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# Difructose Anhydride III Promotes Absorption of the Soluble Flavonoid αG-Rutin in Rats

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A highly soluble quercetin glycoside, αG-rutin, is a glucose adduct of insoluble rutin. We examined the effects of difructose anhydride III (DFAIII; di-D-fructofuranosyl 1,2′:2,3′-dianhydride) on intestinal absorption of αG-rutin by rat experiments. αG-rutin, rutin, quercetin, and the quercetin conjugate appeared in the portal blood after an intubation of αG-rutin (100 μmol), DFAIII effected higher portal concentrations of αG-rutin in portal cannulated rats. In ligated jejunal and ileal loops of rats, αG-rutin, rutin, quercetin, and the quercetin conjugate appeared in the intestinal mesenteric blood at both 30 and 60 min in both loops; the concentration of αG-rutin was 1.5−3 times higher when absorbed in the presence DFAIII. In the isolated mucosae of the jejunum and ileum, mucosal-to-serosal passage of αG-rutin increased with the addition of 100 μmol of DFAIII. These results indicate that αG-rutin is absorbed as the intact form and that DFAIII stimulates its absorption in the small intestine.

**KEYWORDS: Difructose anhydride III; flavonoid; metabolism; absorption; portal cannula**

## INTRODUCTION

Flavonoids are polyphenols that are found in many foods and which function as antioxidants in the body (*1*). However, the mechanisms for absorption of polyphenolic compounds remain unclear. Developments in analytical instrumentation have led to a number of recent studies that have considered the absorption of the polyphenol compounds catechin, anthocyanin, and quercetin (*2*−*4*). Quercetin is a well-known natural flavonoid found in onions, green tea, and sophora (*5*−*7*), and its antioxidant properties are considered beneficial to human health (*8*−*10*). In plants, quercetin is usually glycosylated, occurring predominantly as a β-glucoside (*11*), and the efficiency of absorption is probably influenced by its form of glycosylation (*12*, *13*). In both the small intestine and the liver, absorbed quercetin is rapidly conjugated (*14*). However, no reports have indicated the presence of intact quercetin aglycone or glycosides in the systemic circulation. In addition, quercetin-3-*O*-glucoside was not found in systemic blood following the administration of quercetin-3-glucoside, the most abundant glycoside in plant-derived foods (*15*).

The recently manufactured quercetin glycoside αG-rutin (**Figure 1**) is a water-soluble glucose adduct of rutin (quercetin-3-β-glucosyl-rhamnose), and this compound is 1000 times more dissoluble than rutin. Previously, we reported that αG-rutin was absorbed intact in rats, crossing the mucosa of the stomach and intestines, then appearing in the portal blood (*16*). In addition, we suggested that in the intestine, αG-rutin was absorbed intact, via the paracellular transport pathway (*17*).

It has been recognized that intestinal absorption of flavonoids is not varied, and little is known about enhancers of soluble flavonoid absorption in an in vivo study (*18*). Difructose anhydride III (DFAIII) is a disaccharide comprised of two fructose residues. It is not hydrolyzed by the digestive enzymes of the small intestine but metabolized by microorganisms in the large intestine (*19*, *20*). Mineo et al. reported that a nondigestible saccharide, difructose anhydride III (DFAIII; di-D-fructofuranosyl 1,2′:2,3′-anhydride; **Figure 2**), enhanced paracellular calcium absorption via passage through tight junctions, which are transport pathways for calcium and some water-soluble compounds (*21*, *22*). Using luminally perfused intestinal loops from anesthetized rats, we demonstrated that DFAIII activates this transfer (*23*).

The aims of the present study were to evaluate the role of the paracellular solute passage of αG-rutin by examining the effects of DFAIII on αG-rutin absorption in vivo using portal cannulated rats, in situ using luminally perfused jejunal and ileal loops from anesthetized rats, and in vitro using isolated intestinal mucosae.

## MATERIALS AND METHODS

**Chemicals.** αG-Rutin was donated by Tokyo Sugar Refining Co. Ltd. (Tokyo, Japan). Quercetin and rutin were obtained from Wako Pure Chemical Industries Co. Ltd. (Osaka, Japan). All other reagents

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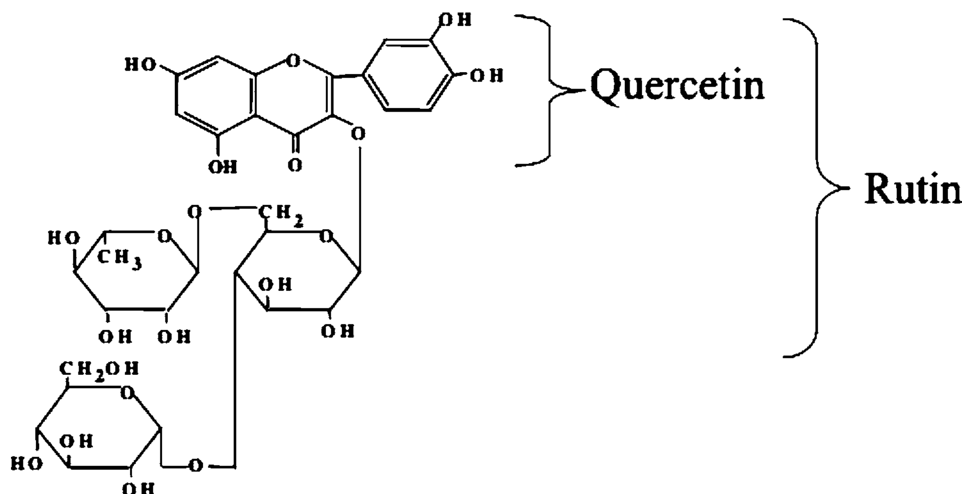


**Figure 1.** Structure of αG-rutin.

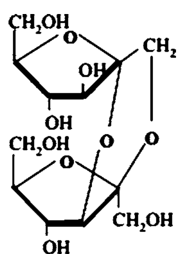

**Figure 2.** Structure of difructose anhydride III.

and chemicals were of the highest commercially available grade. DFAIII is a disaccharide comprised of two fructose residues. It is not hydrolyzed by the digestive enzymes of the small intestine but metabolized by microorganisms in the large intestine. DFAIII was provided by Nippon Beet Sugar Mfg. Ltd. (Obihiro, Japan).

