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The small intestine can both absorb and glucuronidate luminal flavonoids

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Abstract We have studied the perfusion of the jejunum and ileum in an isolated rat intestine model with flavonoids and hydroxycinnamates and the influence of glycosylation on the subsequent metabolism. Flavone and flavonol glucosides and their corresponding aglycones are glucuronidated during transfer across the rat jejunum and ileum and this glucuronidation occurs without the need for gut microflora. Furthermore, this suggests the presence of glycosidases as well as UDP-glucuronyl transferase in the jejunum. In contrast, quercetin-3-glucoside and rutin are mainly absorbed unmetabolised. The results suggest that the more highly reducing phenolics are absorbed predominantly as glucuronides (96.5% ± 4.6) of the amount absorbed, whereas monophenolic hydroxycinnamates and monophenolic B-ring flavonoids are less predisposed to glucuronidation and higher levels of aglycone (88.1% ± 10.1) are detected on absorption through both the jejunum and ileum.

Key words: Flavonoid; Hydroxycinnamate; Absorption; Glucuronide; Jejunum; Ileum

1. Introduction

Flavonoids and hydroxycinnamates are polyphenolic phytochemicals, present in abundance in fruit, vegetables, beverages and grains [1]. The structure-dependent hydrogen-donating abilities of polyphenolics and their propensity for nitration make many of these compounds powerful scavengers of reactive oxygen and reactive nitrogen species [2,3]. Although epidemiological studies based on assessment of intake and clinical endpoints have implicated a role for polyphenolic compounds in disease prevention [4,5], their role as antioxidants in vivo is still unclear. The extent of their antioxidant potential in vivo will be dependent on the metabolism, absorption and excretion of these compounds within the body after ingestion and the reducing properties of the resulting metabolites.

There is much controversy as to whether natural flavonoid glycosides can be absorbed by the gastrointestinal tract, or whether they are hydrolysed in the small intestine prior to absorption. A number of early studies hypothesised that flavonoids would not enter the circulation, either as the natural glycosides or as the aglycone hydrolysis products [6–9]. It was thought that cleavage at the central heterocyclic ring by intestinal bacteria would occur effectively destroying the antioxidant properties of the compounds and generating phenolic acid fission products [6–9]. However, recent studies have detected quercetin-3-rhamnoglucoside (rutin) in the circulation after the consumption of apple or onion [10] and in blood plasma of individuals consuming a generally high fruit and vegetable diet [11]. Anthocyanidin glycoside (anthocyanin) absorption has also been reported in humans [12–14]. The direct intestinal absorption of cyanidin-3-glucoside has been detected in plasma with no appearance of the aglycone after administration to rats, although it was present in the jejunum [15]. In addition, studies on plasma have also demonstrated the direct absorption of cyanidin-3-glucoside in rats and in man derived from red fruit, elderberries and blackcurrants [15].

The purpose of this study was to investigate the absorption and metabolism of flavones, flavonols, flavanones and their glycosides and hydroxycinnamates in the gastrointestinal tract by using an isolated preparation of jejunum and ileum from the small intestine and monitoring resulting native compound, conjugates and metabolites. Naturally occurring dietary glycosides were also compared with free aglycones to investigate the influence of glycosylation on the fate of these compounds.

2. Materials and methods

2.1. Materials

Flavonoids and hydroxycinnamates were obtained from Extrasynthase (ZI Lyon Nord, Genay, France). Quercetin and rutin were obtained from AASC (Southampton, Hampshire, UK). β-Glucuronidase (type L-II from limpets) was purchased from Sigma (Poole, Dorset, UK) and β-glucosidase (from almonds) was purchased from ICN Biomedicals (Thame, Oxon, UK). HPLC grade acetonitrile and methanol were purchased from Rathburn (Walkerburn, UK) and Elgstat UHP double distilled water (18.2 Ω grade) was used throughout the study. HPLC columns were purchased from Waters (Watford, Herts., UK). All other reagents used were of analytical grade and obtained from Sigma.

2.2. Absorption studies

Absorption studies were conducted using the in vitro intestinal preparation of Fisher and Gardner [16] in which the lumen of isolated intestine was perfused with a segmental flow (defined as perfusion of buffer interspersed with bubbles of the gas mixture) of bicarbonate buffer (pH 7.4) equilibrated with 95% O_2:5% CO_2 and containing 28 mM glucose. The bicarbonate buffer consisted of Krebs bicarbonate saline solution (HCO_3^-: 25 mM; Na^+: 143 mM; Cl^-: 133.7 mM; K^+: 5.9 mM; HPO_4^-: 1.2 mM) containing Ca^{2+} (2 mM) and Mg^+ (1.2 mM).

