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Absorption of quercetin-3-glucoside and quercetin-4′-glucoside in the rat small intestine: the role of lactase phlorizin hydrolase and the sodium-dependent glucose transporter

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Abstract

Two hypotheses on absorption mechanisms of flavonoid glucosides across the small intestine have been proposed: active uptake of the quercetin glucoside by the sodium-dependent glucose transporter (SGLT1) with subsequent deglycosylation within the enterocyte by cytosolic β-glucosidase, or luminal hydrolysis of the glucoside by lactase phlorizin hydrolase (LPH) and absorption by passive diffusion of the released aglycone. To test the above hypotheses we employed phlorizin (as an inhibitor of SGLT1) and *N*-(*n*-butyl)-deoxygalactonojirimycin (as an inhibitor of the lactase domain of LPH) in a rat everted-jejunal sac model. Quercetin-4′-glucoside mucosal hydrolysis was 10 times greater than quercetin-3-glucoside hydrolysis in the absence of inhibitors (449 and 47 nmol g^{-1} tissue, respectively), despite the similar amounts (13 ± 4 and 9 ± 1 nmol g^{-1}, respectively) being transferred to the serosal compartment during the 15 min incubation. Apical hydrolysis of both quercetin glucosides was significantly reduced in the presence of NB-DGJ (80%), and transfer of quercetin (measured as quercetin metabolites) to the serosal solution was also significantly reduced (40–50%). In the presence of phlorizin, transfer of metabolites to the serosal solution was only reduced in the case of quercetin-4′-glucoside. Evidently the mechanism of absorption of quercetin-4′-glucoside involves both an interaction with SGLT1 and luminal hydrolysis by LPH, whereas quercetin-3-glucoside appears to be absorbed only following hydrolysis by LPH.

Keywords: Flavonoid; Quercetin glucoside; Intestinal absorption; Deglycosylation; Sodium-dependent glucose transporter 1 (SGLT1); Lactase phlorizin hydrolase (LPH)

1. Introduction

Understanding the absorption of dietary flavonoids is fundamental in determining their biological activity, as the degree of absorption from the diet will profoundly affect bioactivities at flavonoid-responsive sites within the body. In general, dietary flavonoids are attached to sugar residues which affect the mechanism of absorption by altering their physico-chemical properties and thus their ability to enter cells, or to interact with transporters and cellular (lipo)proteins. Deglycosylation is the most likely first step during transfer of flavonoids from the intestinal lumen into the circulation, as indicated by the activity of endogenous β-glucosidases *in vitro* [1–3], by human intervention studies where metabolites have been characterised [4–6], and in a study with ileostomy subjects [7]. The site of deglycosylation and transport across the intestinal enterocytes depends on the nature of the flavonoid aglycone moiety and nature and position of the attached sugar. For example, the contrasting pharmacokinetics of quercetin-3-glucoside and quercetin-3-rhamnoglucoside [8] illustrate the dramatic consequences of structural differences on the site of absorption. Quercetin glucoside may be readily deglycosylated in the small intestine, whereas small intestinal β-glucosidases appear inactive against quercetin rhamnoglucoside [1]. These pharmacokinetic data are also supportive of the

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Abbreviations: LPH, lactase phlorizin hydrolase; SGLT1, sodium-dependent glucose transporter; NB-DGJ, *N*-(*n*-butyl)-deoxygalactonojirimycin; MRP2, multi-drug resistant protein efflux transporter.

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concept of interaction for flavonoid glucosides, but not rhamnoglucosides, with the SGLT1 [9,10].

The Caco-2 cell culture model has been used to study the absorption of some flavonoids, but evidence for transport of quercetin glucosides is confusing, and often contradictory to *in vivo* observations. For example in cell culture, quercetin glucosides are apparently not well absorbed, particularly with respect to the aglycone which is taken up by passive diffusion [11–13]. One explanation results from the activity of MRP2, which pumps the glucosides back out across the mucosal membrane [14]. Indeed by inhibiting MRP2, Walgren *et al.* [15] demonstrated that quercetin-4′-glucoside was transported by SGLT1 into Caco-2 cells, and subsequently confirmed the result in a nonintestinal cell line, Chinese hamster ovary cells, that had been stably transfected with SGLT1 (G6C3 cells). However, expression of LPH (EC 3.2.1.23 and 62) is particularly low in Caco-2 cells [16], and this requires some explanation. If LPH plays an important role in absorption of quercetin glucosides, then low activity of the β-glucosidase would result in a reduced level of quercetin diffusing across the brush-border. The aim of the current study was to investigate the extent to which LPH is involved in absorption of quercetin glucosides by using a previously characterised model, rat everted-jejunal sacs, in the presence of specific inhibitors of (i) the lactase domain of LPH and (ii) transport by SGLT1. Quercetin-3- and 4′-glucosides were chosen as representative substrates due to their abundance in flavonol-rich foods consumed in the diet [17].

