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Comparison of the urinary excretion of quercetin glycosides from red onion and aglycone from dietary supplements in healthy subjects: a randomized, single-blinded, cross-over study

Yuanlu Shi[1], Gary Williamson[1]

[1] School of Food Science and Nutrition, University of Leeds, Leeds LS2 9JT, UK

Key words: quercetin, bioavailability, dietary supplement, human

Abbreviations: SEM, standard error of mean

*** Corresponding Author:** Gary Williamson, Tel: +44(0)113-343-8380; Email: g.williamson@leeds.ac.uk

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ABSTRACT

Some intervention studies have shown that quercetin supplementation can regulate certain biomarkers, but it is not clear how the doses given relate to dietary quercetin (e.g. from onion). We conducted a two-period, two-sequence crossover study to compare the bioavailability of quercetin when administered in the form of fresh red onion meal (naturally glycosylated quercetin) or dietary supplement (aglycone quercetin) under fasting conditions. Six healthy, non-smoking, adult males with BMI 22.7 ± 4.0 kg m^{-2} and age 35.3 ± 12.3 y were grouped to take the two study meals in random order. In each of the 2 study periods, one serving of onion soup (made from 100 g fresh red onion, providing 156.3 ± 3.4 µmol (47 mg) quercetin) or a single dose of a quercetin dihydrate tablet (1800 ± 150 µmol (544 mg) of quercetin) were administered following 3 d washout. Urine samples were collected up to 24 h, and after enzyme deconjugation, quercetin was quantified by LC-MS. The 24-h urinary excretion of quercetin (1.69 ± 0.79 µmol) from red onion in soup was not significantly different to that (1.17 ± 0.44 µmol) for the quercetin supplement tablet ($P = 0.065$, paired t-test). This means that, in practice, 166 mg of quercetin supplement would be comparable to about 10 mg of quercetin aglycone equivalents from onion. These data allow intervention studies on quercetin giving either food or supplements to be more effectively compared.

INTRODUCTION

Quercetin is a flavonoid (class: flavonol) that is present at high levels in onions, apples and tea, in the form of a 3-O-glucoside, 4'-O-glucoside or 3,4'-O-diglucoside. Intervention studies using those foods to examine long term effects are rare, not only because of the extensive food preparation required with consistent composition, but also that volunteers grow tired of the same food for months which limits compliance.

Many studies using quercetin supplements (aglycone) in humans indicate effects on antioxidant status, oxidized LDL, inflammation and metabolism (summarised in Table 1, supplementary information). 500 mg quercetin supplementation twice per day improved the NIH (National Institution of Health) prostatitis symptom score after 30 d in 30 men with chronic pelvic pain syndrome [1] and improved cystitis symptoms after 28 d in 22 interstitial cystitis patients [2]. 150 mg of quercetin significantly affected expression of key genes, glycolipid catabolism, cell proliferation and apoptosis after 42 d intake in 20 subjects with a cardiovascular risk phenotype [3], and decreased systolic blood pressure, serum HDL-cholesterol, and plasma concentrations of atherogenic oxidised LDL in 96 healthy subjects [4]. Daily consumption of 100 mg quercetin for 70 d reduced serum total and LDL/HDL cholesterol, glucose and systolic and diastolic blood pressure in 49 health subjects [5]. 14 d of daily dose of 30 mg quercetin improved the oxidative resistance of LDL [6] and significantly decreased tissue inhibitor of metallopeptidase-1 (TIMP-1) in plasma and lymphocyte mRNA [7] in healthy subjects.

Whether dietary quercetin could achieve the same effects remains unknown since the bioavailability of quercetin aglycone in supplements is much lower than quercetin glucoside [8] and this makes interpretation and comparison of studies using supplements or foods difficult. This randomized, single-blind, two period, two sequence, cross-over intervention study, conducted under fasting conditions with a 3 d washout period, compared different dosages of quercetin from dietary supplements (aglycone) and fresh red onion (naturally conjugated as glucosides). This comparison allows calculation of the dosage of different quercetin sources needed to achieve similar effective absorption in healthy subjects to aid in the design of meaningful intervention studies.

SUBJECTS AND METHODS

Chemicals and enzymes

Absolute methanol, ethanol, acetonitrile (LC-MS grade) and ethyl acetate were from VWR international, France; ascorbic acid was from MP Biomedicals, LLC, France; formic acid, sodium acetate trihydrate, acetic acid, hydrochloric acid, β-glucuronidase from Helix pomatia, and sulfatase from Helix pomatia, were purchased from Sigma-Aldrich, USA. Standards of quercetin dihydrate, quercetin 4'-O-glucoside (spiraeoside), quercetin 3,4'-O-diglucoside, isorhamnetin (3-O-methylquercetin), tamarixetin (4'-O-methyquercetin), daidzein and taxifolin, are all HPLC grade and were purchased from Extrasynthese, France.