**Animals and Diets.** Male Wistar ST rats (230−250 g; Japan SLC, Shizuoka, Japan) were housed in individual wire-bottomed cages at 22 °C in a temperature-controlled room. Rats were acclimated for 1 week with a 25% casein−sucrose diet (25% casein, 60.25% sucrose, 5% cellulose, 3.5% mineral mixture (AIN 93G), 1% vitamin mixture (AIN 93G), 5% corn oil, and 0.25% choline chloride).

This study was approved by the Hokkaido University Animal Committee, and the animals were maintained in accordance with the Hokkaido University guidelines for the care and use of laboratory animals.

**Experiment 1: Portal Absorption of αG-Rutin in Conscious Rats.** Portal and duodenal cannulae were implanted under sodium phenobarbital anesthesia (40 mg/kg body weight, Nembutal, Abbott, Chicago, IL) into 10 acclimated rats. The portal cannula (polyethylene tube, sp 28; i.d. 20.4 mm, o.d. 0.8 mm; Natsume Seisakusho, Tokyo, Japan) was inserted directly into the portal vein (*24*), and the duodenal cannula (silicon tube, Silascon No. 00) was inserted through a duodenal fistula, 1 cm distal to the pylorus. After a 24 h fast, 1 mL of αG-rutin solution (100 $\mu$mol) with either DFAIII (100 $\mu$mol) or NaCl (50 $\mu$mol) was instilled into the duodenum, and portal blood (0.3 mL per time point) was collected prior to and at 15, 30, 60, 90, and 120 min after instillation. At 150 min after instillation of αG-rutin, sodium phenobarbital anesthesia was applied, abdominal aortic blood was collected, and the rats were killed. Both ends of the whole small intestine and the cecum were ligated and removed, and their contents were collected, frozen, and stored at −80 °C for subsequent analyses (*23*).

**Experiment 2: Absorption and Metabolism of Ligated Jejunal and Ileal Loops in Anesthetized Rats.** Ten acclimated rats were placed under phenobarbital anesthesia (sodium phenobarbital, 40 mg/kg), and closed intestinal loops (15 cm) were prepared in the jejunum or ileum using an abdominal midline incision (3−4 cm). The 15 cm jejunal loops were made in the just distal part of the ligament of Treitz to about 15 cm. The 15 cm ileal loops were similarly made in the just proximal part of the ileum from the ileocecal junction. After ligation of both ends of the small intestine, two small cuts were made in the segment, the lumen was washed out by saline, and then 1.5 mL of solution containing αG-rutin (150 $\mu$mol) and DFAIII (150 $\mu$mol) or NaCl (75 $\mu$mol) was instilled into the jejunal and ileal segments. Mesenteric and aortic blood from each segment was collected simultaneously 30 or 60 min following instillation. The rats were then sacrificed, and the jejunum and ileum were removed and frozen. The luminal contents and mucosae were collected by squeezing (*25*), and then the contents were frozen and stored at −80 °C for subsequent analyses.

**Experiment 3: Absorption and Metabolism by the Isolated Mucosa of the Small Intestine.** Under phenobarbital anesthesia, six acclimated rats were killed by withdrawal of aortic blood, and the small intestine from the ligament of Treitz to the ileocecal junction was removed. The outside and inside surfaces of the isolated intestine were washed with ice-cold (4 °C) saline (154 mmol/L NaCl). The jejunum (15 cm segment distal from the Trietz ligament) and ileum (15 cm segment proximal from the ileocecal junction) were collected, cut open along the mesenteric border to form a flat sheet, and rinsed with an ice-cold HEPES-buffered balanced salt solution (HBS; 125 mmol/L NaCl, 4 mmol/L KCl, 10 mmol/L D-glucose, 30 mmol/L HEPES, and 1.25 mmol/L $CaCl_2$, gassed with 100% $O_2$, pH 7.4). The serosal and muscle layers were removed from each segment, and preparations containing the mucosa and submucosal tissue were mounted onto Ussing chambers (Corning Costar Co., Cambridge, UK), exposing a circular area of epithelium (0.64 $cm^2$). The serosal and mucosal sides of the segments were bathed in 1 mL of HBS continuously exposed to 100% $O_2$ gas. After stabilization for 30 min, the medium was replaced on both sides: the serosal side received fresh HBS, and the mucosal side received HBS containing 1 or 10 mmol of αG-rutin/L and 100 mmol/L DFAIII. In addition, 21.8 $\mu$mol/L Lucifer Yellow CH di-lithium salt (LY: FW 457.2; Sigma-Aldrich, St. Louis, MO), a fluorescent dye, was added to the HBS in the mucosal chamber, as a paracellular permeable marker. Following a 30 min incubation at 37 °C, the serosal solution was collected and analyzed.