2. Materials and methods

2.1. Materials

Quercetin, quercetin-3-glucoside, quercetin-4′-glucoside and apigenin of HPLC grade were purchased from Extrasynthese. Phlorizin was purchased from Sigma. Quercetin-3-glucuronide was isolated from green beans [18], and quercetin-7-glucuronide was enzymatically produced as described in an earlier paper [19]. *N*-(*n*-butyl)-deoxygalactonojirimycin (NB-DGJ) was obtained from Calbiochem. Aryl-sulfatase (from *Aerobacter aerogenes*) was purchased from Fluka Chemicals and β-glucuronidase (from *E. coli*) was purchased from Sigma. All other reagents were purchased from Sigma and were of analytical reagent grade or HPLC grade where applicable. Water was purified *via* a Millex Q-plus system (Millipore).

2.2. Animals

Male Wistar rats (∼200 g) were obtained from a licensed commercial animal supplier and housed in an environmentally controlled animal facility prior to use. Animals were fed a standard laboratory chow diet and water *ad lib.* until their body mass reached approximately 300 g. All aspects of animal care complied with the ethical guidelines and technical requirements of the UK Home Office.

2.3. Intestinal preparation

Each rat was deeply anaesthetised with sodium barbiturate (Euthetal; Rhone Merieux) and killed by cervical dislocation immediately prior to removal of the small intestine *via* an abdominal incision. The jejunum was identified (10–50% length) rinsed with 5 mL Krebs bicarbonate buffer (pH 7.2–7.4) and everted. Segments (5 cm) were cut (8 per rat; 8 rats) from the proximal portion and randomised according to a Latin square design. Canulated everted sacs were prepared by ligaturing everted segments at one end and tying the other to tapered disposable syringes (1 mL), pre-charged with Krebs bicarbonate buffer (0.5 mL). The sacs were filled with Krebs buffer by depressing the plunger and emptied, without contamination of the serosal solutions, by withdrawing the plunger at the end of the experiment. The sacs were suspended in organ baths (37°) containing incubation media (8 mL) as described below, and gassed continuously with 5% carbon dioxide in oxygen for 15 min.

2.4. Validation of NB-DGJ

In order to establish the optimum concentration of lactase hydrolase inhibitor, sacs were prepared as above, and incubated in organ baths containing [^{14}C]-lactose (Amersham Biosciences; final concentration 2 mM; 4.63 kBq/mL) in Krebs buffer (8 mL) with 0, 10, 50, 100 or 500 μM NB-DGJ an inhibitor of lactase [20]. After incubation, sacs were rinsed and the serosal solutions drained and transferred to plastic vials (20 mL; Packard). Liquid scintillation cocktail (Zinsser Quicksafe A; 9.5 mL) was added to the serosal solutions and their radioactivity assessed in an automatic scintillation spectrometer. The tissue from each sac was placed in glass vials (2 dram) and dried prior to weighing, acid digestion and liquid scintillation counting [10]. Uptake of hydrolysed lactose was determined by combining the amount of radiolabel in the tissue and serosal solutions and normalising for tissue mass.

Linear inhibition of [^{14}C]-lactose hydrolysis was observed up to 100 μM NB-DGJ, and appeared to have reached maximum but incomplete (60%) inhibition of hydrolysis around 250 μM. Consequently 250 μM NB-DGJ was used in further incubations with flavonol glycosides.