Subjects

Six healthy male volunteers participated in the present study. They were non-smokers, not on any medication, aged 35.3 ± 12.3 y (range 20.0 - 48.9) and had a BMI of 22.7 ± 4.0 kg m^{-2} (range 18.5 - 29.9). Exclusion criteria were metabolic and endocrine diseases, malabsorption syndromes, alcohol abuse, use of dietary supplements or any form of regular medication. All subjects were asked to maintain their normal lifestyle and usual extent of physical activities throughout the study. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the MaPS and Engineering joint Faculty Research Ethics Committee (MEEC 12-019), University of Leeds, UK. Written informed consent was obtained from all subjects.

Study design

The study was conducted with a single-blinded (researcher blind), diet-controlled, cross-over design. Subjects were required to avoid flavonols in the diet for 3 d washout prior to the breakfast and for 1 d during 24-h urine collection. For this purpose, a list of food items rich in flavonols was given to each participant as a guideline. This diet excluded vegetables like onion, spring onion, shallots, leeks, chives, spinach, kale, endive, lettuce, broccoli, asparagus, tomato, olive, pepper, courgette, green beans, broad bean, common bean and galangal; all types of berries and currants, apple, apricot, grape and plum; all types of alcoholic beverages and tea; and propolis supplements. On the morning of the study, baseline urine was collected immediately before breakfast and 24-h urine was collected following the

breakfast. The six participants were randomly assigned to treatment group A or B (n = 5 and 1). Group A ingested one quercetin supplement (1800 ± 150 µmol quercetin equivalents) with a standard breakfast; after another 3 d washout, they ingested onion-enriched soup (156.3 ± 3.4 µmol quercetin equivalents). Group B had treatments in reverse order to Group A. The baseline urine was used as compliance control and no apparent deviation from the low-quercetin diet was observed. Accordingly, the concentrations of quercetin were very low (0.095 ± 0.037 µM, SEM) in baseline urine.

Preparation of standard breakfasts

Red Onion Soup Fresh local red onions were washed, skinned and sliced after removing the top and bottom of the bulb. The slices were frozen at -20℃ for 1 h and quickly minced with a kitchen electronic blender while still frozen. 100 g of the onion mince was stored individually at -20℃ until the day of the human study. A breakfast was freshly made consisting of one portion of instant tomato soup mix 52 g (Slim a Soup, Batchelorsrange, UK) and 100 g of frozen onion by adding hot water and stirring into a soup-paste after heating in a 800 W microwave for 1 min. The standard meal was served with buttered white bread. The soup powder did not contain any quercetin.

Supplement Quercetin dihydrate tablets (500 mg stated, actual measured 544 mg (see Results)) were Purchased from Nature's Best (Kent, UK) without further processing. One tablet was consumed with buttered white bread and instant tomato soup as above.

HPLC Quantification of quercetin in study food

The quercetin content of the red onion soup and of the supplement tablet was determined by HPLC-diode-array analysis. To 5 g of frozen red onion, 5 ml of absolute methanol was added and to 0.4 g soup powder, 5 ml of 70% methanol was added. Extraction was performed using ultra sonication and vortex. The samples were centrifuged (3000 g, 4℃, 10 min) and the supernatant was collected. The extraction was repeated twice with 5 ml of 70% aqueous methanol (containing 0.1 mM ascorbic acid, pH 5.08). 1 ml of the combined extracts was fully dried in a centrifugal evaporator (Genevac Ltd, Ipswich, UK), and then reconstituted with 1 ml of 50% aqueous ethanol containing 100 µM daidzein as internal standard. Before HPLC analysis, the samples were filtered through polytetrafluoroethylene (PTFE) membrane syringe filter (pore size of 0.2 µm). Extraction was performed in duplicate for each food sample.

The reconstituted samples were analyzed on an Agilent HPLC 1200 instrument (Agilent Technologies, Waldbronn, Germany) equipped with C18 column (ZORBAX Eclipse XDB-C18, 4.6×50 mm, 1.8 µm particle size, rapid resolution high throughput, 600 bar column, Agilent, USA) and a pre-column (Eclipse XDB-C18, 4.6×12.5 mm, 5 µm, analytical guard cartridge, Agilent, USA).

