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Human Metabolic Transformation of Quercetin Blocks Its Capacity To Decrease Endothelial Nitric Oxide Synthase (eNOS) Expression and Endothelin-1 Secretion by Human Endothelial Cells

Sandra Tribolo,[†,∥] Federica Lodi,[†,§,∥] Mark S. Winterbone,[†,∥] Shika Saha,[†] Paul W. Needs,[†] David A. Hughes,[†] and Paul A. Kroon*[,†]

[†]Food and Health Programme, Institute of Food Research, Norwich Research Park, Norwich NR4 7UA, United Kingdom

[§]Food Ingredients and Packaging Unit, European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT: The major dietary flavonol quercetin, which has been shown to improve endothelial function and decrease blood pressure, is extensively metabolized during absorption. This study examined the relative abilities of quercetin and its human metabolites to modulate the expression of eNOS and ET-1, which are involved in regulating endothelial homeostasis. Quercetin aglycone significantly reduced both eNOS protein and gene expression in HUVEC, mirroring the effects of the pro-inflammatory cytokine TNFα. In the presence of TNFα the aglycone caused further reductions in eNOS, whereas the metabolites were without effect in either TNFα-stimulated or unstimulated cells. ET-1 expression was significantly reduced by quercetin in both TNFα-stimulated or unstimulated HUVECs. The metabolites had no effect on ET-1 expression with the exception of quercetin-3′-sulfate, which caused a moderate increase in TNFα-stimulated cells. These results suggest that metabolic transformation of quercetin prevents it from causing a potentially deleterious decrease in eNOS in endothelial cells.

KEYWORDS: *flavonoids, flavonols, polyphenols, human metabolism, endothelial function, blood pressure, cardiovascular disease*

INTRODUCTION

Cardiovascular disease (CVD) is a major cause of mortality in Western countries and is likely to increase further due to the increasing prevalence of obesity and type-2 diabetes. Atherosclerosis, a leading cause of CVD mortality, is an inflammatory condition that occurs in all adults to a greater or lesser extent. There is great interest in understanding how dietary components can modify the processes involved in CVD. The benefits derived from dietary factors are likely to be mediated by the vascular endothelium.[1] The endothelium plays a crucial role in maintaining cardiovascular homeostasis, attenuating vascular inflammation, and controlling blood flow and vascular tone. Control of these functions is achieved through the production/release of various mediators, such as the vasoconstrictor endothelin-1 (ET-1)[2] and relaxant nitric oxide (NO)[3] that modify the responsiveness of the underlying vascular smooth muscle. Endothelial dysfunction is characterized by an imbalance in the production of these endothelium-derived mediators and is believed to be an initiating event in atherosclerosis.[4]

Flavonoids are naturally occurring polyphenolic compounds present in fruits and vegetables as glycosylated forms. Data from epidemiological studies and from a meta-analysis of randomized controlled trials with flavonoid-rich foods support the notion that flavonoids are at least partly responsible for the beneficial effects of fruit- and vegetable-rich diets on cardiovascular health.[5−7] The major flavonol in the diet is quercetin,[8] the major dietary sources being tea, onions, apples, and red wine. In human subjects, supplementation with quercetin has been shown to reduce blood pressure, a risk factor for CVD, and lower plasma levels of oxidized low-density lipoproteins (oxLDL) in hypertensive and overweight individuals.[9,10] In healthy men quercetin supplementation reduced plasma levels of the vasoconstrictor ET-1 and increased NO concentrations.[11] Moreover, quercetin has been shown to reduce blood pressure and improve the antioxidant status in a number of animal models of hypertension[12−14] and to attenuate the activity of the superoxide generating enzyme NAPDH-oxidase and enhance the activity of the NO-generating enzyme endothelial nitric oxide synthase (eNOS).[15] Quercetin supplementation has also been shown to reduce inflammation and attenuate atherosclerosis progression in the ApoE mouse.[16] In in vitro studies quercetin induced increases in NO and vasorelaxation in porcine coronary arteries.[17] The relaxant effect of several groups of flavonoids, including quercetin, has been demonstrated in isolated aortic tissue from rats.[18,19] In addition, quercetin induced phosphorylation of eNOS and increased NO in endothelial cells[19,20] and increased gene expression of eNOS and reduced ET-1 gene expression at physiological concentrations.[21] Quercetin thus may have a role in cardiovascular protection by exerting an antioxidant effect (inhibition of LDL oxidation) and in improving vascular tone by increasing eNOS-derived NO and potentially by inhibiting expression of vasoconstrictors such as ET-1.