Male Sprague-Dawley rats (230–260 g) were killed with pentobarbital sodium (90 mg/kg, i.p.) and sections of jejunum (15–30 cm long, beginning 10 cm from the ligament of Treitz) or ileum (20–30 cm long, ending 5 cm from the ileo-caecal junction) were cannulated and the lumen perfused with the buffer described above. The segments of intestine were then removed from the animal and suspended in a chamber containing liquid paraffin at 37°C whilst maintaining the segmental flow of buffer. Before uptake experiments were conducted the segment was perfused for 40 min in order to flush blood from the vasculature and to allow fluid absorption to reach a steady state.

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Thereafter, flavonoids and hydroxycinnamates, at the stated concentrations, were added to the buffer and perfused through the system, in a single pass fashion, for up to 90 min. In some cases the buffer was recirculated through the loop. During perfusion, absorbed fluid dropped through the paraffin to the base of the chamber and was collected at timed intervals. Samples collected from both the jejunum and the ileum were immediately diluted 1:4 with methanol and stored at −70°C until analysis.

2.3. HPLC analysis

Internal standards were added to samples (100 μl) before analysis by HPLC. For hydroxycinnamates the internal standard was salicylic acid (final concentration: 10 μM). For luteolin, luteolin-7-glucoside, quercetin, quercetin-3-glucoside and rutin the internal standard was kaempferol (10 μM). For analysis of kaempferol and its glycosides, quercetin-3-glucoside (10 μM) was used as the internal standard. In all cases 30 μl was injected onto the column for analysis. The presence of both flavonoid and hydroxycinnamate glucuronides in the gut samples was established by treatment with β-glucuronidase, type L-II from limpets. Incubation of samples with enzyme (1000 units) was for 120 min at 37°C in a 0.1 M sodium phosphate buffer, pH 3.8. The 0.1 M phosphate buffer effectively inhibits all sulphatase activity which the enzyme possesses. Samples recovered from perfusion experiments performed with flavonoid glycosides were also treated with β-glucosidase (1000 U/ml; 2 h, 37°C) in order to determine the presence of any absorbed glycosides in the samples. This was important as the β-glucuronidase was observed to cleave glucoside bonds as well as glucuronide bonds.

HPLC analysis to measure compound and metabolite concentrations was carried out using a Waters 626 pump and 600 controller system with an autoinjector 717 and a photodiode array detector 996 linked to the Millennium Software system. Samples were analysed by reverse-phase HPLC using a Nova-Pak C18 column (4.6×250 mm) (Waters Company) with 4 μm particle size. The temperature of the column was maintained at 30°C. The mobile phase consisted of a mixture of aqueous methanol (20%) in hydrochloric acid (0.1%) and acetonitrile (MeCN) and was pumped through the column at 0.5 ml/min. The following gradient system was used (min/% MeCN): 0/5, 10/5, 40/50, 60/5 for detection of all flavonoids, hydroxycinnamates and metabolites. The eluant was monitored by photodiode array detection at 280 and 320 nm and spectra of products obtained over the 220–600 nm range. Calibration curves of the compounds were constructed using authentic standards (0–100 μM) and in each case were found to be linear with correlation coefficients of >0.995.

3. Results

The comparative absorption of flavonoids and their glycosides across the jejunum and ileum in a rat intestinal model was investigated just as the extents of their conjugation and metabolism. Table 1 demonstrates the results for the different families of phenolics studied: hydroxycinnamates, flavones, flavonols and flavanones. The natural dietary forms of flavonoids studied are glycosylated in the 7-position for luteolin and hesperetin, the 3-position for quercetin and kaempferol and the quinic acid ester for chlorogenic acid. The original concentration of compounds perfused is shown (in parentheses) and the cumulative concentrations of the absorbed components are in the form of native compound, aglycone of perfused compound and glucuronide of aglycone. Results are expressed as nmol/20 cm jejunum (ileum)/5 μmol perfused compound, i.e. standardisation to the amount in the lowest concentration perfused, for comparative purposes, but a linear relationship between uptake and concentration perfused cannot necessarily be assumed.