A modified version of the analytical HPLC method from [9] and [10], was used. Solvents A (water with 0.1% v/v of formic acid) and B (acetonitrile with 0.1% v/v of formic acid) were run at a flow rate of 0.5 ml min^{-1}. The chromatographic conditions of elution were as follows: 0 - 2 min, 15% solvent B; 2 - 22 min, increase solvent B from 15% to 40%; 22 - 24 min, isocratic for 2 min. A post-run column clean up procedure was applied by increasing B to 90% in 1 min, isocratic for 3 min and finally rapidly returning to initial conditions with re-equilibration at 29 min for 5 min of 15% B. Each sample (10 µl) was injected and analyzed twice. A column clean-up stage maintained B at 90% (30 min) which was followed by a re-equilibration at 15% B (30 min) to initiate each new batch of analysis. Diode array detection monitored the eluent at 255 nm and 370 nm. A standard curve ranging from 15.6 to 1000 pmol quercetin equivalents was produced using standard solutions of quercetin 3,4'-O-diglucosides (AUC_{370nm} of 0.736/pmol), quercetin 4'-O-glucoside (AUC_{370nm} of 1.49/pmol), daidzein (AUC_{255nm} of 1.68 ± 0.01/pmol), and quercetin (AUC_{370nm} of 1.26/pmol), with retention times of 3.20, 9.44, 12.6 and 14.3 min, respectively. HPLC chromatograms of standard mix, supplement extract and red onion extract are shown in Figure 1.

After HPLC analysis to confirm that the supplement contained pure quercetin (Figure 1), the quantification was performed by spectrophotometry using the extinction coefficient (ε) at λ_{max}(quercetin)/nm 257 (ε/mM^{-1} cm^{-1}, 19.95) and 376 (21.88) against 95% aqueous ethanol [11]. In brief, 5 tablets were finely ground in an electric coffee grinder and about 2 mg of the powder was accurately weighed and fully dissolved in 95% ethanol. Absorbance spectra were compared with quercetin standards prepared in 95% ethanol.

Processing of urine samples and analysis of quercetin in urine

24-h urine was collected into a 3 L sterile urine storage container with 3 g of ascorbic acid added. Once the sample arrived at the laboratory, the weight was measured and two 45 ml aliquots were taken into 50 ml falcon tubes, then centrifuged at 2000 g at 4℃ for 10 min. The supernatant was stored at -20℃ until analysis.

Enzyme hydrolysis of quercetin conjugates and liquid phase extraction

Metabolites of methyl-, glucuronyl-, glucosyl- and sulfo-conjugates of quercetin in human urine were hydrolysed to quercetin and the monomethylated derivatives isorhamnetin (3-O-methylquercetin) and tamarixetin (4'-O-methylquercetin) using β-glucuronidase and sulfatase [12]. To 200 µl of urine, 20 µl of 0.2 M sodium acetate - acetic acid buffer, pH 5.0 containing 200 units β-glucuronidase and 5 units of sulfatase were added; 2 µl of 100 µM taxifolin was added as internal standard, then incubated in a shaking water bath at 37℃, 100 rpm for 1 h. The completion of hydrolysis of all quercetin conjugates was assured by parallel experiments running from 1 h every 0.5 h up to 3 h. Results showed that hydrolysis was complete within 1 h as evidenced by the concentration of quercetin aglycone and isorhamnetin reaching a plateau. The pH of the hydrolysis mixture was adjusted to 2.0 by addition of 30 µl of 0.1 M HCl. To the hydrolysis mixture (about 250 µl), 500 µl of ice-cold ethyl acetate was added, mixed vigorously by vortex for 2 min, followed by standing on ice for 2 min and centrifugation at room temperature at 17,000 g for 2 min. The procedure was repeated twice and 3 supernatants pooled. Extracts were fully dried by nitrogen gas, then reconstituted with 150 µl of 50% ethanol and filtered through 0.2 µm PTFE filters before analysis. An enzyme unit was defined at 37℃ at pH 5.0 according to the manufacturer: one unit of β-glucuronidase liberates 1.0 µg of phenolphthalein from phenolphthalein glucuronide per h; one unit of sulfatase hydrolyzes 1.0 µmol 4-nitrocatechol sulfate per h. Extraction was performed in duplicate for each biological sample.

HPLC-ESI/MS

Analysis of urine concentrations of quercetin and of the monomethylated derivatives: isorhamnetin (3-O-methylquercetin) and tamarixetin (4'-O-methylquercetin) was performed by HPLC with mass spectrometry using a Shimadzu LC-2010C HT with single ion monitoring (Shimadzu, Tokyo, Japan) operated in negative electrospray ionization (-ESI) mode. Nitrogen was used both as drying and nebulizing gas at a flow rate of 15.0 L h^{-1} and 1.5 L h^{-1}. The DL temperature was maintained at 250℃ with detector voltage set at 1.80 kV and interface voltage at -3.5 kV. The standard curve was 0.05 - 2.00 µmol, within-run variance was 6.8 ± 5.6% and between-run variance was 14.5 ± 8.2%. The recovery of quercetin extraction from urine was calculated using the yield of taxifolin (internal standard, 111 ± 14.3%, n = 92). All chromatograms in the same batch were processed automatically by software (Labsolutions, ver. 5, Shimadzu, Tokyo, Japan) using the same processing parameters, such as

integration, peak-to-peak amplitude, and peak detection. Manual integration was performed only rarely when necessary.