Human tissues, except for the lining of the intestinal tract, are exposed to polyphenols via the blood. Flavonoids are modified during absorption and first passage through the liver, so for quercetin the forms reaching the systemic circulation are

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Table 1. Probe and Primer Sequences Used for RT-PCR

gene	probe	sense primer	antisense primer
eNOS	CCATCACCGTGCCCATGAGCG	GCCAACGCCGTGAAGATCT	CATACAGGATTGTCGCCTTCACT
ET-1	AACACTCCCGAGCACGTTGTTCCG	TGCCACCTGGACATCATTTG	GACCTAGGGCTTCCAAGTCCAT
GAPDH	CATGACCACAGTCCATGCCATCACT	TTAGCACCCCTGGCCAAG	GCCATCCACAGTCTTCTGGG

glucuronidated and sulfated conjugates of quercetin and methylquercetin.[22,23] Quercetin glycosides and quercetin aglycone are not present in human plasma.[22,24] The biological activity of the flavonoids is dependent on their structure, and it has been shown that the quercetin conjugates have different activities from those of the parent aglycone.[25−28] Many previous in vitro studies have cultured cells with aglycones or polyphenol-rich extracts of plant material, often at concentrations exceeding those that might be achieved by diet alone.[23] Currently it is not known if the effects of quercetin consumption on markers of endothelial function and blood pressure are due to the phase-II metabolites of quercetin, and if so, which particular conjugates are effective.

In this study, we compared the effects of the biologically relevant sulfated, methylated, and glucuronidated conjugates of quercetin with the quercetin aglycone at physiological concentrations on the expression of ET-1 and eNOS by human endothelial cells (HUVECs). HUVECs were tested under unstimulated (resting) and TNFα-activated conditions. TNFα is present in atherosclerotic plaques[26,29] and was used to mimic the inflammatory conditions present at sites of atherosclerosis. In addition, we examined the ability of blood plasma rich in quercetin metabolites, obtained from volunteers following consumption of an onion meal, to alter the levels of eNOS and ET-1 in HUVECs.

MATERIALS AND METHODS

All reagents were obtained from Sigma (Poole, UK), except for recombinant human tumor necrosis factor-α (TNF-α) from R&D Systems (Abingdon, UK), dissolved in PBS containing 1% BSA, and quercetin from Extrasynthese (Genay, France), dissolved in DMSO. Oligonucleotide primers and fluorogenic probes were purchased from Sigma-Genosys (Poole, UK). Quercetin metabolites [quercetin 3′-sulfate (Q3′S), quercetin 3-glucuronide (Q3GlcA), and 3′-methylquercetin 3-glucuronide (isorhamnetin 3-glucuronide) (IR3GlcA)] were chemically synthesized according to the method we have published previously.[30]

Cell Culture. HUVECs were obtained from Lonza (Wokingham, UK) and grown in an EGM-2 Bullet Kit (Lonza). Cells were seeded onto 6-well plates at a density of 3500 cells/cm^2, and experiments were performed at confluence (day 5 or 6). All of the experiments were carried out between passages 2 and 4 (doubling population $\leq$ 10). Cultures were maintained in a humidified atmosphere at 37 °C and 5% CO_2 in air. Preliminary experiments were performed to determine optimal time of incubation to measure either protein (24 h) or mRNA (6 h) expression of ET-1 or eNOS. The experiments were performed with HUVECs both in an unstimulated (resting) state and in an inflamed condition by treatment with TNFα (10 ng/mL).

Cell Viability Determination. When HUVECs reached 95−100% confluence, cells were incubated with quercetin and quercetin metabolites for 24 h. Following the incubations, cell viability was assessed by trypan blue exclusion. Cells were counted using a dual-chamber hemocytometer under a light microscope.

Determination of ET-1, Endothelial Nitric Oxide Synthase (eNOS), and Inducible Nitric Oxide Synthase (iNOS) Protein Expression. HUVECs were preincubated with TNFα (inflamed) for 24 h or were left untreated (resting) prior to incubation with quercetin or quercetin conjugates for 24 h at 37 °C. ET-1 protein was measured in the cell supernatant by a TiterZyme Enzyme Immunometric Assay (Assay Designs, Cambridge BioSciences, Cambridge, UK) according to the manufacturer's instructions.

eNOS and iNOS proteins were measured in the cell lysate by a commercially available ELISA (R&D Systems, Abingdon, UK) following the manufacturer's protocol. Protein concentrations were normalized using total protein in the lysate, as measured by a bicinchoninic acid protein assay kit (BCA, Sigma).

Gene Expression. For measurement of eNOS gene expression HUVECs were preincubated for 45 min with quercetin or quercetin conjugates. HUVECs were then exposed to TNFα (inflamed) or were left untreated (resting) for 6 h at 37 °C. To measure iNOS or ET-1 gene expression, HUVECs were preconditioned with TNFα (inflamed) for 6 h or left untreated (resting) and then incubated with quercetin or quercetin metabolites for 24 h at 37 °C.

