonols in humans.

Acknowledgements

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