3.1. Absorption of flavones/flavonols

3.1.1. Luteolin and luteolin-7-glucoside. Luteolin was measurable throughout the experiment when the isolated rat jejunum was perfused with either the aglycone (Fig. 1A) or the monoglycoside, luteolin-7-glucoside (Fig. 1B). The amounts of luteolin recovered at each time point were similar and increased in a time-dependent sigmoidal manner (Fig. 1A,B). However, measured levels of the aglycone were small relative to the amount of total luteolin glucuronide recovered after perfusion with either the aglycone or its 7-glucoside (Fig. 1A,B; Table 1). Treatment with β-glucuronidase (1000 units; 37°C, 2 h) indicated the presence of at least six different glucuronides which were detected by our HPLC protocol (Fig. 2). These glucuronides had closely similar retention times, ranging from 32.0 to 38.5 min and spectral characteristics

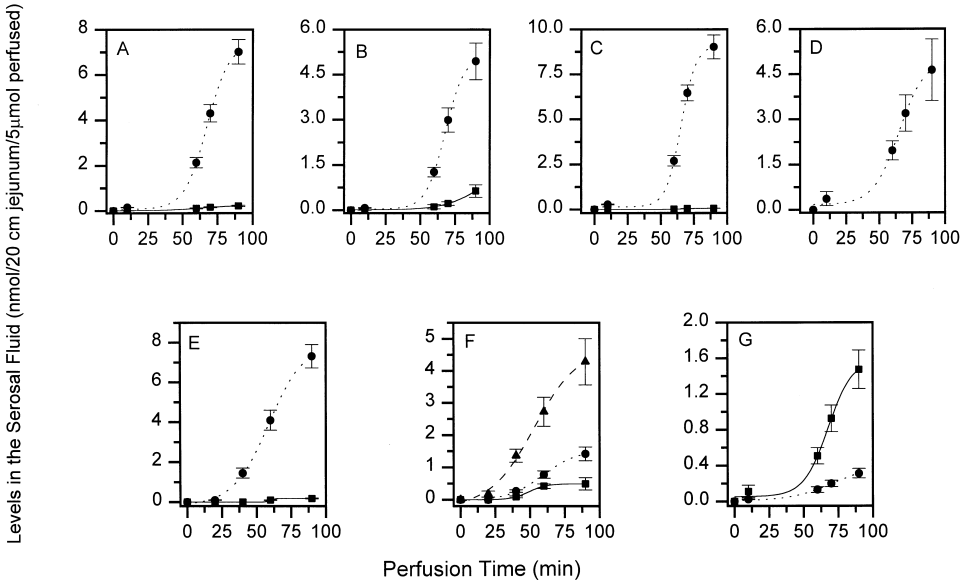

Fig. 1. Cumulative absorption of flavonoids and their glucuronides (nmol/20 cm gut/5 μmol perfused sample) over 90 min through isolated rat jejunum after perfusion with (A) luteolin, (B) luteolin-7-glucoside, (C) kaempferol, (D) kaempferol-3-glucoside, (E) quercetin, (F) quercetin-3-glucoside, (G) hesperetin. Experiments were performed as outlined in Section 2 and in the legend of Table 1. Data are plotted as the mean ± S.E.M. of at least three separate experiments each analysed twice in duplicate. (■) Perfused compound, (●) glucuronide, (▲) aglycone of perfused glycoside.

Table 1
Comparison of cumulative absorption of perfused compounds, aglycones and glucuronides absorption through isolated rat jejunum and ileum (mean value after 90 min)

Perfused compound (concentration of compound perfused μM)	Jejunum (nmol/20 cm jejunum/5 μmol perfused)				Ileum (nmol/20 cm ileum/5 μmol perfused)			
	Perfused compound	Aglycone of perfused compound	Glucuronide	Total	Perfused compound	Aglycone of perfused compound	Glucuronide	Total
Caffeic acid (74.8)	0.695	*	1.220	1.915	0.191	–	0.103	0.294
Ferulic acid (76.0)	6.690	*	1.480	8.170	0.658	–	0.319	0.977
Chlorogenic acid (75.8)	0.115	0.000	0.000	0.115	0.028	–	0.000	0.028
p-coumaric acid (72.7)	3.745	*	0.000	3.745	No data	No data	No data	No data
Quercetin (13.1)	0.185	*	7.317	7.502	0.654	–	3.170	3.824
Quercetin-3-glucoside (47.6)	4.283	0.492	1.419	6.194	No data	No data	No data	No data
Rutin (67.1)	2.059	0.000	0.000	2.059	No data	No data	No data	No data
Luteolin (87.3)	0.222	*	7.014	7.236	0.663	–	4.462	5.125
Luteolin-7-glucoside (81.7)	0.000	0.636	4.941	5.577	No data	No data	No data	No data
Kaempferol (78.9)	0.062	*	9.022	9.084	3.858	–	15.973	19.831
Kaempferol-3-glucoside (5.5)	0.000	0.000	4.633	4.633	0.000	0.000	0.419	0.419
Hesperetin (51.6)	1.473	*	0.313	1.786	2.334	–	0.000	2.334
Hesperidin (hesperetin-3-rhamnoglucoside) (50.4)	0.000	0.000	0.000	0.00	No data	No data	No data	No data