Total RNA was extracted from the cells using a commercially available RNA extraction kit (RNeasy Mini Kit, Qiagen) and quantified using a NanoDrop spectrophotometer ND-1000 (LabTech International, UK), measuring optical densities at 260 and 280 nm. Gene expression was quantified by real-time reverse transcriptase-PCR (RT-PCR) (TaqMan, ABI Prism 7700 Sequence Detection System, Applied Biosystems). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used to normalize the data. Oligonucleotide forward and reverse primers and fluorogenic Taqman probes for eNOS, ET-1, iNOS, and GAPDH (Table 1) were designed using Primer express software (Applied Biosystems). The thermal cycling conditions were as follows: 48 °C for 30 min, 95 °C for 10 min followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Reactions were carried out in triplicate.

Subjects and Study Design. All subjects gave written informed consent to participate in this study. which was approved by the Norwich Research Ethics Committee (06/Q0101/131). Eight apparently healthy men (average age = 31 years, average weight = 82 kg) were recruited to participate in this study. For the first 2 days of the study all volunteers followed a low-polyphenol diet. On day 3 a baseline blood sample (35 mL) was collected into a lithium−heparin tube. Volunteers then consumed a meal consisting of 200 g of fried red onions followed by a standard breakfast of two slices of white toast with 10 g of spread and 200 mL of water. Further blood samples (35 mL) were collected at 1 and 24 h and immediately centrifuged at 1500g for 10 min. Plasma samples were stored at −80 °C until use.

Determination of Effects of Postonion-Plasma on ET-1 and eNOS. HUVECs were incubated with 20% (v/v) of plasma from subjects who had consumed 200 g of red onions. HUVECs in both an untreated and inflamed condition (TNFα) were tested. Levels of gene and protein expression were determined as described previously.

Extraction and Analysis of Quercetin Metabolites from Onion-Plasma. Plasma samples (100 μL) were mixed with 20 μL of an internal standard (20 ng of rhamnetin) and extracted in a double volume of acetonitrile + 1% formic acid. Samples were centrifuged at 4000g for 10 min at 4 °C. The supernatant fraction was collected, and the pellet was re-extracted twice with 100 μL of methanol. The three supernatants were combined, centrifuged, and used for analysis of conjugates.

LC-MS/MS analysis was carried out using a 4000-QTrap mass spectrometer (Applied Biosytems) with a 1200 binary pump, degasser, cooled autosampler, and column oven (Agilent Technologies). Separation was performed using a C18 Gemini 3 μm, 150 × 3 mm, column (Phenomenex) and eluted with a gradient of 10−100%; B (2% tetrahydrofuran, 0.1% acetic acid, 97.9% acetonitrile) and A (10 mM ammonium acetate adjusted to pH 4 with acetic acid). The LC eluent (0.25 mL/min) was sprayed into the MS without splitting. Detection of the conjugates was performed in negative ion mode. The multiple monitoring, declustering potential, collision energy, and collision cell