2.5. Transport studies

Eight randomised sacs from each rat were incubated in Krebs buffer containing: (a) quercetin-3-glucoside (100 μM), (b) quercetin-3-glucoside (100 μM) + phlorizin

(25 μM), (c) quercetin-3-glucoside (100 μM) + NB-DGJ (250 μM), (d) quercetin-3-glucoside (100 μM) + phlorizin (25 μM) + NB-DGJ (250 μM), (e) quercetin-4′-glucoside (100 μM), (f) quercetin-4′-glucoside (100 μM) + phlorizin (25 μM), (g) quercetin-4′-glucoside (100 μM) + NB-DGJ (250 μM), (h) quercetin-4′-glucoside (100 μM) + phlorizin (25 μM) + NB-DGJ (250 μM). At the end of the incubation the mucosal tissue was scraped from each sac and transferred to tubes. Serosal and mucosal solutions and the tissue samples were all immediately frozen on dry ice. A sample of the initial mucosal solution that had not been used in the incubation was also frozen. The muscle layer was not analysed for quercetin conjugates, as previous studies have shown that the overall contribution for the recovery of the flavonoid was negligible [21].

2.6. Preparation of samples

The tissue was homogenised twice with methanol (2 × 0.75 mL) containing ascorbic acid (1 mM; to stabilise the samples during analysis), followed by centrifugation (13,600 *g*, 10 min, 4°). The supernatants were combined and dried under vacuum by rotary evaporation to approximately 300 μL. Methanol was added to give a final volume of 400 μL. Following a further centrifugation (9000 *g*, 4°, 2 min) the supernatant was filtered through 0.22 μm PTFE filter units (Chromos Express, Macclesfield, UK) and analysed by HPLC. Serosal volumes were measured, and an equal volume of methanol containing ascorbic acid (1 mM) was added. Samples were then dried under vacuum by rotary evaporation and methanol added to give a final volume of 400 μL. The samples were then centrifuged (13,600 *g*, 4°, 10 min) and filtered as above for analysis by HPLC. Apigenin (20 μM final concentration) was added to all samples prior to extraction as an internal standard. Mucosal solutions (0.5 mL), including the initial solutions that had been not incubated with the rat intestine, were diluted with methanol (0.5 mL) containing ascorbic acid (1 mM), centrifuged and filtered as described above prior to analysis by HPLC.

Quantitative determination of the conjugates was carried out by hydrolysing sub-samples of mucosal tissue (N = 16) and serosal solution (N = 16) with β-glucuronidase (5 U) and aryl-sulfatase (0.4 U) for 30 min (37°), after which time hydrolysis of metabolites was complete. The amount of quercetin released was calculated and a conversion factor based on peak areas of quercetin-3- and 7-glucuronides was established. As this figure was remarkably consistent ($R^2 = 0.95$) it was used to quantity the conjugates present in all further samples. Data for mucosal solutions were calculated independently from standard curves, as quercetin-3- and 7-glucuronides were the only measurable metabolites in these solutions. Hydrolysis with β-glucuronidase would also release quercetin from the glucoside which is the dominant compound in the mucosal solution.

2.7. Indirect determination of cytosolic β-glucosidase activity in rat small intestine

Cell-free extracts (S9 fraction) of rat small intestine and liver were prepared as described previously [1]. The intestinal extracts (containing 200 μg protein) were incubated for 15 min at 37° with quercetin-3-glucoside (33 μM) in potassium phosphate buffer (50 mM, pH 6, to a final sample volume of 0.5 mL) in the presence or absence of conduritol B epoxide (5 mM). Conduritol B epoxide has previously been shown to inhibit the membrane-bound β-glucosidases glucocerebrosidase [22] and LPH [23]. Quercetin-3-glucoside (33 μM) was also incubated with a cell-free extract of rat liver for 120 min under similar conditions. All reactions were stopped with methanol (0.5 mL) containing ascorbic acid (1 mM), centrifuged (13,600 *g*, 5 min, 4°) and the supernatant filtered prior to analysis by HPLC.

