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Chronic Ingestion of Apple Pectin Can Enhance the Absorption of Quercetin

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The effect of apple pectin (AP) on quercetin and rutin bioavailability was investigated. Rats received a basal (control) or AP-containing diet for 6 weeks followed by orally administered quercetin or rutin. With quercetin administration, the maximum concentration and area under the curve of concentration−time from 0 to 24 h for the sum of quercetin metabolites in the plasma were significantly higher in AP-fed rats than in the control group. However, AP did not significantly affect rutin bioavailability. The crypt depth of the jejunum and ileum and the villus thickness of the ileum of AP-fed rats were significantly greater than those of control rats. These results demonstrate that chronic AP ingestion enhances intestinal absorption of quercetin. This increase in quercetin absorption might be attributed to alteration of the absorptive capacity of the small intestine through AP-induced improvement of its morphological and physiological properties.

KEYWORDS: Apple pectin; quercetin; rutin; bioavailability; intestine

INTRODUCTION

Numerous studies have used plasma concentrations and urinary excretion to examine the kinetics and extent of flavonoid absorption in animals and humans (*1*−*3*). These studies show that the bioavailability of flavonoids is primarily determined by their chemical structure, which depends on various factors such as the degree of glycosylation/acylation, their basic structure, conjugation with other phenolics, molecular size, the degree of polymerization, and solubility (*4*). Furthermore, glucosidase activities and nutrient transport efficiency in the small intestine and metabolization by intestinal microbial flora are also known to affect the bioavailability of flavonoids (*5*−*7*). Through this knowledge, attention has also been focused on whether the bioavailability of flavonoids in the intestine can be influenced by other food ingredients, resulting in a change in flavonoid solubility, modification of nutrient transport efficiency, or alternation of intestinal bacterial flora. For example, Azuma et al. have demonstrated that coingested lipids and emulsifiers enhanced the bioavailability of quercetin glucosides in onion (*8*). It appears that the enhancing function of lipids and emulsifiers is attributable to the increased solubility of quercetin glucosides in lipid micelles. Matsumoto et al. have also reported that difructose anhydride III increases paracellular absorption of the soluble flavonoid αG-rutin in the rat small intestine (*9*). As quercetin and rutin exhibit a strong antioxidant activity in humans (*10, 11*), the enhanced bioavailability of these flavonoids may have a potentially beneficial effect on human health.

Pectin, a major type of dietary fiber, is widely distributed in fruits and vegetables, mainly citrus fruits and apples. Physiologically, it possesses several effects including interaction with lipids and bile acids (*12*), prolongation of the gastric transit time (*13*), alteration of intestinal bacterial flora (*14*), and modification of the morphological parameters of the small intestine (*15*). It has also been reported that pectin has delaying or reducing effects on the intestinal absorption of food due to its physiological properties. For example, pectins interfere with cholesterol absorption (*17*) and have an inhibitory effect on plasma β-carotene levels after ingestion of carotenoid-rich foods (*18*). Thus, it is assumed that flavonoid absorption is also influenced by pectin. Investigation of the effect of pectin on flavonoid absorption is important because flavonoids generally colocalize with pectin in many fruits and vegetables. However, few studies have so far examined the effects of pectin on the intestinal absorption and metabolism of flavonoids. To assess the effects of pectin on flavonoid absorption, it is thought that two different experiments are needed as follows: One is to investigate the effects through morphological and physiological alterations in the intestine by chronic ingestion of pectin, and the other is to investigate the direct effects of pectin on the absorption of a single dose of coingested flavonoid.

In this study, to investigate the possible effect of chronic ingestion of apple pectin (AP) on intestinal flavonoid absorption, we evaluated the plasma concentration and urinary excretion

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Table 1. Formulation of Experimental Diets

ingredient (%)	control diet	pectin diet
casein	20.0	20.0
α-corn starch	13.0	13.0
β-corn starch	40.5	40.5
sucrose	10.0	10.0
corn oil	7.0	7.0
vitamin mixture[a]	3.5	3.5
mineral mixture[a]	1.0	1.0
cellulose	5.0	0.0
AP	0.0	5.0

[a] The vitamin mixture AIN-93 and the mineral mixture AIN-93G were obtained from Oriental Yeast Co.

of quercetin metabolites after single ingestion of quercetin or rutin by rats fed AP for 6 weeks.