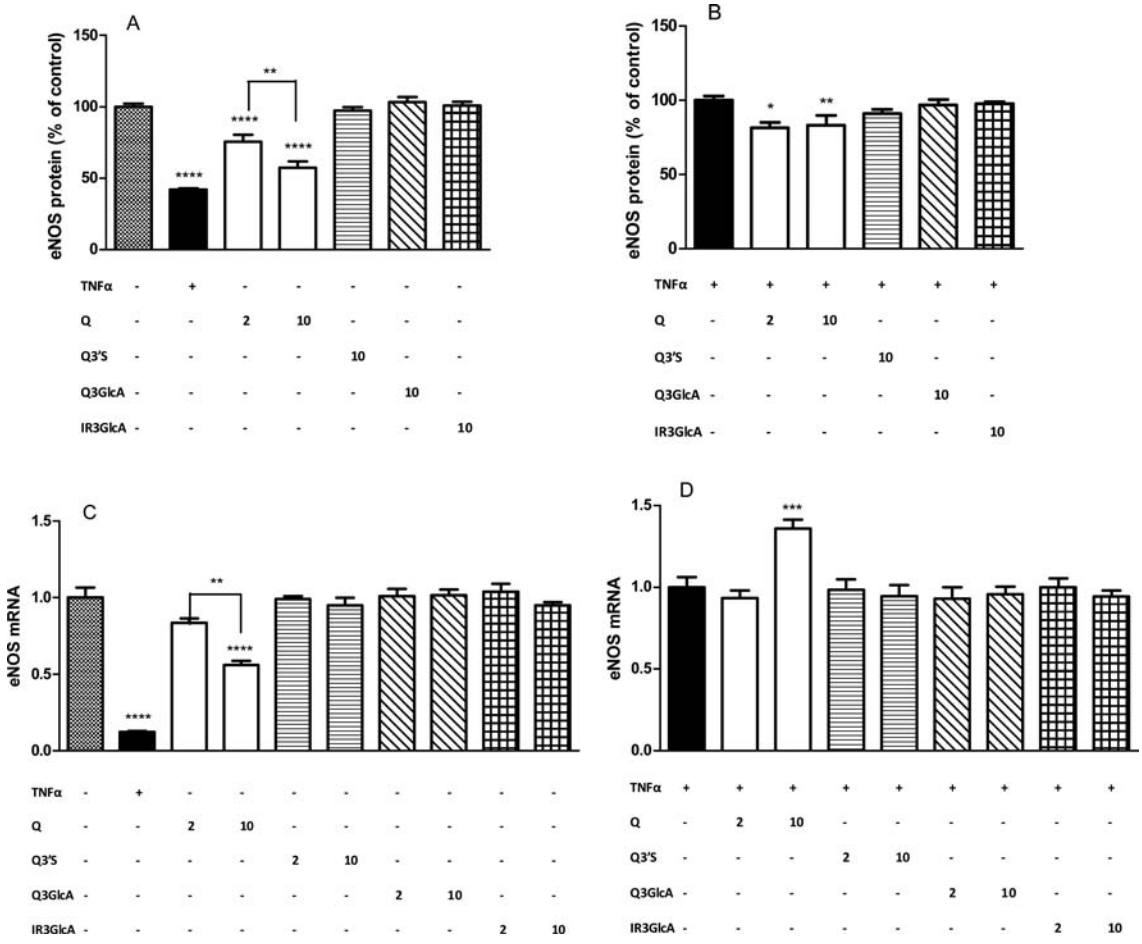

Figure 1. Effect of quercetin and quercetin metabolites on eNOS expression in HUVECs. HUVECs were stimulated with TNFα (10 ng/mL) or left untreated and subsequently treated with pure quercetin or pure metabolites of quercetin. Concentrations of eNOS protein were quantified by ELISA (A, B), and relative mRNA levels were determined by qRT-PCR (C, D). Tables under graphs indicate concentration (μM) of quercetin or quercetin metabolite applied to cells. Q, quercetin; Q3′S, quercetin-3′-sulfate; Q3GlcA, quercetin-3-glucuronide; IR3GlcA, isorhamnetin-3-glucuronide. Data are means ± SEM, n = 5−12. (*) $p < 0.05$; (**) $p < 0.01$; (***) $p < 0.001$.

exit potential for each target analyte were optimized by infusion into the Qtrap. Standard curves of the main quercetin conjugates expected were prepared using authentic standards (Q3GlcA, Q3′S, and IR3GlcA).

Statistical Analysis. Standard ANOVA models were employed to analyze the data. Regression diagnostics were checked to determine if data transformations, outlier omissions, or alternative nonparametric models were required. For post hoc pairwise comparisons, as interest was confined to differences between treatments to the inflamed or resting cells, Dunnett's was the preferred method. Results are expressed as the mean ± SEM of independent experiments. Changes were considered significant for $p < 0.05$.

RESULTS

Cell Viability. Cell viability was measured in resting and inflamed cells. The percentage of viable cells after treatment with quercetin, quercetin metabolites, or 20% (v/v) onion-plasma was <95%, indicating that none of the treatments were cytotoxic.

Effect of Quercetin and Quercetin Metabolites on Protein and Gene Expression. *eNOS.* Treatment of HUVECs with inflammation-inducing TNFα resulted in a significant decrease of eNOS expression at both protein and gene levels (Figure 1A,C). In resting cells, quercetin treatments also decreased eNOS protein expression in a concentration-dependent manner ($p < 0.001$), with 10 μM quercetin producing a reduction in eNOS expression similar to that seen with TNFα alone (Figure 1A). In contrast, quercetin metabolites did not exert any significant effect on eNOS protein expression. Under pro-inflammatory conditions, eNOS protein levels were reduced to ∼40% of control (Figure 1A). Quercetin further reduced eNOS protein levels in TNFα-treated cells at both 10 μM ($p < 0.01$) and 2 μM ($p < 0.05$), whereas the quercetin metabolites did not affect eNOS protein under the same conditions (Figure 1B).

eNOS transcripts were reduced 8-fold under pro-inflammatory conditions (Figure 1C). Quercetin, at the higher concentration of 10 μM, resulted in a small but significant (∼30%; $p < 0.001$) increase in eNOS transcripts in inflamed cells, but no significant changes were observed with quercetin at 2 μM or with the metabolites at both concentrations tested (Figure 1D). In resting cells quercetin reduced eNOS transcript expression in a dose-dependent manner (Figure 1C), whereas no effects of the quercetin metabolites were observed at either concentration tested.

iNOS. iNOS expression was not detected in either resting or inflamed HUVECs at either the protein or transcript level (data not shown).