2.8. HPLC analysis

A Hewlett-Packard 1100 HPLC system comprising of an autosampler and quaternary pump coupled to a photodiode array detector was used, controlled by Chemstation software. The column was packed with Prodigy 5 μm ODS3 reversed-phase silica, 250 mm by 4.6 mm i.d., with a guard column, 30 mm × 4.6 mm (Phenomenex). 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^{-1}, 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 90% B (5 min). A column clean-up stage maintained B at 90% (5 min) followed by a re-equilibration at 17% B (15 min). Each sample was injected on to the column (20–30 μL) for analysis, with the diode array monitoring the eluent at 270 and 370 nm. Quercetin-3-glucoside, quercetin-4′-glucoside and quercetin-3-glucuronide were run as external standards after every eight injections. On occasion samples were re-run under different conditions to clearly separate the hydrophilic quercetin metabolites from quercetin-3-glucoside [24]: gradient was 17% B (2 min), increasing to 20% B (10 min), 27% B (35 min), and then to 90% B (5 min).

2.9. Statistical methods

Results are expressed as means with their standard error. The significance of differences between means was assessed using ANOVA, with Tukey's test for the secondary analysis. All calculations were carried out using Minitab statistical software.

3. Results

Quercetin was detected in the mucosal solution after incubation of the rat intestine with quercetin-4′-glucoside

Table 1
Concentration of quercetin recovered as free quercetin or quercetin metabolites in different compartments of the rat everted sac after incubation with quercetin-4′-glucoside or quercetin-3-glucoside

	No inhibitor	Phlorizin (Pz)	NB-DGJ	Pz + NB-DGJ
Quercetin-4′-glucoside (quercetin equivalent; nmol/g tissue)[a]				
Mucosal sol^N				
Quercetin	449 ± 67[b] (8.0)	180 ± 13[b,*] (3.8)	76 ± 15[b,*] (1.5)	83 ± 37[b,*] (1.5)
Q metabolites	264 ± 56[b] (4.7)	74 ± 12[b,*] (1.6)	188 ± 31[b] (3.7)	162 ± 52[b] (3.0)
Tissue				
Quercetin	15 ± 3 (0.3)	22 ± 3 (0.5)	7 ± 3[*] (0.1)	7 ± 3[*] (0.2)
Q metabolites	23 ± 4 (0.4)	22 ± 6 (0.4)	10 ± 1[*] (0.2)	9 ± 1[*] (0.2)
Serosal sol^N				
Q metabolites	13 ± 4 (0.2)	8 ± 2 (0.2)	7 ± 2[*] (0.1)	7 ± 2 (0.1)
Quercetin-3-glucoside (quercetin equivalent; nmol/g tissue)[a]				
Mucosal sol^N				
Quercetin	47 ± 9 (0.7)	45 ± 8 (0.7)	10 ± 2[*] (0.2)	19 ± 1[b] (0.3)
Q metabolites	211 ± 14 (3.6)	172 ± 19 (2.8)	100 ± 11[*] (1.8)	136 ± 17[b] (2.5)
Tissue				
Quercetin	7 ± 2 (0.1)	6 ± 2 (0.1)	4 ± 2 (0.1)	3 ± 1 (0.1)
Q metabolites	12 ± 1 (0.2)	11 ± 1 (0.2)	4 ± 0[*] (0.1)	4 ± 1[*] (0.1)
Serosal sol^N				
Q metabolites	9 ± 1 (0.2)	10 ± 1 (0.2)	3 ± 1[*] (0.1)	2 ± 1[*] (0.1)

Q metabolites represents quercetin present in compartment as quercetin glucuronide or sulfate metabolites. Value in parentheses represents the percentage of initial quercetin glucoside recovered in each compartment.

[a] Value ± SE, where N = 7.

[b] Value ± SE, where N = 3.

[*] Significant difference compared to no inhibitor, $P < 0.05$.

and quercetin-3-glucoside. The concentration of free quercetin was approximately 10 times higher after incubation for 15 min with quercetin-4′-glucoside (449 nmol g^{-1} tissue) rather than quercetin-3-glucoside (47 nmol g^{-1} tissue) as shown in Table 1. NB-DGJ significantly inhibited the deglycosylation of both quercetin glucosides (83% quercetin-4′-glucoside, 79% quercetin-3-glucoside). Phlorizin, although not having any effect on quercetin-3-glucoside hydrolysis, reduced quercetin-4′-glucoside hydrolysis (60%). However, for both glycosides, the presence of both inhibitors had a similar effect to NB-DGJ alone.