Results are expressed as nmol/20 cm jejunum or ileum/5 μmol perfused compound in order to standardise to the amount in the lowest concentration perfused. Experiments were performed as described in Section 2. * indicates that the perfused compound is the aglycone.

which would suggest they were mono-glucuronidated at different sites and possibly di- or tri-glucuronidated (Fig. 2A). Recoveries of both the luteolin aglycone and its glucuronides were lower across the ileum relative to the jejunum (Table 1). Samples of the buffer solution containing the compounds of interest before and after the experiment were analysed by HPLC to ascertain that the compounds were present and stable throughout the course of the experiment. Luteolin, luteolin-7-glucoside, kaempferol and kaempferol-3-glucoside were stable over the duration of the 90 min experiment.

3.1.2. Kaempferol and kaempferol-3-glucoside. Perfusion of the jejunum or the ileum with kaempferol resulted in the transfer of large amounts of kaempferol glucuronides and a small amount of the aglycone across the gastrointestinal membrane to the serosal fluid (Fig. 1C; Table 1). Levels of both the glucuronide and the aglycone were higher when the ileum was perfused with kaempferol compared to the jejunum (Table 1). However, no kaempferol was detected in samples at any time point when either the jejunum or the ileum was perfused with kaempferol-3-glucoside, although, relatively large amounts of kaempferol glucuronide were detected after perfusion with kaempferol-3-glucoside (Fig. 1D). This may have been due to the lower concentration of kaempferol-3-glucoside to which the jejunum was exposed. Three glucuronidated forms of kaempferol were detected with retention times ranging from 33.5 to 36.0 min on perfusion with the aglycone but only one when the 3-glucoside was perfused as evidenced by the effects of β-glucuronidase. There was no significant loss of kaempferol or its 3-glucoside over the 90 min experimental period.

3.1.3. Quercetin, quercetin-3-glucoside and rutin. The amount of quercetin aglycone absorbed through the isolated rat jejunum versus time, after perfusion with quercetin, is presented in Fig. 1E. No quercetin was detected in samples at time points earlier than 60 min, however, small amounts were present at later times. As with kaempferol, higher quercetin levels were measurable in ileum perfused samples (Table 1). By contrast to the aglycone, relatively large amounts of quercetin glucuronides were detected in the serosal fluid over the 90 min perfusion time (Fig. 1E). Perfusion of the jejunum with quercetin-3-glucoside resulted in the detection of quercetin-3-glucoside, the hydrolysis product quercetin as well as quercetin glucuronides (Fig. 1F) with quercetin-3-glucoside being the major absorbed species. In the case of rutin (quercitrin-3-rhamnoglucoside) there was no quercetin aglycone or glucuronide detected but rutin was detected (Table 1) suggesting that hydrolysis did not occur but that the diglycoside was absorbed in the native form. Measurement of quercetin, in pre- and post-buffer samples indicated that some oxidation of the compound may be occurring over the course of the experiment, although quercetin-3-glucoside and rutin appeared more stable.

3.2. Absorption of flavanones

3.2.1. Hesperetin and hesperidin. Perfusion of the jejunum and ileum with hesperetin resulted in its detection at all time points (Fig. 1G). Small amounts of glucuronide were detected after 60 min of perfusion but the major component was the native perfused form. In contrast, no hesperidin (hesperetin-7-rhamnoglucoside), its hydrolysis nor glucuronidation products were detected at any time point after perfusion with hesperidin (Table 1). Both compounds were stable over the course of the experiments.

3.3. Absorption of hydroxycinnamates

The amounts of caffeic acid, ferulic acid, *p*-coumaric acid and chlorogenic acid (nmol/20 cm gut/5 μmol perfused compound) absorbed through rat jejunum versus time are presented in Fig. 3A–D, respectively. Large amounts of ferulic acid and *p*-coumaric acid aglycone were detected in gut samples cumulating after 60 min perfusion (Fig. 3A,B, respectively). However, the amounts of caffeic acid (Fig. 3C) and its quinic ester, chlorogenic acid (Fig. 3D), were low at all time points. In addition, in contrast to the flavonoids the absorption of the hydroxycinnamates through the ileum was much lower than that observed through the jejunum (Table