MATERIALS AND METHODS

Chemicals. Quercetin, rutin (quercetin-3-β-D-rutinoside), highly methylated AP (70−75%, P8471), and β-glucuronidase/sulfatase (*Helix pomatia*, G1512) were purchased from Sigma (St. Louis, MO). Diosmetin, isorhamnetin (quercetin-3′-methyl ether), and tamarixetin (quercetin-4′-methyl ether) were purchased from Extrasynthese (Genay, France). Carboxymethyl cellulose sodium salt and Eosin Y were purchased from Wako Pure Chemical Industries (Osaka, Japan). Hematoxylin was purchased from Merck (Darmstadt, Germany). Other chemicals were commercial products of the highest grade available.

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

Male Wistar rats (7 weeks old; 140−160 g) were obtained from Charles River Japan Co., Ltd. (Atsugi, Japan). They were kept individually in stainless wire netting cages in a room maintained at 22 ± 1 °C with 12 h light/dark cycles with free access to tap water and a commercial diet (type MF; Oriental Yeast Co., Tokyo, Japan) for 1 week before the experiments.

Dietary compositions are shown in **Table 1**. The compositions of the basal diet were based on the AIN-93 dietary compositions (*19*) as a control diet, and in the pectin diet, 5% AP was replaced with 5% cellulose.

Single Oral Administration of Quercetin and Rutin. Twenty rats were randomly divided into four groups of five. The CQ (control diet plus quercetin) and CR (control diet plus rutin) groups received the control diet, and the PQ (pectin diet plus quercetin) and PR (pectin diet plus rutin) groups received the pectin diet ad libitum for 6 weeks. Body weight and food intake were checked daily. After an experimental period of 6 weeks, all rats were orally administered with quercetin or rutin under fasting conditions.

Quercetin and rutin were prepared at concentrations of 50 and 200 mg/3 mL, respectively, in 1% carboxymethyl cellulose sodium salt suspension. All rats were fasted for 17 h before administration. The CQ and PQ groups were orally administered with quercetin at a dose of 50 mg/kg, and the CR and PR groups were orally administered with rutin at a dose of 200 mg/kg by stomach intubation. In a preliminary test, the plasma level of quercetin metabolites in rats administered with rutin was lower than in rats administered with quercetin at equal molar amounts; thus, in this study, a double dose of rutin (on a molar basis) was administered. In the CQ and PQ groups, blood was collected at 0, 1, 2, 4, 8, and 24 h after administration, and urine was collected for 24 h after administration using a metabolic cage. In the CR and PR groups, additional collection of blood at 48 h and urine from 24 to 48 h after administration was also performed. Blood was collected from the tail artery into a heparinized tube, and plasma was immediately isolated by centrifugation at 9000*g* for 5 min. The plasma and urine samples were immediately frozen and kept at −80 °C until use.

Determination of Quercetin Metabolites in Rat Plasma. Quercetin metabolites in rat plasma were quantitatively determined by high-performance liquid chromatography (HPLC) according to Azuma et al. (*8*)with some modifications. Briefly, 50 μL of plasma was mixed with 50 μL of 10000 units/mL type H5 β-glucuronidase containing 500 units of β-glucuronidase, 25 units of sulfatase, and 5 μL of 200 mM ascorbic acid in 0.1 mM sodium acetate buffer (pH 5.0). The mixture was incubated at 37 °C for 2 h. After incubation, 5 μL of 30 μM diosmetin as an internal standard and 900 μL of acetone were added to the mixture and thoroughly mixed. Metabolites were extracted into the acetone layer by centrifugation at 12000*g* for 10 min. The acetone layer was then evaporated to dryness and redissolved in 50 μL of acetonitrile/0.5% phosphoric acid 35/65 (v/v) for HPLC determination. Recovery by this method was checked (>90%) using pure flavonoids (quercetin, isorhmnetin, and diosmetin).

HPLC analysis was performed under the following conditions: column, CAPCELL PAK C_{18} MG (5 μm, 250 mm × 4.6 mm i.d.; Shiseido Co., Tokyo, Japan); mobile phase, acetonitrile and 0.5% phosphoric acid 35/65 (v/v); flow rate, 1 mL/min; column temperature, 40 °C; and detection, UV at 370 nm. Quercetin and isorhamnetin were determined by an internal standard method. The quantification limits for quercetin and isorhamnetin were 0.3 μM.