**Plasma Sample Treatment.** Plasma samples (100 $\mu$L) were obtained from portal and aortic blood by centrifugation, then acidified (pH 4.9) with 10 $\mu$L of acetic acid (0.58 mol/L) and treated at 37 °C for 30 min in the presence or absence of 10 $\mu$L of *Helix pomatia* extract (Sigma G-0876, 106 U/L, $\beta$-glucuronidase and 2.5 and 105 U/L sulfatase), to measure total flavonoid and unconjugated forms of flavonoid, respectively. Then, methanol (MeOH; 100 $\mu$L) was added to the reaction mixture, which was heated at 100 °C for 1 min and centrifuged at 9000*g* for 3 min, and the supernatant was collected. This extraction procedure was repeated a further three times, without the heating step. The combined supernatant was applied to oasis HLB cartridges (Waters Co. Ltd., Milford, MA). The eluent (1 mL) was dried and dissolved in 100 $\mu$L of 50% MeOH solution (sample solution).

**Analysis.** αG-Rutin and its metabolites were identified and quantified in the sample solution using a mass spectrometer (LC-MS) equipped

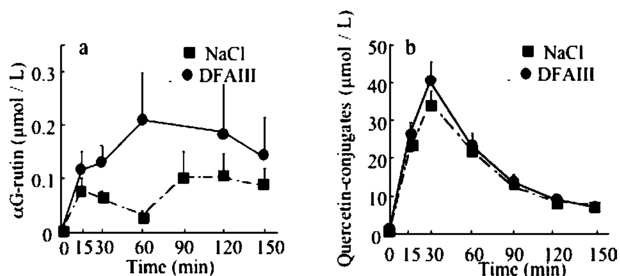


**Figure 3.** Concentration of αG-rutin in plasma from blood in the portal vein of rats instilled with αG-rutin (**a**) or quercetin conjugates (**b**), with or without DFAIII. Portal blood was collected at the time points indicated, before and following instillation of 1 mL of αG-rutin (100 $\mu$mol/rat) and DFAIII solution (100 $\mu$mol/rat) or NaCl (50 $\mu$mol/rat). Values represent means ± SEM, $n = 6$. $P$ values were estimated by two-way ANOVA: (**a**) DFAIII, $P = 0.029$; min, $P = 0.480$; and DFAIII × min, $P = 0.480$ and (**b**) DFAIII, $P = 0.210$; min, $P < 0.001$; and DFAIII × min, $P = 0.885$.

with an electric spray ionization (ESI) interface operating in positive ionization mode (ZQ 2000, Waters Co. Ltd., Milford, MA) as described previously (*16*). The temperatures of the capillary and vaporization heaters were maintained at 100 and 300 °C, respectively. The flow rate of the sheath gas (nitrogen) was 70 arb unit. LC/ESI-MS was carried out in scan mode from ($m/z$) +50 to 2000 amu or in selected ion monitoring (SIM) mode ($m/z$) +303 for quercetin, ($m/z$) +611 for rutin, and ($m/z$) +773 for αG-rutin, respectively. The HPLC system was fitted with a 5 $\mu$m $C_{18}$ Waters Puresil column (150 mm × 4.6 mm; Waters Co. Ltd., Milford, MA), and the temperature was

**Table 1.** αG-Rutin, Rutin, and Quercetin Remaining in Small Intestine[a]

| | αG-rutin ($\mu$mol) | rutin ($\mu$mol) | quercetin ($\mu$mol) |
|---|---|---|---|
| | Small Intestine | | |
| NaCl | 0.94 ± 0.17[b] | 64.2 ± 6.7 | 2.13 ± 0.29 |
| DFAIII | 0.47 ± 0.09 | 59.6 ± 7.1 | 1.90 ± 0.25 |
| | Cecum | | |
| NaCl | 0.31 ± 0.13 | 16.5 ± 5.1 | 0.23 ± 0.07 |
| DFAIII | 0.17 ± 0.07 | 20.4 ± 6.5 | 0.15 ± 0.03 |

[a] Rats were instilled with 1 mL of αG-rutin (100 $\mu$mol/rat) and NaCl (50 $\mu$mol/rat) or DFAIII (100 $\mu$mol/rat), and the intestinal contents were collected after 150 min. Values represent means ± SEM, $n = 10$. Statistical analyses were performed using one-way ANOVA. [b] Differences between treatment groups were analyzed using Duncan's multiple range test and were considered significant at $*P < 0.05$.