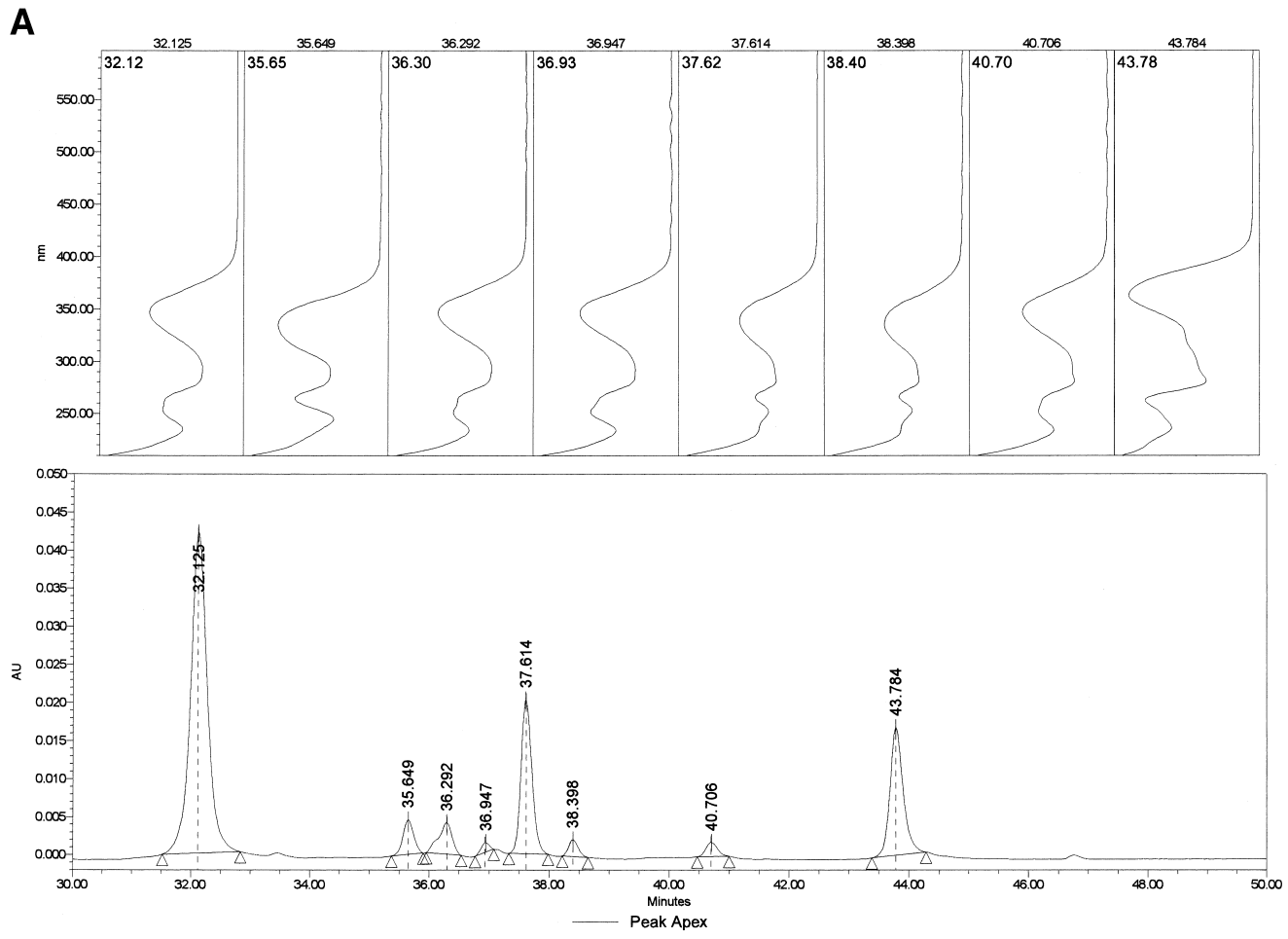

Fig. 2. Representative HPLC chromatogram after perfusion of isolated rat jejunum with luteolin. A: No enzyme treatment. B: Treatment with β-glucuronidase (1000 units) for 2 h at 37°C. Peaks at retention times of ~40.7 min and ~43.8 min are luteolin and kaempferol (internal standard), respectively.

1). For example, over the 90 min perfusion period the absorption of ferulic acid through the ileum was approximately 10-fold lower than through the jejunum (0.658 and 6.690 nmol/20 cm gut/5 μmol perfused sample, respectively) (Table 1).