Determination of the Quercetin Metabolites in Rat Urine. The quercetin metabolites in rat urine were quantitatively determined by HPLC. Briefly, 50 μL of urine was mixed with 50 μL of 10000 units/mL type H5 β-glucuronidase containing 500 units of β-glucuronidase, 25 units of sulfatase, and 5 μL of 200 mM ascorbic acid in 0.1 mM sodium acetate buffer (pH 5.0). After incubation of the mixture at 37 °C for 2 h, metabolites were extracted twice with 200 μL of ethyl acetate. The ethyl acetate phase was collected and evaporated to dryness and then redissolved in 50 μL of acetonitrile/0.5% phosphoric acid (35/65, v/v) for HPLC determination. Recovery by this method was checked (>90%) using pure flavonoids (quercetin, isorhamnetin, tamarixetin, and diosmetin).

HPLC analysis was performed using similar apparatus as in the plasma determination. The mobile phases and elution profile (1 mL/min) were as follows: 0−10 min, isocratic elution 25% acetonitrile/75% 0.5% phosphoric acid; 10−45 min, linear gradient from acetonitrile/0.5% phosphoric acid (25/75) to those (45/55); and 45−60 min, acetonitrile/0.5% phosphoric acid (25/75). Quercetin isorhamnetin and tamarixetin were determined by an external standard method. The quantification limits for quercetin isorhamnetin and tamarixetin were 0.3 μM.

Pharmacokinetic Parameters. The maximum concentration in the plasma (C_{max}) and time for the maximum concentration to be reached (t_{max}) were determined in individual rats by a direct method. The area under the curve (AUC) of the time−course concentration was calculated according to the trapezodial method from 0 to 24 h for quercetin administration and from 0 to 48 h for rutin administration.

Histological Studies. Twelve rats were randomly divided into two groups of six. Rats then received a control diet or pectin diet ad libitum for 6 weeks, and their body weight and food intake were checked daily. After an experimental period, rats were sacrificed by anesthetizing with sodium pentobarbital. The intestine from the pylorus to the cecum was then immediately excised and rinsed in cold saline before being placed in 10% formalin for histological analysis.

Jejunal and ileal specimens, fixed in 10% formalin, were embedded in paraffin, and then, histological sections were cut and stained with hematoxylin and eosin. Ten villi were selected randomly from each tissue section for measurement. Villus length, crypt depth, villus thickness, and intervillus separation were measured. Villus length (crypt to tip) was determined on the right side of each villus. Villus thickness and intervillus separation were measured at a point one-third from the villus tip.

Statistical Analysis. Results are expressed as means ± standard deviations (SDs). Statistical analysis was carried out using Student's *t* test, and $P < 0.05$ was considered significant.

RESULTS

Food Intake and Body Weight. There were no significant differences in the final body weight after fasting for 17 h or the total weight gain and daily food intake among the four groups (CQ, PQ, CR, and PR).

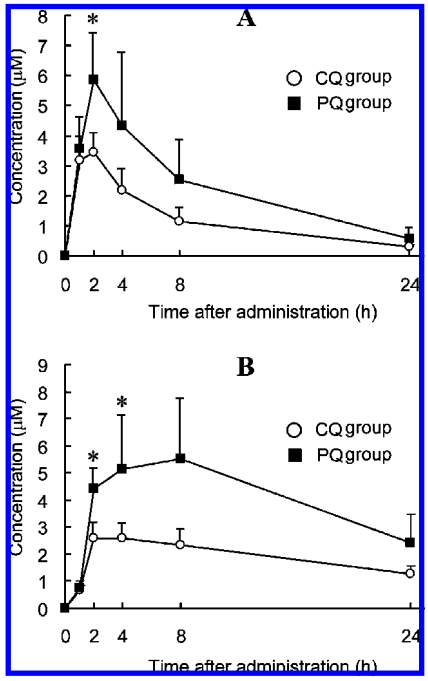

Figure 1. Time course of deconjugated quercetin (**A**) and isorhamnetin (**B**) in the plasma of rats after single administration of quercetin. Rats were orally administered with quercetin (50 mg/kg bw) after receiving a control diet (CQ) or pectin diet (PQ) for 6 weeks. Plasma was collected at 0, 1, 2, 4, 8, and 24 h after administration. Values represent the means ± SDs ($n = 5$). *A significant difference between CQ and PQ at $P < 0.05$ according to Student's t test.