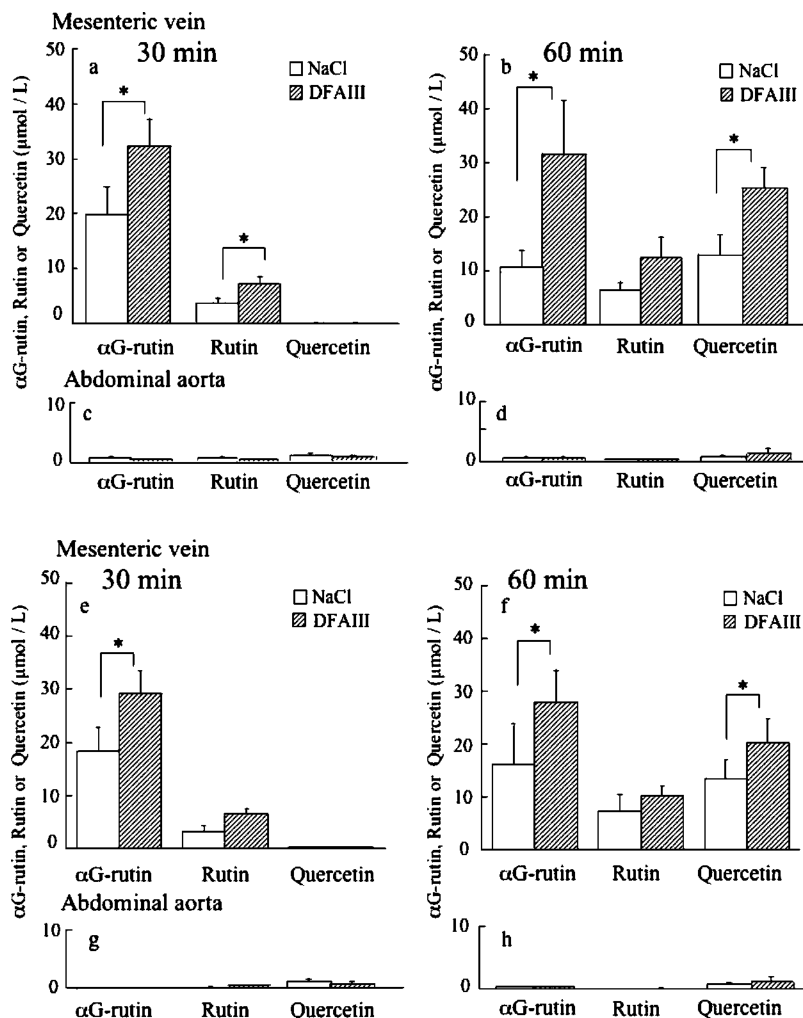


**Figure 4.** Concentration of αG-rutin, rutin, and quercetin in plasma from blood in the mesenteric vein and abdominal aorta of rats instilled with 1.5 mL of αG-rutin (150 $\mu$mol/rat) and DFAIII (150 $\mu$mol/rat) or NaCl (75 $\mu$mol/rat) at 30 or 60 min following instillation. (**a**) Mesenteric blood in jejunum at 30 min and (**b**) at 60 min. (**c**) Abdominal aortic blood in jejunum at 30 min and (**d**) at 60 min. (**e**) Mesenteric blood in ileum at 30 min and (**f**) at 60 min. (**g**) Abdominal aortic blood in ileum at 30 min and (**h**) at 60 min. Values represent means ± SEM, $n = 6$. Statistical analyses were performed using one-way ANOVA. Differences between treatment groups were analyzed using Duncan's multiple range test and were considered significant at $*P < 0.05$.

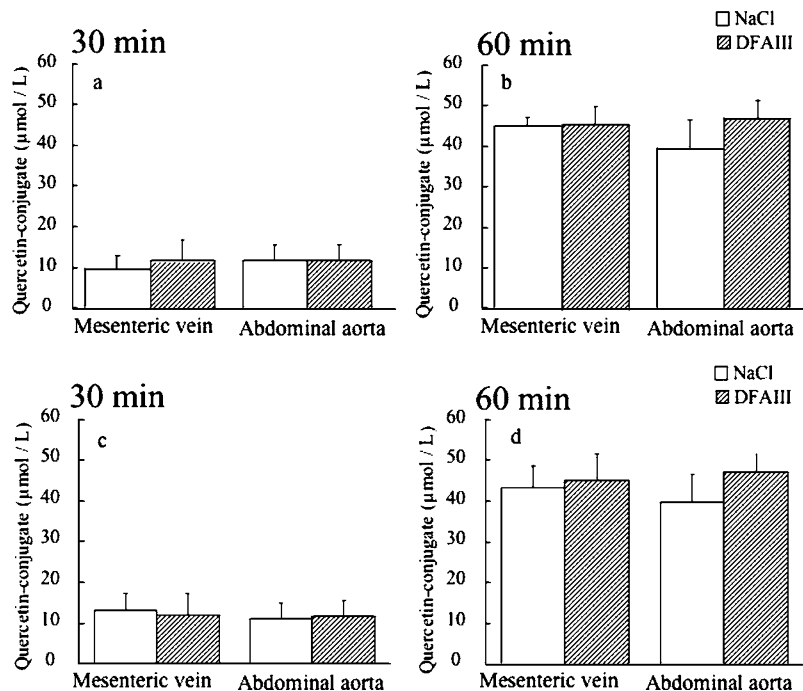


**Figure 5.** Concentration of quercetin conjugates in the mesenteric vein plasma and abdominal aortic plasma of rats instilled with 1.5 mL of αG-rutin (150 $\mu$mol/rat) and DFAIII (150 $\mu$mol/rat) or NaCl (75 $\mu$mol/rat) at 30 or 60 min following instillation. (**a**) Jejunum at 30 min and (**b**) at 60 min. (**c**) Ileum at 30 min and (**d**) at 60 min. Values represent means ± SEM, $n = 6$. Statistical analyses were performed using one-way ANOVA. Differences between treatment groups were analyzed using Duncan's multiple range test and were considered significant at $^*P < 0.05$.

maintained at 40 °C by the column oven. Solvents A (water/methanol/trifluoroacetic acid, 70:30:0.1) and B (methanol/trifluoroacetic acid, 100:0.1) were run at a flow rate of 1 mL/min using linear gradients of solvent B as follows: from 10 to 30% for 20 min, back to 10% for 5 min, then maintained at 10% for a further 5 min with 0.3 mL/min among total flow injected into the MS detector. UV chromatograms were recorded at 360 nm. Concentrations of flavonoids were estimated using calibration curves of quercetin, rutin, and αG-rutin standard solutions. LY in the serosal solution was diluted with purified water, and its concentration was determined fluorometrically (excitation, 430 nm; emission, 540 nm; FP-550; Jasco, Tokyo, Japan).