ET-1. Treatment of HUVECs with TNFα resulted in significant increases of ET-1 expression, at both the protein and gene levels (Figure 2A,C). In resting cells, 24 h of

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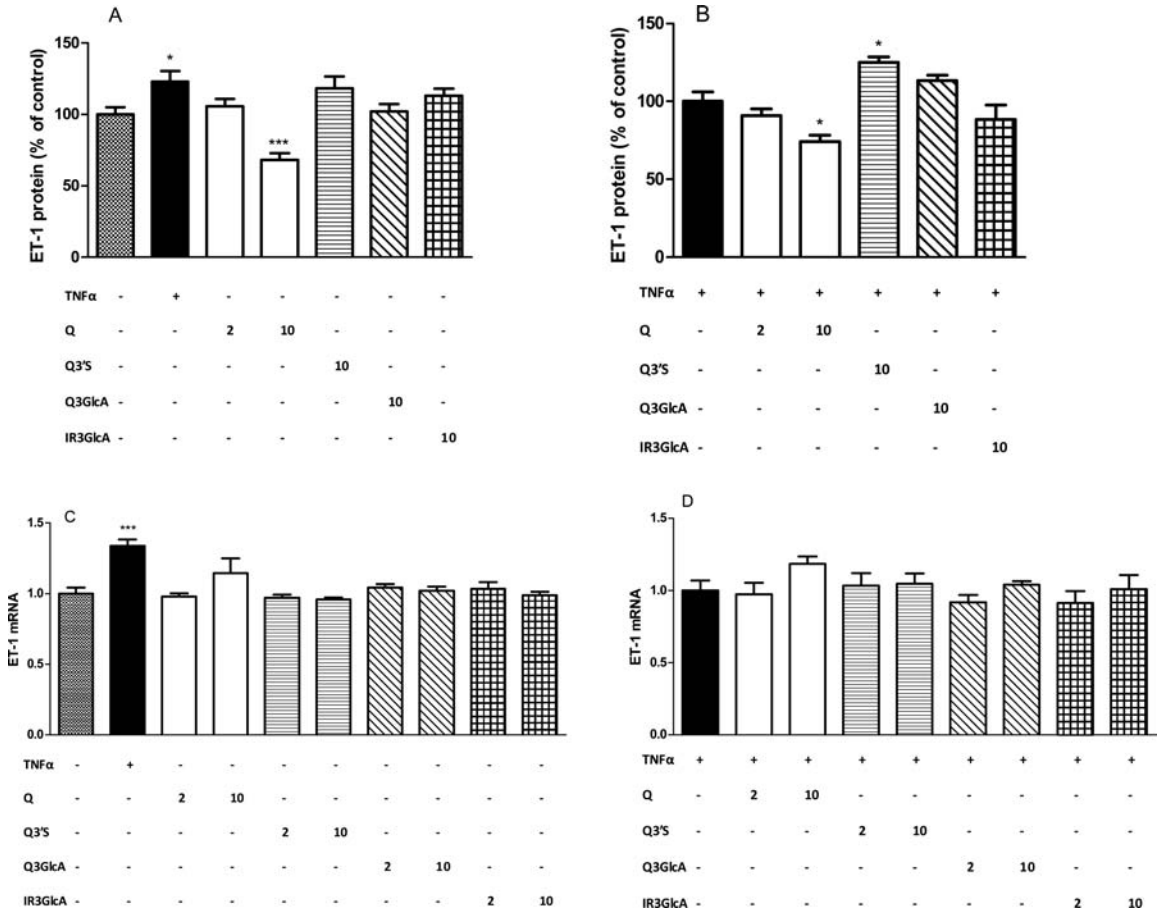

Figure 2. Effect of quercetin and quercetin metabolites on ET-1 expression in HUVECs. HUVEC were stimulated with TNFα (10 ng/mL) or left untreated and subsequently treated with pure quercetin or pure metabolites of quercetin. Concentrations of ET-1 protein were quantified by ELISA (A, B), and relative mRNA levels were determined by qRT-PCR (C, D). Tables under graphs indicate concentration (μM) of quercetin or quercetin metabolite applied to cells. Q, quercetin; Q3′S, quercetin-3′-sulfate; Q3GlcA, quercetin-3-glucuronide; IR3GlcA, isorhamnetin-3-glucuronide. Data are means ± SEM, n = 5−12. (*) $p < 0.05$; (**) $p < 0.01$; (***) $p < 0.001$.