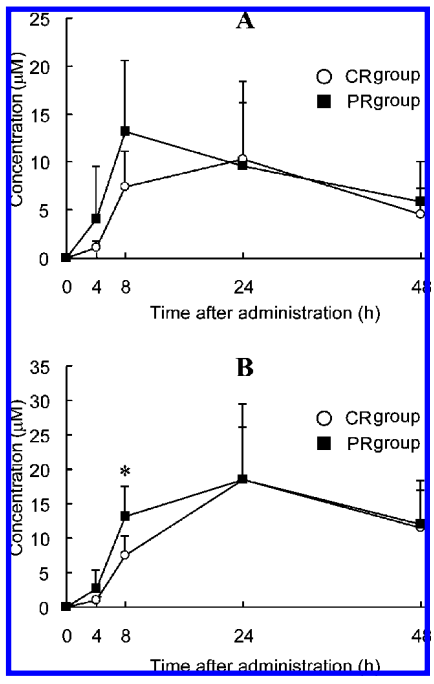

Figure 2. Time course of deconjugated quercetin (**A**) and isorhamnetin (**B**) in the plasma of rats after single administration of rutin. Rats were orally administered with rutin (200 mg/kg bw) after receiving a control diet (CR) or pectin diet (PR) for 6 weeks. Plasma was collected at 0, 1, 2, 4, 8 24, and 48 h after administration. Values represent the means ± SDs ($n = 5$). *A significant difference between CR and PR at $P < 0.05$ according to Student's t test.

Quercetin and Rutin Administration. Figures 1 and **2** show the changes in plasma concentrations of deconjugated quercetin and isorhamnetin in rats fed a control diet or AP diet for 6 weeks followed by oral administration of 50 mg/kg quercetin or 200 mg/kg rutin, respectively. The pharmacokinetic parameters, C_{max}, t_{max}, and AUC, calculated from the data and urinary excretion are shown in **Table 2**. Two hours after administration of quercetin, the plasma concentration of deconjugated quercetin from the tail artery was higher in the AP-fed PQ group (5.84 ± 1.60 μM) than the control diet-fed CQ group (3.45 ± 0.67 μM). At 2 and 4 h, the plasma concentrations of deconjugated isorhamnetin were also higher in the PQ group (4.40 ± 0.78 and 5.14 ± 2.00 μM, respectively) than in the CQ group (2.60 ± 0.56 and 2.57 ± 0.58 μM, respectively). Additionally, the concentrations of deconjugated quercetin over 4 and 8 h and deconjugated isorhamnetin over 8 and 24 h also tended to be higher in the PQ group. The t_{max} values of quercetin, isorhamnetin, and total metabolites were not significantly different between the two groups, while the C_{max} values of quercetin and total metabolites were significantly higher in the PQ group than in the CQ group. No significant difference in the ratio of quercetin/isorhamnetin was observed. The plasma AUC for isorhamnetin and total metabolites was about two times higher in the PQ group than the CQ group. The AUC for deconjugated quercetin was also two times higher in the PQ group, although this was not significant. The recovery of quercetin from urine in the first 24 h also tended to be higher in the PQ group.

On the other hand, the plasma concentrations of deconjugated quercetin and isorhamnetin did not differ between the two groups, except for deconjugated isorhamnetin at 8 h after rutin administration. Moreover, there was no significant difference in C_{max}, t_{max}, AUC, and urinary excretion.

Histological Studies. The crypt depths of the jejunum and ileum of the AP-fed group were significantly greater than those of the control group (**Table 3**). The villus thickness and intervillus separation of the AP-fed group were also greater than those of the control group but only in the ileum. There was no significant difference in the villus length between the AP-fed group and the control group.

DISCUSSION

In this study, the effect of AP on the absorption of quercetin and rutin in rats was investigated. To evaluate the effect of AP on quercetin and rutin absorption, we measured plasma quercetin and isorhamnetin levels and the cumulative urinary excretion. With a single administration of quercetin (50 mg/kg bw) by stomach intubation, the C_{max} and AUC values of plasma quercetin metabolites in rats fed a diet containing AP for 6 weeks were greater than in those fed the control diet. Furthermore, urinary excretion over 24 h in AP-fed rats tended to be higher than in the control rats. In contrast, in rats given rutin (200 mg/kg bw), AP did not significantly affect the bioavailability of rutin. These results show that chronic ingestion of AP promotes the absorption of quercetin but not that of rutin. It is known that the sugar moiety of quercetin is a major determinant of the absorption and metabolism of dietary quercetin derivatives (*20*). Rutin is reportedly absorbed by the large intestine, where it is hydrolyzed to aglycone by the intestinal microbial flora, while quercetin is absorbed by both the small and the large intestines (*21*). Thus, the results showing that AP enhances the absorption of quercetin, but not rutin, suggest that AP influences the absorption of flavonoids from the small intestine. Tamura et al. have reported that pectin increased the bioavailability of rutin in mice fed a pectin−rutin-supplemented diet for 2 weeks as compared with mice fed a cellulose−rutin-supplemented diet (*22*). This report mentions that the alteration of intestinal bacterial flora by ingestion of