Figure 2 shows a typical LC-MS Chromatogram of quercetin and conjugates after enzymatic hydrolysis of urine. The retention times of quercetin (m/z 301), isorhamnetin (m/z 315), tamarixetin (m/z 315) and taxifolin (m/z 303) are 16.1 min, 20.4 min, 20.6 min and 8.8 min, respectively.

Statistical analysis

All statistical analyses were performed using the SPSS statistics software (version 21; International Business Machines Corp., New York, USA). Normality of data distribution was checked with the Shapiro-Wilk test and data are normally distributed; independent samples t test was used to compare means between treatments. All calculations were carried out with CI 95%, and differences were considered significant at $P < 0.05$. Unless otherwise indicated, the results were reported as mean values with their standard deviations.

RESULTS

Control variables and intervention compliance

The baseline urine was used as compliance control and no deviation from the low-quercetin diet was observed. Accordingly, the concentration of quercetin was very low 0.095 ± 0.037 µM (SEM) in baseline urine.

Quercetin content of the study meals

Based on individual analysis of compounds, red onion soup contained 156.3 ± 3.4 µmol quercetin equivalents per portion made from 100 g fresh red onion (quercetin 3, 4'-O-diglucoside 59.3% and quercetin 4'-O-glucoside 40.7%, molar equivalents). Quercetin dihydrate tablets contained 1800 ± 150 µmol of quercetin (100% quercetin aglycone).

Urinary excretion of quercetin

The 24-h urinary excretion of quercetin for each individual after consuming a meal of 100 g red onion or a single study tablet is shown in Figure 3.

24-h urinary excretion of quercetin after consuming red onion soup, made from 100 g fresh red onion, was 1.69 ± 0.79 µmol (of which 72.9 ± 6.0% of quercetin, 7.70 ± 5.92% of isorhamnetin and 19.4 ± 5.95% of tamarixetin), and that from the 500 mg quercetin supplement was 1.17 ± 0.44 µmol (71.4 ± 11.1%, 7.54 ± 6.38% and 21.0 ± 11.7%). No significant difference in quercetin excretion was observed within subject (P = 0.065, paired t test) or among groups (P = 0.189, independent t test, n = 6) for the total quercetin.

DISCUSSION

The aim of the present randomized, single-blind, two-period, two-sequence, cross-over intervention study, conducted under fasting conditions with a 3 d washout period, was to compare the absorption of quercetin from fresh red onion (156.3 ± 3.4 µmol, naturally conjugated) and dietary supplements (1800 ± 150 µmol, aglycone) in healthy subjects. This resulted in similar amounts of quercetin being absorbed as assessed by quantifying 24-h urinary excretion of quercetin.

Quercetin supplementation dose-dependently increases plasma quercetin concentrations in healthy humans [13], and incorporation of the washout period was designed to diminish the impact of carryover effects. According to other reports, the plasma concentrations after quercetin-4'-O-glucoside supplementation (equivalent to 100 mg quercetin) reached a peak after 0.7 ± 0.3 h and the apparent elimination half-life was about 11 h [14]. Quercetin accumulated in plasma after repeated intake of onion (elimination half-life of 28 h), apples (elimination half-life of 23 h) and tea [8], but a steady state concentration in plasma was reached after about 4 d [15] and so plasma concentrations would reflect the intake of only the previous 3 d. For this reason, the length of the washout period was designed to be 3 d.

24-h urinary excretion of quercetin after consumption of red onion (mainly glucoside conjugated quercetin) and supplement (quercetin aglycone) was significantly different when compared by percentage dose (P < 0.0001, paired t test, 1.08 ± 0.51% and 0.065 ± 0.024%). These values are consistent with other human studies. For example, 24-h urinary excretion of quercetin as a proportion of intake after consumption of conjugated quercetin from fried onion was 0.8 ± 0.4% [16] and 1.1 ± 0.5% [17]. 13-h urinary excretion of quercetin as a proportion of intake from onion was 0.31 ± 0.14% and that from 100 mg quercetin aglycone was 0.12 ± 0.08% [18]. A systematic review confirmed that the