Quercetin-3- and 7-glucuronide metabolites were detected in the mucosal solutions at the end of the incubation, demonstrating apical efflux from the enterocyte. The overall concentration of conjugates was similar regardless of initial substrate, suggesting saturation of the efflux transporter (Table 1). Presence of NB-DGJ in the incubation medium resulted in reduced efflux of quercetin glucuronides when quercetin-3-glucoside was the substrate, but had no effect on the efflux of quercetin glucuronides formed from quercetin-4′-glucoside. Conversely, phlorizin did not affect the appearance of quercetin-3-glucoside metabolites in the mucosal solution, but did significantly inhibit efflux of quercetin-4′-glucoside metabolites (note: only three samples of mucosal solutions from incubations with quercetin-4′-glucoside were available for analysis).

Free quercetin and quercetin conjugates, but not quercetin glucosides, were detected within the small intestinal tissue at the end of the incubation. Total quercetin equivalents from quercetin-4′-glucoside were twice those seen in incubation with quercetin-3-glucoside (Table 1). NB-DGJ decreased the concentration of free quercetin and quercetin metabolites, regardless of substrate, but the concentration of total quercetin from quercetin-4′-glucoside in the presence of NB-DGJ was similar to the total concentration of quercetin from quercetin-3-glucoside in the absence of any inhibitor. Phlorizin appeared to increase the amount of free quercetin in the tissue when quercetin-4′-glucoside was incubated with the rat intestine, but had little affect on the concentration of quercetin metabolites within the tissue. Phlorizin did not affect the concentration of quercetin-3-glucoside metabolites or free quercetin within the mucosa. When sacs were exposed to phlorizin and NB-DGJ simultaneously, results were similar to those observed with NB-DGJ alone.

Following incubation of rat everted-jejunal sacs with either quercetin-4′-glucoside or quercetin-3-glucoside, the major compounds detected in the serosal solutions were quercetin metabolites, mainly quercetin glucuronides. The metabolites were qualitatively the same as in our previous study [10] and were identical for both quercetin-3- and 4′-glucoside (Fig. 1A and B). Further, the qualitative metabolic profile was not affected by the presence of phlorizin or NB-DGJ (Fig. 1C and D). Quercetin glucosides were not detected in the majority of serosal solutions, although trace levels (<1%) of quercetin-3-glucoside were present when the incubation media contained NB-DGJ (with or without phlorizin). Data from one rat was excluded due to lack of intestinal integrity as evidenced by absence of substrate metabolism in any compartment.

Although there was a tendency towards a higher serosal transfer of metabolites derived from quercetin-4′-glucoside