treatment with 10 μM quercetin caused significant decreases in ET-1 protein expression (Figure 2A; $p < 0.05$). In contrast, the quercetin metabolites did not alter ET-1 protein expression in resting HUVECs. In inflamed cells, quercetin attenuated the TNFα-induced increase of ET-1 protein expression after 24 h (significant for 10 μM treatment ($p < 0.05$); Figure 2B). Neither Q3GlcA nor IR3GlcA treatment caused significant changes in ET-1 protein expression in inflamed cells, but Q3′S significantly increased ET-1 protein expression (~23%; $p < 0.05$) in the inflamed HUVECs (Figure 2B). Neither quercetin nor its metabolites caused any significant change in ET-1 transcript concentrations in either resting or inflamed cells (Figure 2C,D)

Analysis of Quercetin Metabolites in Plasma. Plasma levels of Q3′S, Q3GlcA, and IR3GlcA were quantified before (baseline) and at 1 and 24 h following ingestion of a meal containing 200 g of fried red onion. None of the conjugates were detected in the baseline samples of any volunteer. Table 2 shows the mean concentration of quercetin metabolites in plasma at 1 and 24 h. At 24 h, conjugates were detected in plasma of only two volunteers. Quercetin aglycone was not detected in plasma of volunteers at any time point.

Effect of Postonion Plasma on eNOS and ET-1 Expression. HUVECs were incubated with the baseline plasma samples, and these were compared with 1 and 24 h samples following ingestion of 200 g of a quercetin-rich meal (red onions). No significant changes were detected in the expression of eNOS or ET-1 at either the protein or transcript level in HUVECs in response to 1 or 24 h postonion plasma (Figure 3).

Table 2. Mean Concentration of Quercetin Metabolites in Plasma at 1 and 24 h Postingestion of an Onion Meal

	1 h, μM ± SEM (range, μM)	24 h, μM ± SEM (range, μM)
Q3′S	0.88 ± 0.235 (0.451−2.435)	0.010 ± 0.008 (0−0.063)
Q3GlcA	0.340 ± 0.049 (0.170−0.591)	0.017 ± 0.006 (0−0.041)
IR3GlcA	0.129 ± 0.025 (0.053−0.261)	0.002 ± 0.001 (0−0.013)

DISCUSSION

This study has shown that whereas the aglycone form of quercetin can induce significant changes in eNOS expression and ET-1 secretion by vascular endothelial cells, the major human phase-II metabolites of quercetin are largely ineffective. Importantly, this study has provided evidence that the aglycone form of the dietary flavonoid quercetin can induce pro-inflammatory changes in eNOS protein and gene expression in endothelial cells but that the normal efficient phase-II

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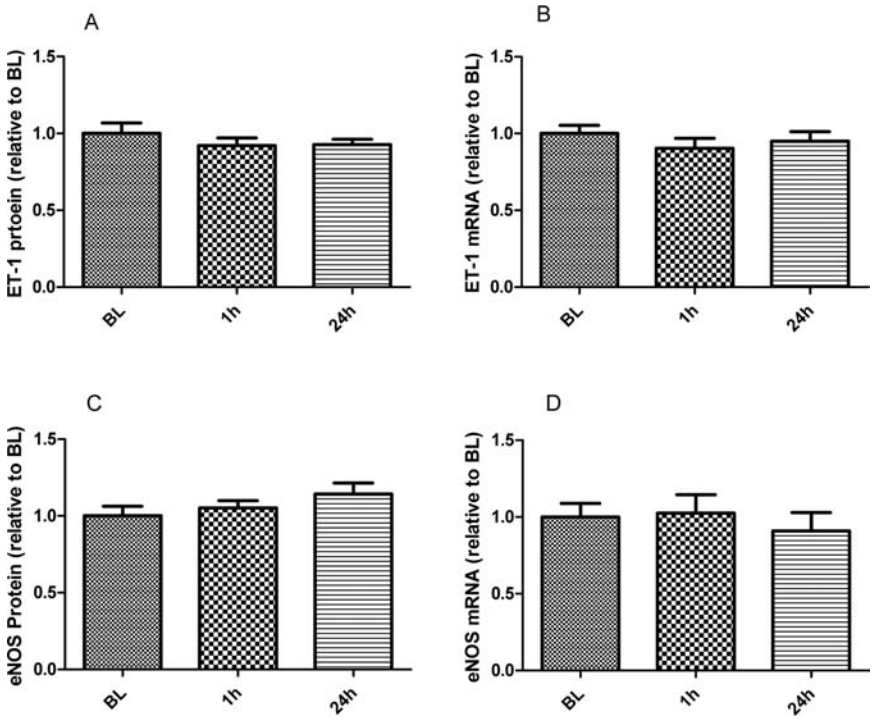