Table 2. Pharmacokinetic Parameters of the Quercetin Metabolites after Single Administration of Quercetin or Rutin[a]

	quercetin administration		rutin administration	
	CQ	PQ	CR	PR
C_{max} (μM)[b]				
quercetin	3.48 ± 0.72	5.93 ± 1.66[f]	11.59 ± 7.56	14.86 ± 7.22
isorhamnetin	3.25 ± 0.91	5.59 ± 2.18	18.47 ± 11.04	18.95 ± 7.15
total	6.30 ± 1.03	11.03 ± 3.20[f]	28.96 ± 18.74	31.47 ± 13.06
t_{max} (h)[c]				
quercetin	1.8 ± 0.4	2.5 ± 1.0	14.4 ± 8.8	11.2 ± 7.2
isorhamnetin	4.4 ± 3.3	5.5 ± 3.0	24.0 ± 0.0	20.8 ± 7.2
total	3.2 ± 2.7	3.6 ± 2.6	20.8 ± 7.2	14.4 ± 8.8
AUC (μM × h)[d]				
quercetin	25.30 ± 6.41	50.58 ± 24.66	339.4 ± 212.4	409.5 ± 245.7
isorhamnetin	48.90 ± 15.16	94.76 ± 37.11[f]	587.0 ± 310.2	654.4 ± 239.5
total	74.20 ± 21.50	145.34 ± 61.69[f]	926.4 ± 515.5	1063.9 ± 479.1
urinary excretion (μmol)[e]				
quercetin	0.11 ± 0.04	0.20 ± 0.07	1.99 ± 0.89	1.78 ± 1.37
isorhamnetin	0.05 ± 0.02	0.11 ± 0.04	1.61 ± 0.76	1.42 ± 1.10
tamarixetin	0.07 ± 0.04	0.11 ± 0.05	1.32 ± 0.76	1.48 ± 0.82
total	0.24 ± 0.09	0.42 ± 0.15	4.93 ± 2.38	4.67 ± 3.10

[a] Rats were orally administered with quercetin (50 mg/kg bw) or rutin (200 mg/kg bw) after receiving a control diet (CQ or CR, respectively) or pectin diet (PQ or PR, respectively) for 6 weeks. In the CQ and PQ groups, plasma was collected at 0, 1, 2, 4, 8, and 24 h after administration, and urine was collected for 24 h after administration. In the CR and PR groups, additional collection of plasma at 48 h and urine from 24 to 48 h after administration was also performed. Values represent the means ± SDs ($n = 5$). [b] C_{max}, maximum concentration in the plasma. [c] t_{max}, time for the maximum concentration to be reached. [d] AUC, area under the curve of the time−course concentration, calculated from 0 to 24 h after quercetin administration and from 0 to 48 h after rutin administration. [e] Urinary excretion was calculated from 0 to 24 h after quercetin administration and from 0 to 48 h after rutin administration. [f] A significant difference between CQ and PQ at $P < 0.05$ according to Student's t test.

Table 3. Effect of AP on Villus Height, Crypt Depth, Villus Thickness, and Intervillus Separation[a]

	dietary group	
	control diet	pectin diet
no. of animals	6	6
jejunum		
villus height (μm)	510 ± 44	520 ± 68
crypt depth (μm)	170 ± 13	197 ± 20[b]
villus thickness (μm)	121 ± 21	119 ± 9
intervillus separation (μm)	9 ± 4	12 ± 8
ileum		
villus height (μm)	297 ± 25	318 ± 47
crypt depth (μm)	136 ± 11	173 ± 22[b]
villus thickness (μm)	100 ± 9	121 ± 12[b]
intervillus separation (μm)	26 ± 11	12 ± 6[b]

[a] Values represent the means ± SDs ($n = 6$). [b] A significant difference between the control diet group and pectin diet group at $P < 0.05$ according to Student's t test.

both pectin and rutin improved the productivity of quercetin from rutin, resulting in an increase in the bioavailability of rutin. In contrast, our results showed that pectin did not affect the bioavailability of rutin, whereas the bioavailability of quercetin, which does not undergo hydrolyzation, significantly increased. It appears that this disagreement is perhaps attributed to the manner in which rutin was administered, since the composition of the intestinal flora could be affected not just by pectin but also by rutin.