**Calculations and Statistics.** Concentrations of αG-rutin, rutin, and quercetin were calculated from the peak area of each mass spectrum in combination with calibration curves. The concentrations of conjugated derivatives were estimated as the difference between quercetin concentrations before and after $\beta$-glucuronidase/sulfatase treatment. Statistical analyses were performed using one- and two-way ANOVA, and differences between treatment groups were determined with Duncan's multiple range test. Differences were considered significant at $P < 0.05$.

## RESULTS

**Effect of DFAIII on Portal Absorption of αG-Rutin in Conscious Rats.** Intact αG-rutin appeared within 15 min in the portal blood of rats instilled with αG-rutin and either DFAIII or NaCl (control). When compared to the control group, animals receiving DFAIII in addition to αG-rutin exhibited a higher concentration of the latter in the portal blood, reaching a peak at 60 min (**Figure 3a**). In addition, the average concentrations of αG-rutin in the portal blood of DFAIII treated animals tended to be higher than those of the control group (two-way ANOVA, $P = 0.029$). Quercetin conjugate concentration in the portal plasma was markedly increased and reached a peak value at 30 min (control and DFAIII groups were 34.4 and 40.2 $\mu$mol/L, respectively; **Figure 3b**). Although quercetin aglycone and rutin were also detected in the portal blood, the levels were low and were not significantly different between groups (data not shown).

The luminal contents remaining in the small intestine and cecum 150 min after the instillation of αG-rutin consisted primarily of rutin, only the remaining αG-rutin was significantly higher in the DFAIII group than in the control group, but there was no significant difference between groups in the cecum (**Table 1**). Levels of αG-rutin remaining of combined αG-rutin, rutin, and quercetin in the whole intestine were 67.3 ± 7.2 $\mu$mol in the DFAIII group and 62.0 ± 7.5 $\mu$mol in the NaCl control.

**Effect of DFAIII on Absorption of αG-Rutin in the Ligated Jejunal and Ileal Loops.** Concentrations of αG-rutin, rutin, and quercetin in the intestinal mesenteric and aortic blood were determined for the jejunal and ileal loops (**Figure 4**). The intact αG-rutin concentration in the intestinal mesenteric blood of both loops was higher for the DFAIII group than the control (NaCl) at 30 and 60 min. The concentration of quercetin in the intestinal mesenteric blood of the jejunum and ileum was higher in the DFAIII group than for the NaCl control at 60 min, although it could not be detected at 30 min in either group. Rutin concentrations were not influenced by DFAIII; neither was the concentration of the quercetin conjugate in the mesenteric blood, which was detected at 30 min and had increased by 60 min for both jejunal and ileal loops (**Figure 5**).

αG-Rutin and rutin, but not quercetin or quercetin conjugates, remained in the ligated jejunal and ileal loop contents 30 and 60 min after the instillation of αG-rutin (150 $\mu$mol). The percentage disappearance rate of αG-rutin and its metabolites in the jejunum and ileum loops was higher in the DFAIII group than in the controls (two-way ANOVA was significant, $P = 0.004$ and 0.006; **Figure 6**). Remaining of αG-rutin or rutin in the jejunum and ileum loops at both 30 and 60 min after instillation were the DFAIII group 98.3 ± 4.8 and 12.1 ± 0.8 (jejunum 30 min), 86.9 ± 2.9 and 21.2 ± 1.2 (jejunum 60 min), 98.9 ± 4.2 and 15.9 ± 2.7 (ileum 30 min), and 91.6 ± 6.0 and 21.1 ± 1.2 (ileum 60 min) and the control group 108.6 ± 2.3 and 12.7 ± 0. 6 (jejunum 30 min), 94.6 ± 3.3 and 24.8 ± 1.5

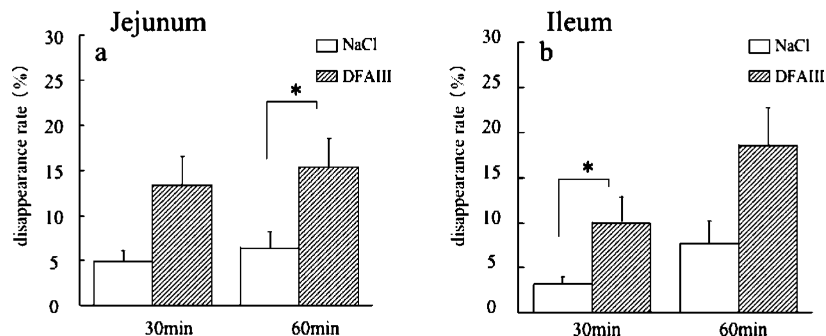


**Figure 6.** Rate of disappearance of αG-rutin and rutin in ligated jejunal (**a**) and ileal (**b**) loops. Rats were instilled with 1.5 mL of αG-rutin (150 μmol/rat) and DFAIII (150 μmol/rat) or NaCl (75 μmol/rat), and the intestinal contents were collected after 30 or 60 min. Values represent means ± SEM, $n = 6$. Statistical analyses were performed using one- and two-way ANOVA. *P* values were estimated by two-way ANOVA: (**a**) DFAIII, $P = 0.004$; min, $P = 0.335$; and DFAIII × min, $P = 0.921$ and (**b**) DFAIII, $P = 0.006$; min, $P = 0.175$; and DFAIII × min, $P = 0.251$. Differences between treatment groups were analyzed using Duncan's multiple range test and were considered significant at $^*P < 0.05$.