Figure 3. Effect of postonion meal plasma on eNOS and ET-1 in HUVECs. Human plasma was obtained from eight volunteers who had consumed 200 g of fried red onions. HUVECs were incubated with human plasma collected at baseline (t = 0 h), 1 h postonion meal, or 24 h postonion meal. Total eNOS protein in HUVEC lysates was quantified following a 24 h incubation period (A) and eNOS mRNA after a 6 h incubation (B). Secretion of ET-1 protein into cell culture supernatants was quantified following a 24 h incubation period (C), and mRNA levels in HUVECs were quantified after a 6 h incubation (D). BL, baseline. Data are expressed as changes relative to baseline samples (t = 0 h) and are means ± SEM, n = 8. Each sample was measured in triplicate.

conjugation of quercetin during absorption abolishes this pro-inflammatory activity. We also showed that conjugates of quercetin lack the potentially beneficial ability of the aglycone to attenuate the TNFα-induced increases in secretion of ET-1.

Endothelial cells in the vascular system provide protection against the development of CVD. A key regulator of endothelial cells is NO generated by eNOS within the endothelial layer. Vascular NO regulates vascular tone and blood pressure and can control the activity of platelets and the underlying smooth muscle cells.[31] Endothelial dysfunction occurs in regions of metabolic stress, and endothelial damage and a reduction in NO bioavailability are characteristic of CVD.[31,32] Although many studies have shown potentially positive effects of quercetin, negative effects of quercetin have also been reported; for example, quercetin and related flavonoids have been shown to generate superoxide ($O_2^{\bullet-}$) and have cytotoxic effects on human lymphocytes.[33] Quercetin also reduced glutathione and glutathione reductase activity in rats fed quercetin[34] and caused an increase in serum LDL.[35] Other authors have also described a reduction in eNOS expression in response to quercetin treatment.[36,37] Quercetin is able to act as a pro-oxidant and generate reactive oxygen species (ROS) by several mechanisms, for example, through intracellular metabolism to *o*-quinones[38] and via interactions with transition metals such as Cu(II).[39,40] We have also observed an ability of quercetin to undergo autoxidation and generate $O_2^{\bullet-}$, a property not shared by the glucuronidated metabolites.[41] The ability of quercetin to act as a prooxidant may depend upon the oxidative environment. Lopez-Lopez et al., for example, showed that at physiological pH and O_2 quercetin reduced NO bioavailability; however, when $O_2^{\bullet-}$ concentrations were increased, quercetin improved NO bioavailability by preferential scavenging of $O_2^{\bullet-}$.[42] We have observed that quercetin improved the NO response in rat aorta under conditions of high oxidative stress; in addition, the quercetin metabolites were also effective, although less so than the aglycone.[43] Therefore, damaging prooxidant actions of quercetin may become antioxidant and protective in regions of high oxidative stress such as may be expected in atherosclerotic lesions. We were unable to detect iNOS under any of the conditions we employed; this is likely to be due to epigenetic silencing of the iNOS promoter.[44]

Quercetin reduced ET-1 expression in both resting and inflamed HUVEC as has been previously reported.[45] The quercetin metabolites, however, did not alter ET-1 protein expression with the exception of Q3′S, which caused an increase in ET-1 expression in inflamed cells. A divergent effect of quercetin and its metabolites on endothelial proliferation has been described,[46] whereby Q3′S stimulated vascular endothelial growth factor (VEGF) induced proliferation and angiogenesis, which was associated with enhanced stimulation of VEGF receptor-2. VEGF has been shown to enhance the expression of ET-1 in endothelial cells,[47] and this may explain the increase in ET-1 we observed.

Recent studies have demonstrated that human blood plasma, in addition to the three major purified conjugates tested in the cell culture model, contains a diverse mixture of quercetin metabolities, which peak around 1 h after consumption of an onion meal and which decline to near baseline levels by 24 h.[48,49] Therefore, we utilized an ex vivo approach similar to that of Canali et al. in which human volunteers fed an onion meal act as "bioreactors" to provide metabolite-rich blood plasma that was used to enrich the HUVEC culture media, giving a

more physiologically relevant quercetin−metabolite mix.[50] Exposure of HUVEC to this metabolite-rich blood plasma resulted in no changes to eNOS or ET-1 in either the resting or inflamed cells, reflecting the results obtained with the pure metabolites. To avoid adverse effects on the HUVEC, we diluted the onion metabolite rich blood plasma (20% v/v) with culture media before exposure to the cells. This was the same dilution used by Canali et al. to treat HUVEC with red wine enriched blood plasma and was able to affect mRNA expression of several CVD-associated genes. This dilution resulted in the concentration of the individual metabolites in the exposure medium being lower than when HUVECs were exposed to the pure metabolites in isolation and could account for the lack of effect observed.