After perfusion with caffeic acid for 90 min two glucuronides were detected in the serosal fluid throughout the experiment. The levels of glucuronide detected were approximately two-fold higher than caffeic acid (Table 1; Fig. 3C), the amount of native caffeic acid absorbed through the jejunum being 0.695 nmol/20 cm gut/5 μmol perfused compound, compared to the amount of caffeic acid glucuronides of 1.220 nmol/20 cm gut/5 μmol perfused compound. Significant amounts of ferulic acid glucuronide were observed (1.480 nmol/20 cm gut/5 μmol perfused compound), however, this was low compared to the amount of the ferulic acid itself (6.690 nmol/20 cm gut/5 μmol perfused compound). In contrast to the other hydroxycinnamates, no glucuronide of chlorogenic acid was observed at any time point. Data for the absorption of hydroxycinnamate glucuronides across the ileum followed a similar pattern to that seen across the jejunum but was much lower (Table 1).

4. Discussion

The purpose of this study was to determine the forms in which the flavonoids and phenolics studied are absorbed and to obtain information on their metabolism in the small intestine. In order for the glycosides luteolin-7-glucoside, kaempferol-3-glucoside, quercetin-3-glucoside and rutin to appear as glucuronides of their aglycones, the glycosidic groups must first be cleaved by an intestinal enzyme prior to glucuronidation. The studies reported here show that luteolin-7-glucoside, kaempferol-3-glucoside and quercetin-3-glucoside are cleaved by rat jejunal or ileal mucosa, suggesting the presence of a glucosidase, and that a UDP glucuronyl transferase is present glucuronidating the phenolics before efflux into the serosal fluid. The findings here concerning quercetin-3- and kaempferol-3-glucosides are in contrast with the study of Day et al. [17] describing a β-glucosidase in cell free extracts of the human small intestine hydrolysing some monoglucosides, e.g. quercetin-4′-glucoside, naringenin-7-glucoside, but not quercetin-3-glucoside, kaempferol-3-glucoside, nor the diglycosides such as rutin and quercetin-3,4′-diglucoside. Prior to this, it had been assumed that the glycosides could not be absorbed from the small intestine and cleavage of the β-glycosidic bond will not occur until the compound reaches the microflora of the large intestine.

The advantage of the isolated preparation used in this study to assess intestinal transfer of both flavonoids and hydroxycinnamates and their glycosides is that serial measurements of