correlation between the dose of quercetin ingested and its recovery in 24-h urine samples in humans is on average 0.43% but with recovery ranging from 0.07 to 8.4% with this range at least partially due to the nature of the sugar conjugated to quercetin [19]. It should be noted that the amount in urine reflects the minimum amount of quercetin absorbed, and other experiments such as intestinal perfusion show that the actual amount absorbed is considerably higher [20]. Nevertheless, the amount in urine is a suitable biomarker for some polyphenols since it allows comparisons between different foods or supplements, and between individuals for the same compound [8, 21]. The low amount of compounds such as quercetin in the urine means that the remainder of the dose is either excreted in the bile, in the faeces or may end up as chemically-altered microbial metabolites, which can then be absorbed in the colon [22]. Typical microbial metabolites of quercetin are 3,4-dihydroxyphenylacetic acid, 3,4-dihydroxybenzoic acid and 3-hydroxyphenylacetic acid [23]. After absorption, these compounds participate in metabolism and so may ultimately contribute to the physiological effects of quercetin [24]. Even though the amount of intact quercetin in urine after these dosages of supplementation and onion intake were similar, it is likely that the supplement will deliver higher concentrations of microbial metabolites to the blood.

Supplements have consistent quality and a relatively long shelf life, and are preferred in many intervention studies since they remove the complication of the activity of other components in the food, and are well tolerated long-term by volunteers. However, it is important to know the “equivalence” of quercetin-containing foods and supplements, to allow for future design and to compare existing studies. According to the result of this study in practical terms, 100 g of onion gives a comparable amount of quercetin in the urine to a 500 mg quercetin aglycone supplement. Based on this data, we can compare reported intervention studies on quercetin from onions and from supplements (Table 2, supplementary information), which lists the human intervention studies using dietary sources of quercetin. The obvious difference between the dose ranges between Table 1 and Table 2 (supplementary information) may explain, for example, why plasma LDL/HDL reduction after 14 d administration was observed by Kim et al. [25] but not by Egert et al. [13] or Chopra et al. [6]. This pilot study provides a guideline for design of future human studies when using supplements and foods, and also facilitates comparison of studies in existing literature.

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Conflict of interest: None

Authorship: YS planned and performed experiments; GW initiated and planned the work. Both of the authors contributed equally to the writing of this manuscript and share responsibility for the final content. Both authors have read and approved the final manuscript.

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Supplementary information: Table 1 Human intervention studies on quercetin supplementation[1]

Dose per day[2]	Days	No. of subjects per group[3]	Biomarkers significantly affected	Biomarkers not significantly affected	Ref
500 mg x 2	30	30 men with chronic pelvic pain syndrome	Improvement in NIH prostatitis symptom score		1
500 mg x 2	28	22 interstitial cystitis patients	Improvement in cystitis symptoms	No side effects or adverse reactions	2
250 mg x 4	21	63		Blood antioxidant capacity or plasma lipid during ultramarathon	26
150 mg	14	12		Serum uric acid, plasma α- and γ - tocopherols, oxidized LDL, tumour necrosis factor-α, serum lipids and lipoproteins, plasma antioxidant capacity, body composition, or resting energy expenditure supplementation	13
150 mg	42	20 with cardiovascular risk phenotype	Gene expression of C1GALT1, O-glycan biosynthesis; GM2A, glycolipid catabolism; HDGF, cell proliferation; SERPINB9, apoptosis	Gene expression of the other target genes	3
150 mg	42	96	Decrease of systolic blood pressure, serum HDL, plasma concentrations of atherogenic oxidised LDL	Total cholesterol, TAG, LDL/HDL, TAG/HDL, TNF-α, C-reactive protein, nutritional status, blood parameters of liver and kidney function, haematology or serum electrolytes	4
100 mg	70	49	Increase of HDL; decrease of serum total cholesterol and LDL; decrease of systolic and diastolic blood pressure, blood glucose	Inflammatory IL-6, sVCAM-1	5
30 mg	14	10	Improved oxidative resistance of LDL	Plasma triglycerides, HDL or LDL	6
30 mg	14	4	Decrease in TIMP-1 plasma protein and lymphocyte mRNA	TIMP-2 and matrix metalloprotein-2 lymphocyte mRNA or plasma protein	7
500 mg quercetin-3-O-glucoside	7	15		Repeated-sprint performance, percent fatigue decrement, blood xanthine oxidase activity, IL-6 or uric acid	27

[1] Some of the entries were derived from [28]

[2] Quercetin aglycone, unless otherwise stated.

[3] Healthy subjects, unless otherwise stated.