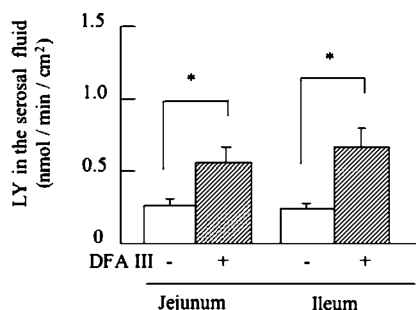


**Figure 7.** Effect of DFAIII on the transport of LY in isolated jejunal and ileal mucosae. HBS containing LY (21.8 μmol/L) and DFAIII (0 or 100 mmol/L) was applied to the mucosal chamber and incubated for 30 min at 37 °C. Values represent means ± SEM, $n = 5$. Statistical analyses were performed using one-way ANOVA. Differences between treatment groups were analyzed using Duncan's multiple range test and were considered significant at $^*P < 0.05$.

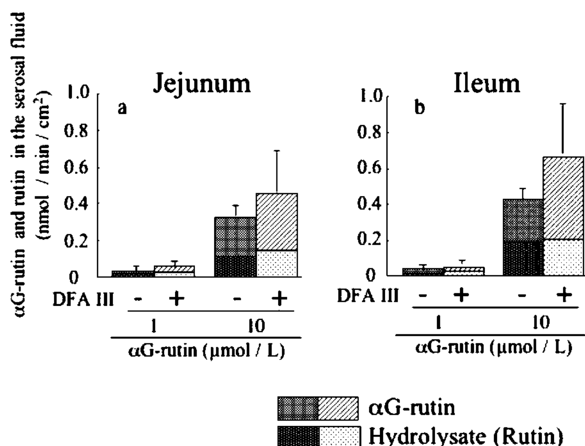


**Figure 8.** Effect of DFAIII on transport of αG-rutin in isolated jejunal (**a**) and ileal (**b**) mucosae. HBS containing αG-rutin (1 or 10 mmol/L) and DFAIII (0 or 100 mmol/L) was applied to the mucosal chamber and incubated for 30 min at 37 °C. Values represent means ± SEM, $n = 5$. Statistical analyses were performed using one-way ANOVA. Differences between treatment groups were analyzed using Duncan's multiple range test and were considered significant at $^*P < 0.05$.

(jejunum 60 min), 104.8 ± 4.8 and 18.6 ± 2.6 (ileum 30 min), and 99.8 ± 2.9 and 12.9 ± 2.4 (ileum 60 min), respectively.

**Effect of DFAIII on Absorption and Metabolism by Isolated Mucosae.** LY transport increased in the jejunal and ileal mucosae following application of DFAIII (**Figure 7**). Transport of αG-rutin and hydrolysate (rutin) by the isolated mucosae of jejunum and ileum was examined at 30 min (**Figure 8**). αG-Rutin and rutin, but not quercetin, were detected in the serosal fluid from all intestinal segments 30 min after incubation with αG-rutin in the mucosal fluid (**Table 1**). The transport rate of αG-rutin (but not rutin) to the serosal fluid tended to be higher in the DFAIII groups than in the controls, in both jejunum and ileum. Neither quercetin nor quercetin conjugates were detected in the serosal or mucosal fluid.

## DISCUSSION

In this study, we examined the intestinal absorption and metabolism of αG-rutin in ex vivo (portal and duodenal cannulated rats), in situ (ligated jejunal and ileal loops), and in vitro (isolated mucosae of the intestines) experiments. Recently, we reported that αG-rutin was transported through the intestinal mucosa both in vivo and in vitro and demonstrated that a considerable proportion of the αG-rutin was absorbed as the intact glycoside via a paracellular pathway (*16*, *17*). In addition, we found that in organs other than the intestine, most of the absorbed glycosides were hydrolyzed rapidly to aglycone and its conjugates. Our present results from in vivo, in situ, and in vitro experiments demonstrate that DFAIII increases αG-rutin absorption. These findings support our previous suggestion that αG-rutin is absorbed via a tight junction since DFAIII activates paracellular passage through tight junctions in the small intestine.

An in vivo experiment using portal cannulated rats indicated that the intact glycoside (αG-rutin) appeared in the portal blood and that its concentration was higher in DFAIII groups than in controls, at all time points investigated. The lower concentration of αG-rutin remaining in the intestinal lumen following treatment with DFAIII strongly suggests that DFAIII enhances αG-rutin absorption. A possible mechanism for higher absorption of αG-rutin is the inhibition of αG-rutin to rutin by DFAIII because rutin is hardly absorbable with insolubility. However, the levels of rutin in the lumen were very similar between groups, suggesting that DFAIII does not affect the hydrolysis of αG-rutin. Thus, αG-rutin may be absorbed in its intact soluble form prior to hydrolysis to insoluble rutin. LY is an indicator of paracellular passage, and its transport increases with DFAIII, strongly supporting the suggestion that DFAIII promotes αG-rutin absorption by activation of the paracellular pathway in the small intestine (*19*).

Hara and Kondo demonstrated that DFAIII enhanced calcium absorption in the ligated small intestine of anesthetized rats, suggesting that DFAIII can activate the paracellular pathway for solute transport in situ (*23*). The present study with ligated

intestinal loops demonstrated that DFAIII increases the uptake of intact αG-rutin into the mesenteric blood of both jejunum and ileum. The lower concentrations of αG-rutin remaining in the loops of the DFAIII treated group indicate a greater absorption of αG-rutin; furthermore, its concentration in the mesenteric blood was considerably higher than that of rutin, quercetin aglycone, or the conjugate form. These findings suggest that αG-rutin is absorbed as the intact glycoside and that DFAIII increases intact αG-rutin absorption in both upper and lower parts of the small intestine. At 30 min following luminal instillation of αG-rutin, the mesenteric levels of quercetin conjugates were much lower than those of αG-rutin and were very similar to the aortic conjugate levels. These results indicate that in the small intestine, there was neither production nor addition of conjugate. In contrast, at 60 min, the levels of conjugates had increased in the mesenteric and aortic blood and were found to be similar for both groups. These levels of conjugate were comparable to the portal concentrations measured in vivo, and taken together, these findings strongly suggest that the quercetin conjugate is produced from absorbed αG-rutin, after which it accumulates in the systemic blood. Thus, the high level of portal conjugates presented in **Figure 3** may have occurred as a result of recirculation of accumulated conjugates in systemic blood.