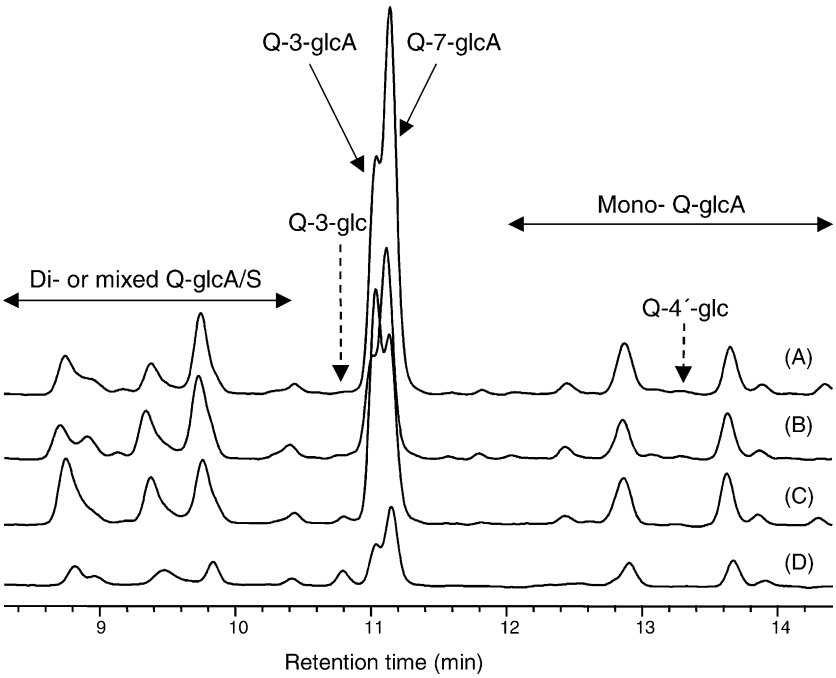

Fig. 1. Chromatograms showing the metabolic profile of (A) quercetin-4′-glucoside, (B) quercetin-3-glucoside, (C) quercetin-3-glucoside in the presence of phlorizin, and (D) quercetin-3-glucoside in the presence of *N*-(*n*-butyl)-deoxygalactonojirimycin, after transport across the rat everted-jejunum. Absorbance was monitored at 370 nm. Only the section of chromatograms, where major metabolites elute are shown for clarity. Q is quercetin, glc is glucoside, glcA is glucuronide, S is sulfate, dotted vertical arrows indicates the elution time for quercetin-3- or 4′-glucoside.

compared to metabolites derived from quercetin-3-glucoside, this difference was not significant (Table 1). For both parent compounds amounts transferred to the serosal solution were significantly reduced by the presence of NB-DGJ in the incubation medium (quercetin-4′-glucoside, 50%; quercetin-3-glucoside, 39%; $P < 0.05$). Phlorizin appeared to reduce the transfer of metabolites from quercetin-4′-glucoside to a similar extent as NB-DGJ, but had no affect on the transfer of metabolites from quercetin-3-glucoside to the serosal solution. The combined co-incubation of both inhibitors had no additional effect to NB-DGJ alone on quercetin glucoside metabolite transfer.

Fig. 2 shows the total amount of quercetin glucoside metabolised (deglycosylated and/or conjugated) by the rat small intestine over the course of the incubation. The total amount of quercetin-4′-glucoside metabolised during the experiment was at least 2-fold greater than in incubations with quercetin-3-glucoside, or quercetin-4′-glucoside in the presence of either inhibitor. The difference is accounted for mainly in terms of higher levels of free quercetin in the mucosal solution. Metabolism of quercetin-4′-glucoside was significantly reduced by the presence of phlorizin and/or NB-DGJ in the incubating media (∼58% in all cases, $P < 0.05$). In contrast quercetin-3-glucoside metabolism was significantly reduced in the presence of NB-DGJ (53%, $P < 0.05$) or NB-DGJ + phlorizin (36%, $P < 0.05$).

Quercetin-3-glucoside was deglycosylated by rat small intestine cell-free extract (28% hydrolysed in 15 min incubation), but not rat liver cell-free extracts (0% in 120 min incubation). Conduritol B epoxide, a competitive inhibitor of membrane-bound β-glucosidases but not of broad-specificity cytosolic β-glucosidase, significantly reduced quercetin-3-glucoside hydrolysis by 87%. These results indicate that quercetin-3-glucoside is not a substrate for rat cytosolic β-glucosidase (EC 3.2.1.21), which is in agreement with previous studies on cytosolic β-glucosidase from other sources [25,26]. Furthermore, the results demonstrate that deglycosylation of quercetin-3-glucoside is facilitated by a membrane-bound β-glucosidase, such as

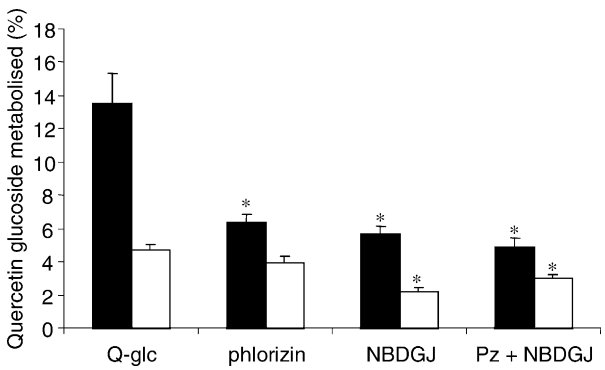

Fig. 2. Percentage of quercetin glucoside (800 nmol) metabolised by rat everted-jejunal sacs over a 15 min incubation in the presence of absence of phlorizin (Pz; 25 μM) or *N*-(*n*-butyl)-deoxygalactonojirimycin (NB-DGJ; 250 μM). Each value represents aglycone plus conjugates recovered from mucosal, tissue and serosal compartments combined. Solid bars represent quercetin-4′-glucoside, open bars represent quercetin-3-glucoside. Data are means ± SEM, N = 7. Asterisk (∗) indicates significant difference compared to no inhibitor, $P < 0.05$.