Pectins, derived from apple, citrus, and other fruits, reportedly possess several effects including interactions with lipids and bile acids (*12*), prolongation of the gastric transit time (*13*), alteration of intestinal bacterial flora (*14*), and modification of the morphological parameters of the small intestine (*15*). These effects are attributed to the specific properties of pectin, such as its dispersibility, viscosity, ability to adsorb and bind compounds, and its fermentability. In the small intestine, it is known that pectin increases brush border membrane enzyme activities (*15*), the concentration of intestinal bile acids (*16*), the overall length (*23*), and the crypt depth and villus height of the jejunum and ileum (*15, 24*). We also observed a significant increase in the crypt depth of both the jejunum and the ileum, as well as villus thickness and intervillus separation in the ileum in the AP-fed group. The mechanism by which pectin enhances the absorption of quercetin in the small intestine has not yet been determined. It is possible that the promotion of quercetin absorption with pectin consumption could be ascribed to improvement of the nutrient transport efficiency of the enterocytes, an increment in the concentration of intestinal bile acids, or augmentation of the absorptive surface area resulting from alteration of the function and morphology of the small intestine. Sigeleo et al. also suggested that the absorptive surface area of the intestinal villus determines changes in nutrient influxes (*25*). In addition, the physical properties of pectin, such as its viscosity and gelling characteristics, cause a prolongation of gastric transit time and are also considered to be a cause of quercetin absorption enhancement. However, in this study, flavonols were administered after fasting for 17 h, and moreover, AP had little impact on the t_{max} of plasma quercetin metabolites in rats administered both quercetin and rutin. This suggests that the physical properties of pectin are not involved in the enhancement of quercetin absorption. Thus, we speculate that an improvement in physiological functions including nutrient transport efficiency and the secretion of bile acids in the intestine or an increase in the absorptive surface area of the epithelial tissue in the small intestine contributed to the enhancing effect. In this study, we did not examine whether an alteration in intestinal bacterial flora composition or the concentration of intestinal bile acids could have affected the bioavailability of flavonoids. However, it is well-known that rutin reaches the large intestine, where it is metabolized to phenolic acids via aglycone by the intestinal microflora (*26, 27*). These phenolic acids such as 3,4-dihydroxyphenylacetic acid also have the ability to perform various biological activities (*26*). Therefore, to fully understand the effect of AP on the bioavailability of quercetin and rutin, these phenolic acids will also need to be determined.

It has been reported that pectin has various inhibitory effects on the intestinal absorption of food. For example, Fernandez et al. reported that pectin interferes with cholesterol and bile acid absorption (*17*), while Rock et al. demonstrated that pectin has an inhibitory effect on the plasma β-carotene response after ingestion of carotenoid-rich foods (*18*). These reductions in the bioavailability of lipophilic compounds might be caused by interaction with pectin. In our study, pectin increased the absorption of quercetin, one type of lipophilic compounds, although unlike β-carotene and cholesterol, quercetin was not coingested with pectin. It was shown, however, that chronic ingestion of pectin could affect intestinal flavonoid absorption. Our findings therefore suggest that, depending on the nature of the food or the method of ingestion, pectin may play a facilitatory role in the absorption of other food components. Further study is thus needed to investigate directly the effects of pectin on absorption of a single dose of coingested flavonoid and the effects of pectin on the absorption of quercetin glycosides generally occurring in fruits and vegetables.

In conclusion, the present study demonstrated that ingestion of AP for 6 weeks significantly enhanced the intestinal absorption of quercetin. In contrast, AP did not affect the bioavailability of rutin. It appears likely that the enhancing effect of AP on quercetin absorption is attributable to improvement of the quercetin absorptive capacity of the small intestine. Investigation of the mechanism by which AP enhances quercetin absorption is now in progress.

ABBREVIATIONS USED

AP, apple pectin; C_{max}, maximum concentration; t_{max}, time for the maximum concentration; AUC, area under the curve of the time−course concentration

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