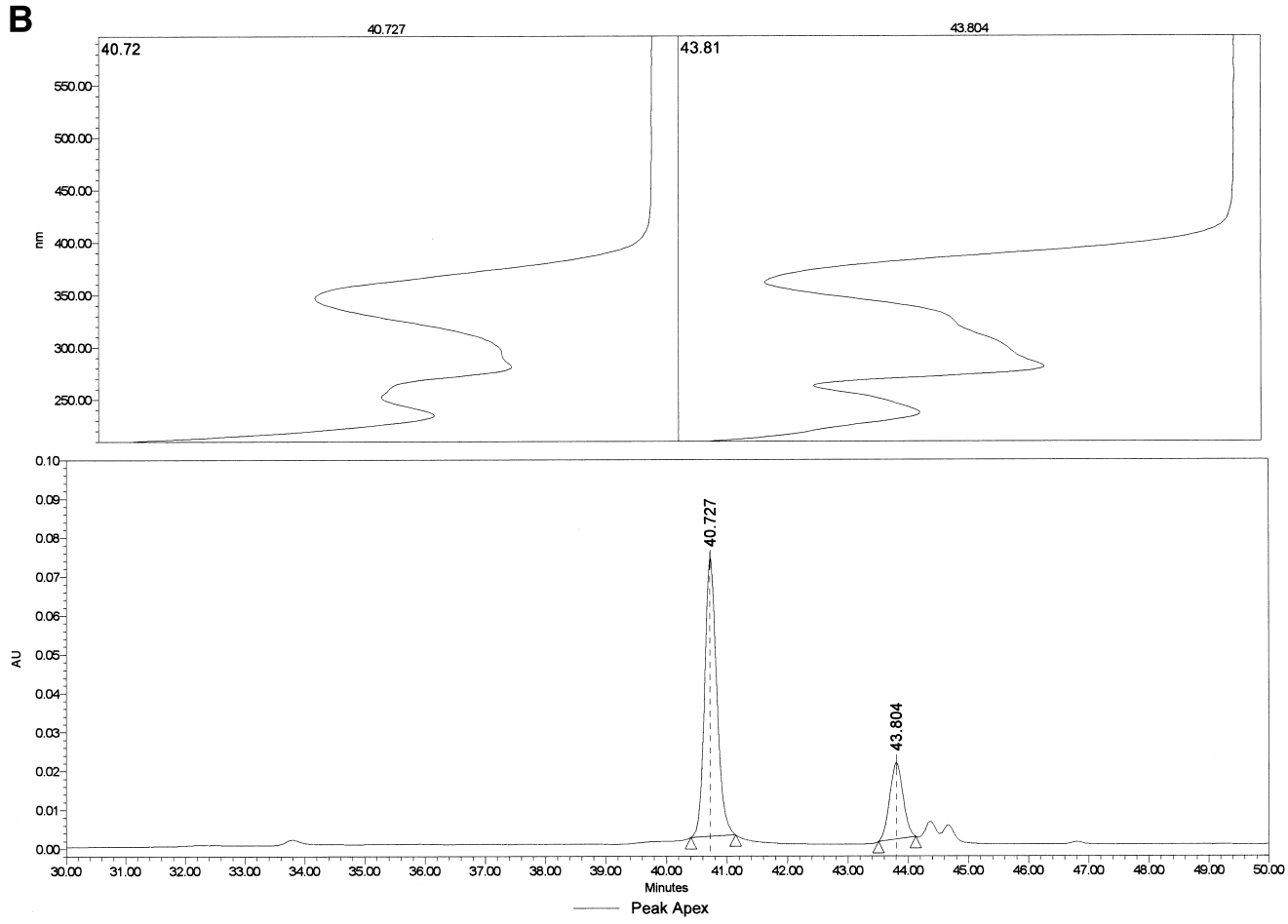

Fig. 2 (*continued*).

the rate of absorption from the lumen can be made using defined intestinal segments at short intervals. In addition the solute under study appears on the serosal surface in the same form as if it were transferred to the mesenteric circulation. Therefore, enterocyte metabolism of the flavonoids and hydroxycinnamates, as well as their rate of transfer across specific gut regions, may be studied. As reported previously [16], tissue viability in our studies was confirmed by the finding that fluid transfer continued at a constant rate for the 90 min collection period, and glucose concentration in the absorbed fluid was over double that initially present in the perfused buffer (65.4 ± 3.7 mM versus 28 mM [$n = 5$]), indicating the ability for active solute transport. Although other intestinal preparations have been used to study flavonoid uptake, the results obtained often need to be interpreted with caution and may explain some discrepancies of conclusions compared to this present work. As an example, the recent report by Crespy et al. [18] using in vivo perfused intestine compares the concentrations of flavonoid in the inflow and outflow buffer in order to calculate uptake of the substrate under study. However, this method of analysis takes no account of fluid absorption from lumen to blood which is likely to be considerable in view of the rate of perfusion, length of intestine used and the presence of glucose in the buffer. In contrast to the present investigation, the failure of Crespy et al. [19] to detect uptake of rutin is likely to have a methodological basis.

Our results suggest that the major products transferred across the intestinal epithelium are glucuronides of the parent aglycone or of the hydrolysed glycoside. Whether perfused with the aglycone or the glycoside, the dominant forms present in the serosal fluid (90–100% of total) were the glucuronides of kaempferol and luteolin. However, while a small proportion of aglycone was also absorbed from perfusion with aglycone, the native glycosides were not detected after perfusion of the glycosides. While the same situation pertained for quercetin, their glycosides (the 3-monoglucoside and the 3-rhamnoglucoside) were absorbed to a major extent in these glycosylated forms. Indeed, previous studies have detected rutin and quercetin-3-glucoside in human plasma [10,11]. It has been proposed that glycoside linkages are less stable than, for example, glucuronide linkages and might not withstand the acidic environment of the stomach [19]. In the case of quercetin glycosides, at least, this has been shown not to be the case from in vivo studies.

Hydroxycinnamate absorption in the jejunum seems to be related to the oxidisability of the compound in question. For example, relatively high amounts of ferulic acid are absorbed through the jejunum over the 90 min (6.69 nmol/20 cm gut/5 μmol perfused) but only low amounts of both the catechol-containing monophenolics caffeic acid (0.695 nmol/20 cm gut/5 μmol perfused) or its quinic ester (0.115 nmol/20 cm gut/5 μmol perfused) are detected after exposure for the same length of time. This difference may be a result of the higher susceptibility to oxidation of the two latter compounds at the intes-