LPH, that is present in the small intestine but not present in liver.

4. Discussion

Inhibiting the lactase domain of LPH in a rat *in vitro* intestinal model significantly reduced both luminal deglycosylation of quercetin-3- and 4′-glucoside and the transfer of metabolites to the serosal solution. LPH is an enzyme with two active sites and by using controlled inactivation of phlorizin hydrolase we previously showed that quercetin glucosides were hydrolysed *in vitro* mainly (75–85%) at the lactase active site [2]. This was confirmed here by the significant suppression (around 80%) of quercetin-3- and quercetin-4′-glucoside hydrolysis in the presence of a lactase-specific inhibitor. Cytosolic β-glucosidase, from rat small intestine, appears unable to deglycosylate quercetin-3-glucoside, providing evidence that the hydrolytic route for quercetin-3-glucoside is almost certainly *via* the action of LPH at the apical surface of the enterocyte. Only when lactase activity was suppressed did intact quercetin-3-glucoside appear in the serosal solution, and then to a very limited extent. These observations are in agreement with the results of Wolffram *et al.* [27], whereby quercetin-3-glucoside was not detected within the mucosal tissue or after serosal transport in a similar rat intestine model. However, these authors attributed the presence of quercetin in all compartments to be a result of the action of cytosolic β-glucosidase, which we, and others [25,26], have shown not to be active on quercetin-3-glucoside. Quercetin-4′-glucoside, however, is a substrate for cytosolic β-glucosidase and, therefore, absence of the 4′-glucoside in the tissue or serosal solution would be expected.

Phlorizin was shown to have a negligible effect on quercetin-3-glucoside hydrolysis, tissue uptake and transfer into the serosal solution. In contrast, when quercetin-4′-glucoside was subjected to similar incubation conditions there was a significant reduction in hydrolysis of the glucoside, and the concentration of quercetin metabolites was lower in the serosal compartment. The reason for the inhibition (60%) of quercetin-4′-glucoside deglycosylation by phlorizin is not clear, as it would suggest a large contribution to activity from the phlorizin hydrolase domain of LPH. This would be surprising, given the 80% inhibition of hydrolysis from NB-DGJ, but cannot be ruled out.

The degree of quercetin-4′-glucoside hydrolysis was 10 times the level of quercetin-3-glucoside hydrolysis suggesting that quercetin-4′-glucoside is a better substrate than quercetin-3-glucoside for LPH in the rat intestine. Although variation between species may account for the differences observed, the catalytic efficiency of purified sheep LPH has been shown to be similar for both compounds [2]. We also speculate that the interaction of quercetin-4′-glucoside with SGLT1 somehow increases the rate of deglycosylation by LPH, to an extent not seen with quercetin-3-glucoside, and that phlorizin could reduce such an interaction. Previous reports [28,29] have shown that glucose liberated from the hydrolysis of phlorizin at the mucosal surface appears to have a kinetic advantage for uptake *via* the sodium-dependent transport pathway, despite the blockade of SGLT1 by phlorizin. This was attributed to a proximity effect of transporter and hydrolase enzyme within the confines of the unstirred mucosal layer. Interestingly, a similar concentration of quercetin metabolites from quercetin-4′-glucoside and quercetin-3-glucoside was observed in the serosal compartment, despite the higher concentration of quercetin in the mucosal solution derived from quercetin-4′-glucoside. One explanation for this is that both the apical and the baso-lateral transporters may be close to saturation, which would slow diffusion into the enterocyte due to a reduced concentration gradient.

The effects of phlorizin within the intestine are complex, and hence inferences based on a specific inhibitory mechanism of phlorizin require caution. Phlorizin is a competitive inhibitor of SGLT1 with an extremely high affinity, but is itself not transported [30]. Phlorizin is also a substrate for the phlorizin hydrolase domain of LPH. Phloretin, released from the hydrolytic step, will compete with other phenolics for both UDP-glucuronosyltransferase (UGT) during the process of conjugation to glucuronic acid, and apical and basolateral transporters [31]. Fig. 3 represents the potential competition between quercetin and phloretin within the small intestine enterocyte.