We observed high levels of quercetin aglycone in the mesenteric blood, but not aortic blood, 60 min after the instillation of αG-rutin (**Figure 4**). This indicates that by this time, αG-rutin was hydrolyzed to quercetin aglycone in the intestine, whereas it had not been hydrolyzed by 30 min. Previously, we reported that the hydrolysis of αG-rutin to aglycone could be detected in intestinal homogenates, but not in the intestinal sacs (rings), suggesting that this activity is intracellular (*16*, *17*). In addition, we were unable to detect quercetin or quercetin conjugates in the ligated intestinal loops of either the jejunum or ileum, suggesting that a part of the αG-rutin or rutin molecule becomes incorporated into the intestinal mucosal cells, then released into the mesenteric blood as quercetin aglycone. This possibility must be clarified in the future.

Finally, we observed the effect of DFAIII on paracellular passage and αG-rutin absorption, using mucosae isolated from the small intestine. Macromolecules that have been studied with regard to their paracellular transport across either the intestinal epitherium or a monolayer of Caco-2 cells include intact proteins (*26*) and high molecular weight probes, such as polyethylene glycol 4000, inulin (5500 Da), and dextran 4000 (*27*, *28*). Additionally, the permeation of poly-D-glutamate across both Caco-2 monolayer and rat intestinal membrane occurs (*29*). We already showed a linear increases in αG-rutin transport from the mucosal side to the serosal side in the isolated mucosa with the application of αG-rutin from 10 $\mu$mol/L to 100 mmol/L (*17*). The linear and non-saturable increases dependent on mucosal αG-rutin demonstrate that the glycoside was transported through a simple diffusional pathway, which may be the paracellular route via tight junction. Furthermore, our results clearly demonstrate that DFAIII activates paracellular passage of molecules and effects increased transport of quercetin glycosides. This additional in vitro study supports the suggestion that DFAIII increases paracellular αG-rutin absorption in the small intestine.

We conclude that DFAIII stimulates paracellular absorption of intact αG-rutin from the intestine and enhances the bioavailability of αG-rutin in vivo, in situ, and in vitro. As quercetin and rutin exhibit a strong antioxidant activity in humans (*9*, *30*), the enhanced bioavailability of αG-rutin resulting from DFAIII treatment may have beneficial effects.

## LITERATURE CITED


(1) Vinson, J. A.; Su, X.; Zubik, L.; Bose, P. Phenol antioxidant quantity and quality in foods: fruits. *J. Agric. Food Chem.* **2001**, *49*, 5315−5321.

(2) Li, C.; Meng, X.; Winik, B.; Lee, M. J.; Lu, H.; Sheng, S.; Bucklev, B.; Yang, C. S. Analysis of urinaly metabolites of tea catechins by liquid chromatography/electrospray ionization mass spectrometry. *Chem. Res. Toxicol.* **2001**, *14*, 702−707.

(3) Felgines, C.; Talavera, S.; Texier, O.; Besson, C.; Fogliano, V.; Lamaison, J. L.; Fauci, L.; Galvano, G.; Remesy, C.; Galvano, F. Absorption and metabolism of red orange juice anthocyanins in rats. *Br. J. Nutr.* **2006**, *95*, 898−904.

(4) Mullen, W.; Edwards, C. A.; Crozier, A. Absorption, excretion, and metabolite profiling of methyl-, glucuronyl-, glucosyl-, and sulpho-conjugates of quercetin in human plasma and urine after ingestion of onions. *Br. J. Nutr.* **2006**, *96*, 107−116.

(5) O'Reilly, J. D.; Mallet, A. I.; McAnlis, G. T.; Young, I. S.; Halliwell, B.; Sanders, T. A.; Wiseman, H. Consumption of flavonoids in onions and black tea: lack of effect on F2-isoprostanes and autoantibodies to oxidized LDL in healthy humans. *Am. J. Clin. Nutr*. **2001**, *73*, 1040−1044.

(6) Rechner, A. R.; Wagner, E.; van Bure, L.; van de Put, F.; Wiseman, S.; Rice-Evans, C. A. Black tea represents a major source of dietary phenolics among regular tea drinkers. *Free Radical Res.* **2002**, *36*, 1127−1135.

(7) Liu, I. M.; Sheu, S. J. Analysis and processing of Chinese herbal drugs. VIII: The study of sophorae floe. *Am. J. Chin. Med*. **1989**, *17*, 179−187.

(8) Gee, J. M.; Hara, H.; Johnson, I. T. Suppression of intestinal crypt cell proliferation and aberrant crypt foci by dietary quercetin in rats. *Nutr. Cancer* **2002**, *43*, 193−201.

(9) Grinder-Pedersen, L.; Rasmussen, S. E.; Bugel, S.; Jorgensen, L. V.; Dragsted, L. O.; Gundersen, V.; Sandstrom, B. Effect of diets based on foods from conventional versus organic production on intake and excretion of flavonoids and markers of antioxidative defense in humans. *J. Agric. Food Chem*. **2003**, *51*, 5671−5676.