Abbreviation: NIH, national institution of health; C1GALT1, Core 1 synthase, glycoprotein-N-acetylgalactosamine 3-beta-galactosyltransferase; GM2A, ganglioside monosialic 2 activator; HDGF, hepatoma-derived growth factor; SERPINB9, Serpin B9; IL-6, Interleukin 6; sVCAM-1, soluble vascular cell adhesion molecule 1; TIMP-1, tissue inhibitor of metallopeptidase -1; TIMP-2, tissue inhibitor of metallopeptidase-2.

Supplementary information: Table 2 Human intervention studies on dietary quercetin[1]

Dose per day[2]	Quercetin equivalent[3]	Days	No. of subjects per group[4]	Biomarkers significantly affected	Biomarkers not significantly affected	Ref
76-110 mg quercetin and other flavonols from 400 g onion (with tomato sauce) + 6 cups of tea	1200-1800 mg with other	14	10 type 2 diabetic patients	Decrease oxidative damage to lymphocyte DNA	Fasting plasma glucose, fructosamine, vitamin C, carotenoids, α-tocopherol, urate, albumin and bilirubin	29
200 g onion	1500 mg	1	6 female	Increase resistance of lymphocyte DNA to strand breakage, decrease in urinary 8-hydroxy-2'-deoxyguanosine	Urinary malondialdehyde	30
21 mg dietary quercetin, 9 mg dietary kaempferol	350 mg with other	1	19 female	Increase in erythrocyte superoxide dismutase activity, decrease in lymphocyte DNA damage (tail moment)	Plasma α-tocopherol or β-carotene	31
51 mg quercetin from 4.3 g onion extract	850 mg	30	23 male with oral maltose load induced postprandial endothelial dysfunction	Increase postprandial flow-mediated vasodilation (FMD) responses	Fasting FMD systemic or forearm hemodynamic	32
100 mg quercetin + 128 mg other flavonoids, onion peel extract	1660 mg with other	14	12 female	Decrease total cholesterol level, LDL cholesterol and atherogenic index	Erythrocyte antioxidant enzymes, lipid peroxidation markers, plasma antioxidant vitamin (retinol, tocopherol, carotenoids, coenzyme Q10), ex vivo H_2O_2-provoked oxidative DNA damage	25

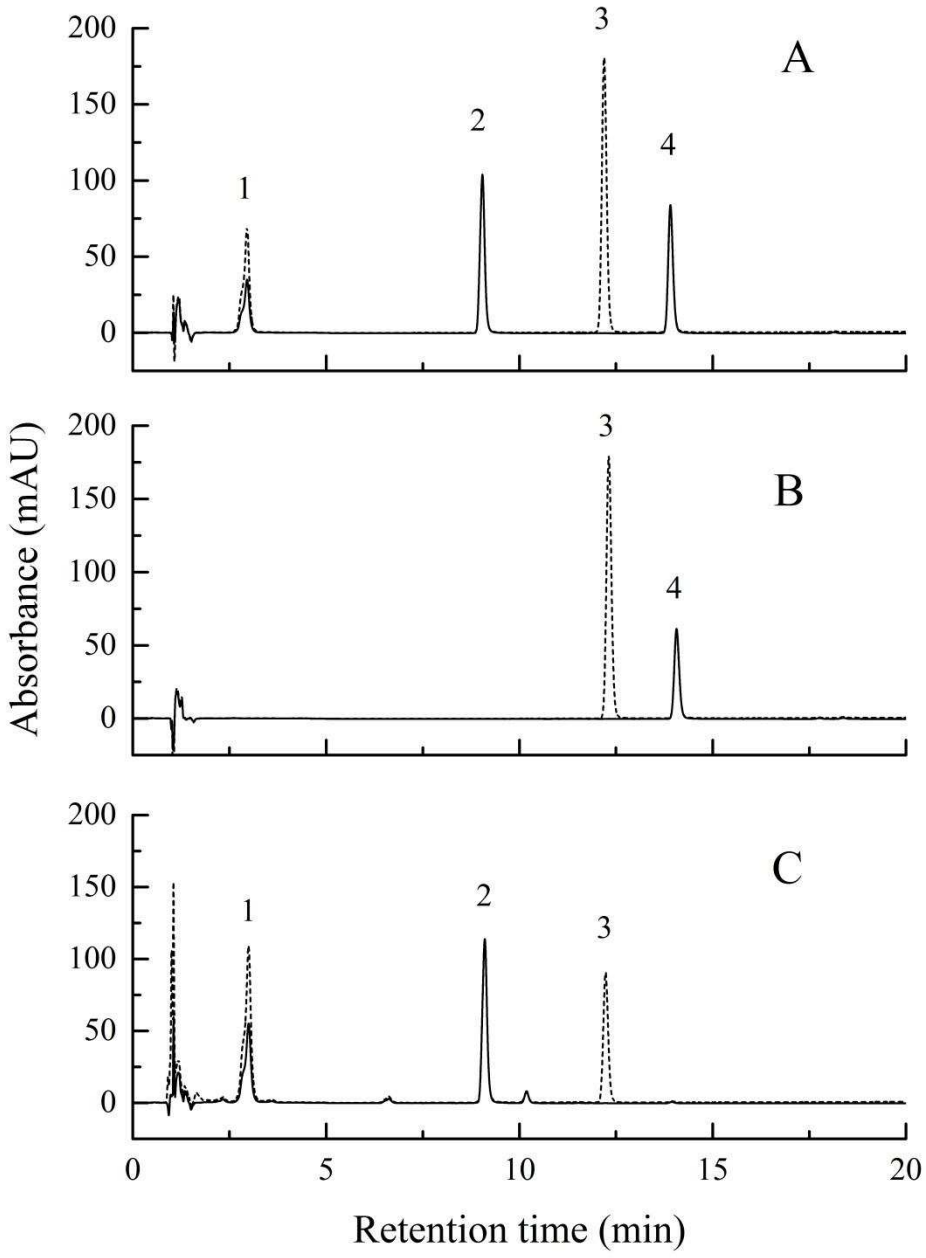
[1] Some of the entries were derived from [28]
[2] Quercetin aglycone, unless otherwise stated.
[3] Calculation is based on 16.6-fold since 166 mg quercetin aglycones from supplements would be comparable to 10 mg quercetin aglycone equivalents from onions according to this study.
[4] Healthy subjects, unless otherwise stated.