In previous studies, we [10], and others [32], reported an increased absorption of quercetin glucosides compared to

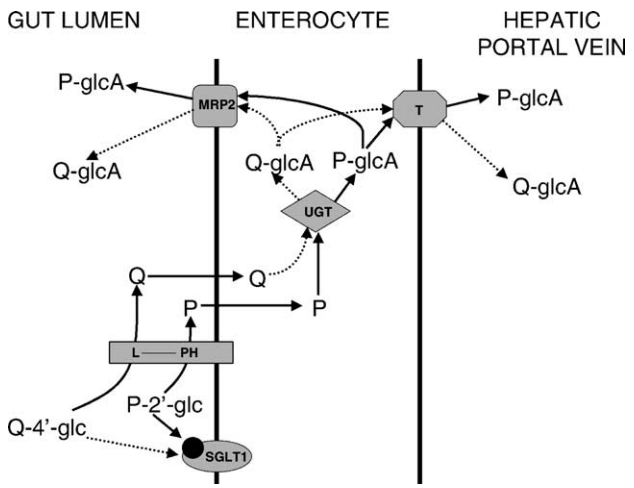

Fig. 3. Potential interactions of phlorizin and quercetin-4′-glucoside within the enterocyte. Q-4′-glc is quercetin-4′-glucoside, Q is quercetin, Q-glcA are quercetin glucuronide metabolites, P-2′-glc is phlorizin, P is phloretin, P-glcA are phloretin glucuronide metabolites, SGLT1 is the sodium-dependent glucose transporter 1, LPH is lactase phlorizin hydrolase (domains L and PH are indicated), MRP2 is the multi-drug transporter 2, T is an unidentified transporter, UGT is UDP-glucuronosyltransferase. Solid arrows represent proposed major metabolic pathways. Where these are competitively inhibited, routes are indicated by dotted lines.

Table 2
Relative activities of lactase phlorizin hydrolase (LPH) in cells and tissues

	Typical specific activity on lactose (mU mg^{-1} protein)	Reference
Caco-2 cells	0.1	[16]
Caco-2 cells clone PD7	0.3	[16]
Human small intestine (lactose absorbers)	20–80	[34]
Human small intestine (lactose malabsorbers)	2–10	[34]
Rat small intestine	10–20	[35]
	28	[36]

the aglycone in rat model systems. We attributed this to the quercetin released by deglycosylation having a kinetic advantage in absorption due to an increased localised concentration in the unstirred layer stimulating diffusion. Crespy *et al.*, using a rat perfused intestinal model, have recently shown that although the net absorption of quercetin-3-glucoside is twice that of the aglycone the total amounts absorbed into the enterocyte (including mucosally transported metabolites) were similar [33]. These experiments suggest an alternative hypothesis: the glucoside does not stimulate uptake of quercetin into the enterocyte, but slows apical efflux of the glucuronide, thereby increasing the transfer of metabolites to the serosal compartment. Contrasting data from studies with Caco-2 cell models [11,12], showing that the flavonoid aglycone was absorbed better than the glycosides, can be attributed to the dramaticallly lower expression of LPH in this cell line compared with intact intestine, as shown in Table 2. Due to very low expression of LPH in Caco-2 cells, SGLT1 appears to play a proportionately greater role in uptake of flavonoid glucosides and, as in the rat model, quercetin-4′-glucoside is more efficiently absorbed than quercetin-3-glucoside.

We conclude that although flavonol glycosides are transported to a small extent by SGLT1, deglycosylation by LPH and subsequent diffusion of the aglycone is the major route of absorption for both quercetin glucosides *in vivo*. In support of this hypothesis, there was no additive effect when tissue was incubated with a combination of phlorizin and NB-DGJ on quercetin-4′-glucoside absorption, which would also be expected if SGLT1 played a major role. It seems that quercetin-3-glucoside utilises the LPH hydrolytic pathway almost exclusively, whereas two potential routes of passage into the enterocyte appear to be available to quercetin-4′-glucoside: luminal hydrolysis by LPH to quercetin followed by diffusion of the aglycone, or transport by SGLT1 with subsequent deglycosylation by cytosolic β-glucosidase. Although SGLT1 and LPH are critical for absorption of quercetin glucosides, the role these proteins play with absorption of other flavonoid sub-classes has yet to be determined. A greater understanding of absorption early in the gastrointestinal tract will help identify the extent to which putative bioactive compounds may reach flavonoid-responsive sites within the body.

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