(10) Togna, G. I.; Togna, A. R.; Franconi, M.; Marra, C.; Guiso, M. Olive oil isochromans inhibit human platelet reactivity. *J. Nutr*. **2003**, *133*, 2532−2536.

(11) Crespy, V.; Morand, C.; Besson, C.; Manach, C.; Demigne, C.; Remesy, C. Comparison of the intestinal absorption of quercetin, phloretin, and their glucosides in rats. *J. Nutr*. **2001**, *131*, 2109−2114.

(12) Booth, A. N.; Murray, C. W.; Jones, F. T.; Deeds, H. The metabolic fate of rutin and quercetin in the animal body. *J. Biol. Chem*. **1956**, *97*, 233−241.

(13) Nakagawa, Y.; Shtlar, M. R.; Wender, S. H. Urinary products from quercetin in neomycin treated rats. *Biochem. Biophys. Acta* **1965**, *97*, 233−241.

(14) Hollman, P. C.; de Vries, J. H.; van Leeuwen, S. D.; Mengelers, M. J.; Katan, M. B. Absorption of dietary quercetin glucosides and quercetin in healthy ileostomy volunteers. *Am. J. Clin. Nutr*. **1995**, *62*, 1276−1282.

(15) Morand, C.; Manach, C.; Crespy, V.; Remesy, C. Quercetin 3-D-$\beta$-glucoside is better absorbed than other quercetin forms and is not present in plasma. *Free Radical Biol. Med.* **2000**, *33*, 667−676.

(16) Matsumoto, M.; Matsukawa, N.; Chiji, H.; Hara, H. A soluble flavonoid glycoside, αG-rutin, is absorbed as glycosides in the isolated gastric and intestinal mucosa. *Biosci., Biotechnol., Biochem.* **2004**, *68*, 1929−1934.

(17) Matsumoto, M.; Chiji, H.; Hara, H. Intestinal absorption and metabolism of a soluble flavonoid, αG-rutin, in portal cannulated rats. *Free Radical Res.* **2005**, *39*, 1139−1146.

(18) Zafar, T. A.; Weaver, C. M.; Jones, K.; Moore, D. R.; Barnes, S. Inulin effects on bioavailability of soy isoflavones and their calcium absorption enhancing ability. *J. Agric. Food Chem.* **2004**, *52*, 2827−2831.
(19) Minamida, K.; Sujaya, I. N.; Tamura, A.; Shigematsu, N.; Sone, T.; Yokota, A.; Asano, K.; Benno, Y.; Tomota, F. The effect of di-fructofuranose 1,2′:2,3′-dianhydride (DFAIII) administration on human intestinal microbiota. *J. Biosci. Bioeng*. **2004**, *98*, 244−250.
(20) Minamida, K.; Shiga, K.; Sujaya, I. N.; Sone, T.; Yokota, A.; Hara, H.; Asano, K.; Tomita, F. Effect of difructose anhydride III (DFAIII) administration on rat intestinal microbiota. *J. Biosci. Bioeng*. **2005**, *99*, 230−236.
(21) Mineo, H.; Hara, H.; Shigematsu, N.; Okuhara, Y.; Tomita, F. Melibiose, difructose anhydride III, and difructose anhydoride IV enhance net calcium absorption in rat small and large intestinal epithelium by increasing the passage of tight junctions in vitro. *J. Nutr*. **2002**, *132*, 3394−3399.
(22) Mitamura, R.; Hara, H.; Aoyama, Y.; Chiji, H. Supplemental feeding of difructose anhydride III restores calcium absorption impaired by ovariectomy in rats. *J. Nutr*. **2002**, *132*, 3387−3393.
(23) Hara, H.; Kondo, K. Difructose anhydrides III and IV equally promote calcium absorption from the luminally perfused rat small intestine. *Biosci., Biotechnol., Biochem.* **2005**, *69*, 839−841.
(24) Hara, H.; Kiriyama, S. In vivo evaluation of free chymotrypsin activity in the lumen using benzoyl-L-tyrosyl-D-aminobenzoic acid in portal cannulated rats. *J. Nutr. Sci. Vitaminol*. **1991**, *37*, 379−388.
(25) Kasai, T.; Tanaka, T.; Kiriyama, S.; Sonoyama, K. Facile preparation of rat intestinal mucosal enzyme activity. *J. Nutr. Sci. Vitaminol*. **1993**, *39*, 399−403.
(26) Gardner, M. L. G. Intestinal assimilation of intact peptides and proteins from the diet-A neglected filed? *Biol. Rev.* **1984**, *59*, 289−331.
(27) Tanaka, Y.; Taki, Y.; Sakane, T.; Nadai, T.; Sezaki, H.; Yamashita, S. Characterization of drug transport through tight-junctional pathway in Caco-2 monolayer: comparison with isolated rat jejunum and colon. *Pharm. Res*. **1995**, *12*, 523−528.
(28) Lane, M. E.; O'Driscoll, C. M.; Corrigam, O. I. The relationship between rat intestinal permeability and hydrophilic probe size. *Pharm. Res*. **1996**, *13*, 1554−1558.
(29) Salamat-Miller, N. T.; Chittchang, M.; Mitra, A. K.; Johnston, T. P. A randomly-coiled, high-molecular weight polypeptide exhibits increased paracellular diffusion in vitro and in situ relative to the highly ordered α-helix conformer. *Pharm. Res*. **2005**, *22*, 245−254.
(30) Hollman, P. C.; van Trijp, J. M.; Buysman, M. N.; van der Gaag, M. S.; Mengelers, M. J.; de Vries, J. H.; Katan, M. B. Relative bioavailability of the antioxidant flavonoid quercetin from various foods in man. *FEBS Lett.* **1997**, *418*, 152−156.

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