Quercetin undergoes extensive metabolism following absorption in the gastrointestinal tract such that the only forms of quercetin detectable in the systemic circulation are sulfated and glucuronidated conjugates of quercetin and methylquercetin. Modification of quercetin alters its biological activities. For example, we have previously reported that quercetin conjugates are less effective than the aglycone or not effective at all in reducing adhesion molecule expression in TNFα-treated HUVECs and vascular smooth muscle.[26,28] Other papers provide evidence that the metabolites have reduced anti-inflammatory activities,[51] lack the vasorelaxant effect of the aglycone,[43] and are less potent inhibitors of neutrophil-mediated LDL oxidation and myeloperoxidase (MPO) activity.[52] Nevertheless, in the latter example, all three major quercetin human conjugates significantly inhibited LDL oxidation and MPO activity at physiological concentrations (1 μM).[52] Our data suggest that quercetin can reduce expression of the vasoconstrictor ET-1, a potentially beneficial effect. However, quercetin also reduced expression of eNOS in HUVECS, a potentially negative effect that could lead to reduced NO bioavailability and endothelial dysfunction. The phase-II conjugates of quercetin were, however, largely inactive. Therefore, data presented here show that metabolic conjugation of quercetin may provide protection from the negative effects of the aglycone on NO synthesis and endothelial function.

It has been suggested that deconjugation of flavonoid metabolites at the site of inflammation by β-glucuronidase may be a mechanism by which flavonoids could exert protective affects in vivo.[53,54] Recently, Kawai et al., using immunohistochemical techniques, showed that Q3GlcA accumulates in atherosclerotic lesions, but not in normal tissue, and was associated with macrophage-like foam cells.[55] In addition, they also demonstrated that Q3GlcA could be taken up by activated macrophages and deconjugated to quercetin. Mendez et al. have shown that rat mesenteric bed perfused with Q3GlcA accumulated quercetin, and this accumulation was inhibited by a β-glucuronidase inhibitor and that inhibition of rat aortic ring contractile response by Q3GlcA was also prevented by β-glucuronidase inhibition.[56] Hence, at least some quercetin metabolites may accumulate within diseased tissues and undergo deconjugation to the more active aglycone. Our data indicate that metabolic transformation of quercetin would prevent potentially deleterious effects on healthy tissue, allowing the conjugates to be transported to diseased tissue, where, following deconjugation, in an environment of increased oxidative stress, they have the potential to exert protective antioxidant/anti-inflammatory effects.

We have previously reported that treatment of HUVECS with the major human metabolites of quercetin can induce anti-inflammatory responses, for example, significant reductions in the cell-surface expression of two cell adhesion molecules.[26] We have also reported that these quercetin metabolites can beneficially modulate inflammation-induced responses in porcine tissue.[57] On the other hand, there were no significant effects of the quercetin conjugates when tested in a rat tissue model for investigating potential vasorelaxant effects.[43] These differences in effects most likely reflect differences in the species and models chosen to study the quercetin metabolites.

In conclusion, data presented here show that gastrointestinal metabolic transformation abolishes the negative effects of quercetin aglycone on eNOS expression but also negates beneficial effects of the aglycone, which was able to reduce ET-1 secretion. In demonstrating that conjugation of flavonoids can block potentially deleterious effects of the aglycone, it is important that human conjugates are used in studies examining the biological activity of flavonoids.

AUTHOR INFORMATION

Corresponding Author

*(P.A.K.) E-mail: paul.kroon@ifr.ac.uk. Phone: +44 (0)1603 255000. Fax: +44(0)1603 507123.

Author Contributions

‖S.T., F.L., and M.S.W. contributed equally.

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Notes

The publication was drafted under the sole responsibility of the author (F.L.) and is not considered as an EFSA output. The positions and opinions presented are those of the author (F.L.) alone and are not intended to represent the views of EFSA. The authors declare no competing financial interest.

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ABBREVIATIONS USED

eNOS, endothelial nitric oxide synthase; ET-1, endothelin-1; HUVEC, human umbilical vein endothelial cells; Q3′S, quercetin-3′-sulfate; Q3GlcA, quercetin-3-glucuronide; IR3GlcA, isorhamnetin-3-glucuronide

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