FIGURE LEGENDS

Figure 1 HPLC chromatograms of A) quercetin standards B) supplement extracts and C) onion extracts at 255 nm (dash line) and 370 nm (solid line): (1) quercetin 3,4'-O-diglucoside; (2) quercetin 4'-O-glucoside; (3) daidzein (i.s.); (4) quercetin.

Figure 2 LC-MS chromatogram of quercetin and methylquercetin after β–glucuronidase and sulfatase hydrolysis of urine.

Figure 3 Urinary excretion of quercetin and methyl quercetin (mean ± SEM). 1800 ± 150 µmol quercetin from supplements or 156.3 ± 3.4 µmol quercetin from red onion soup was provided to each individual on separate occasions.



341

342 Figure 1

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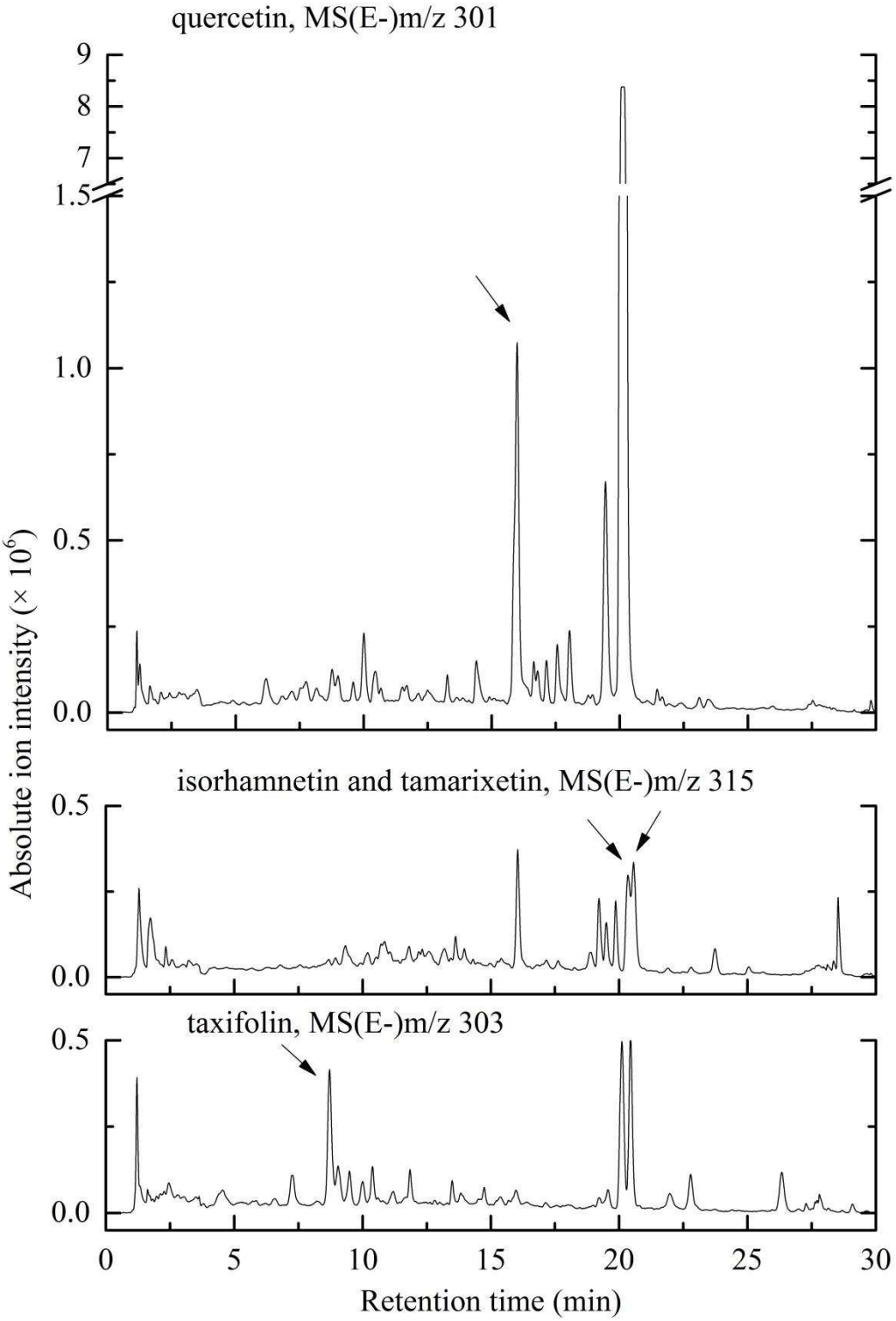
quercetin, MS(E-)m/z 301
9
8
7
1.5
1.0
0.5
0.0
Absolute ion intensity (× 10^6)
isorhamnetin and tamarixetin, MS(E-)m/z 315
0.5
0.0
taxifolin, MS(E-)m/z 303
0.5
0.0
0
5
10
15
20
25
30
Retention time (min)