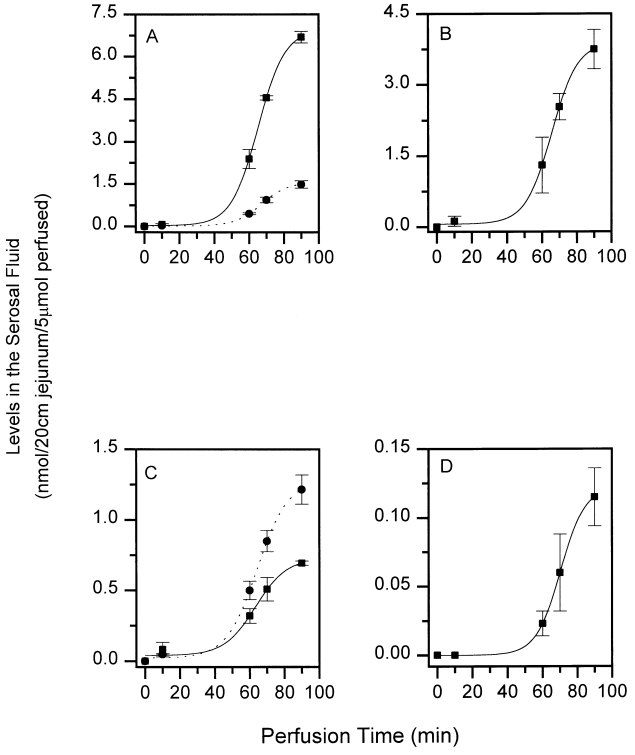

Fig. 3. Cumulative absorption of hydroxycinnamates and their glucuronides (nmol/20 cm gut/5 μmol perfused sample) over 90 min through isolated rat jejunum after perfusion with (A) ferulic acid, (B) *p*-coumaric acid, (C) caffeic acid, (D) chlorogenic acid. Experiments were performed as outlined in Section 2 and in the legend of Table 1. Data are plotted as the mean ± S.E.M. of at least three separate experiments analysed in duplicate. (■) Perfused compound, (●) glucuronide.

tinal epithelium wall. The extent of glucuronidation is clearly dependent on the available hydroxyl groups. It is worthwhile emphasising the observation that the monophenolic hydroxycinnamates and the flavonoid with a substituted hydroxyl group on the B-ring (ferulic acid, *p*-coumaric acid and hesperetin, respectively) are also less predisposed to glucuronidation and relatively high levels of the aglycone (80–100% of total) are detected after absorption through both the jejunum and the ileum. On the other hand, the flavonoids studied containing a 3′,4′-ortho-dihydroxy (or catechol) B-ring were absorbed predominantly as glucuronides (88–100%), with the exception of quercetin-3-glucoside and rutin which are absorbed as glycosides.

Bioavailability studies on catechins, quercetin (and its glycosides) and other catechol-containing flavonols and flavanols have demonstrated very low levels of the native compounds and their conjugates both in the circulation and excreted in the urine of both rat and man [20–23]. Studies comparing the urinary excretion of quercetin and its glycosides after oral versus intravenous administration to rats detect low levels of native compounds or conjugates, suggesting that the majority is cleaved and metabolised by the liver [23]. In contrast, the major non-catechol containing phenolic components of the diet, such as ferulic acid and naringenin compounds are absorbed to much greater extents as shown particularly in urinary elimination studies in rat and man [12,23]. The studies described here indicate that the total absorption of a ferulic acid, quercetin and quercetin-3-glucoside, for example, across the jejunum of the isolated rat intestine are reasonably comparable and indicate that in vivo more extensive metabolism (especially of the more oxidizable phenolics) takes place in the tissues, circulation and the liver after absorption across the intestinal wall.

Glucuronidation of the flavonoids was observed to occur at different, and possibly, multiple hydroxyl groups within the structure. The position at which glucuronidation is occurring will be of importance when considering the resulting antioxidant potential of the absorbed glucuronides especially since the reduction potential of the B-ring of phenolics is lower than that of the A-ring [24]. For example, glucuronidation at 3′- or 4′-OH groups on the B-ring of flavonoids would increase the reduction potential and therefore decrease its antioxidant activity. However, if glucuronidation occurs at hydroxyl groups in the A-ring of the flavonoid then the antioxidant potential would be less influenced. Indeed, recent studies have identified the 5-*O*-β-glucoside of catechin and epicatechin excreted in the urine of rats post-ingestion and that this does not interfere with their antioxidant properties [21,22]. Our perfusion studies with luteolin are consistent with those of Shimoi et al. [25] who studied transfer of compounds from the mucosa to the serosal side of a rat everted intestine model. However, although they also observed glucuronidation of luteolin and the hydrolysis of luteolin-7-glucoside to luteolin during transfer across the gut, their studies only revealed the presence of two glucuronidated/sulphated metabolites whereas we have identified six distinct luteolin glucuronides (Fig. 3). An investigation into what the major sites of glucuronidation are on flavonoids, whether this glucuronidation is reversible in vivo and how effective the major absorbed glucuronides are as antioxidants would provide valuable information about the potential of flavonoids to act as antioxidants in vivo.

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