Figure 2

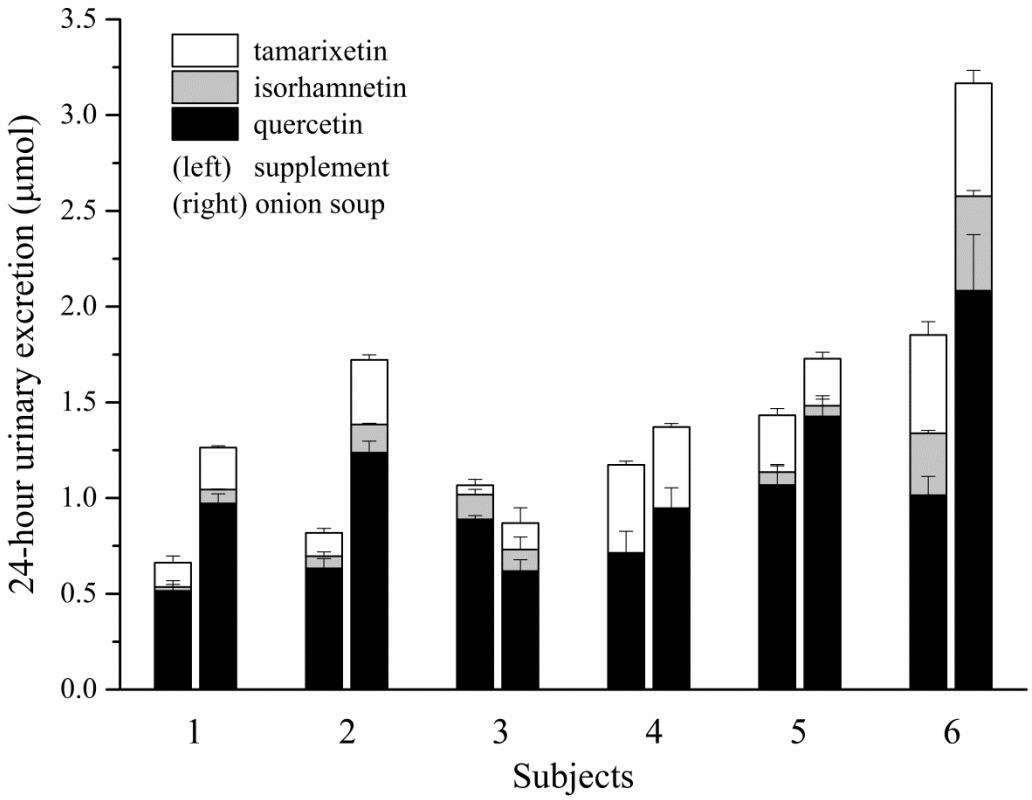
tamarixetin
isorhamnetin
quercetin
(left) supplement
(right) onion soup
24-hour urinary excretion (µmol)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1
2
3
4
5
6
Subjects

Figure 3