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Combination of Lipids and Emulsifiers Enhances the Absorption of Orally Administered Quercetin in Rats

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The effects of lipids, emulsifiers, and ethanol on the absorption of orally administered quercetin in rats were investigated for its efficient intestinal absorption. Rats were administered 150 μmol/kg quercetin in water supplemented with lipids and/or emulsifiers, or ethanol, and blood was collected from the tail for 6 h after administration. Co-administration of lipids such as lecithin and soybean oil or emulsifiers including sucrose fatty acid ester, polyglycerol fatty acid ester, and sodium taurocholate had no statistically significant effects on quercetin absorption, although these constituents rather increased the accumulation of conjugated forms of quercetin and those of isorhamnetin in rat plasma. However, the combination of lipids and emulsifiers enhanced the absorption of quercetin significantly. Thirty and fifty percent (v/v) of the ethanol in the vehicle raised the efficiency of quercetin absorption in a concentration-dependent manner. Quercetin absorption-enhancing effects of these constituents seemed to be affected by quercetin's solubility in respective vehicles used for the administration. Ethanol is not helpful for the effective absorption of quercetin, as a high concentration is required. In conclusion, a combination of lipids and emulsifiers is necessary for enhancing quercetin absorption.

KEYWORDS: Absorption; quercetin; rats; lipids; emulsifiers

INTRODUCTION

Flavonoids, which are widely distributed in the plant kingdom, are mostly present as glycosides where phenolic hydrogen or hydrogens are substituted to sugar moiety (*1*, *2*). The total dietary intake of flavonoids by a human is estimated to reach several hundred milligrams per day (*3*). Quercetin (3,3′,4′,5,7-pentahydroxyflavone), a flavonol type flavonoid, is found in a variety of fruits and vegetables including onions, lettuce, and apples and composes the largest percent of flavonoid intake. We have reported that *Corchorus olitorius* L., a vegetable named "moroheiya" in Japan, contains a large amount of quercetin glycosides, which are responsible for its remarkable antioxidative activity (*4*).

In recent years, flavonoids have attracted much attention for their physiological function in vivo, because of their antioxidative activity. In particular, flavonoids are expected to have a role in the prevention of coronary heart disease by inhibiting oxidative modification of low-density lipoprotein from occurring during the initial process of atherosclerosis (*5*−*8*). Epidemiological studies have also shown an inverse relationship between the intake of flavonoids and the risk of coronary heart disease (*9*−*11*).

A number of reports on absorption and metabolic conversion of flavonoids have been published recently (*12*−*20*). Quercetin, which is abundant in foods of plant origin, is one of the most studied flavonoids. Although the intestinal absorption and metabolism of ingested flavonoid glycosides has been at issue, it is suggested that the main pathway for quercetin glycosides is its conversion to conjugate metabolites through aglycone by deglycosylation activity and following conjugation activity (*21*). The metabolic fate of orally administered (−)-epicatechin, another flavonoid commonly present in foods, has been reported to be similar to that of quercetin. It is suggested to enter the common blood circulation in the glucuronized form and then sulfate in the liver and methylate in the liver and kidney (*14*).

There are some reports indicating that the efficiency of intestinal absorption of quercetin is strongly affected by its solubility in the vehicles (*22*−*24*). It has also been found that quercetin was absorbed when this compound was given as a food additive (*25*) or as an inherent component (mostly as glycosides) (*26*−*28*). However, it is not yet clear how various coexisting food constituents affect the intestinal absorption of quercetin.

This study aimed to investigate the effects of co-administration of lipids, emulsifiers, and ethanol on the efficiency of intestinal absorption of quercetin in rats. Because quercetin glycosides, the most abundant form for quercetin in our diets, are suggested to be hydrolyzed in the intestine by β-glucosidases

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Table 1. List of Food or Bile Constituents Supplemented to Water as a Vehicle for Quercetin Administration

rat group no.	constituent supplemented
1	none (control group)
2	10% (w/w) lecithin
3	20% (w/w) soybean oil
4	3% (w/v) sucrose fatty acid ester
5	3% (w/v) polyglycerol fatty acid ester
6	3% (w/v) sodium taurocholate
7	10% (w/w) lecithin and 3% (w/v) sucrose fatty acid ester
8	10% (w/w) lecithin and 3% (w/v) polyglycerol fatty acid ester
9	10% (w/w) lecithin and 3% (w/v) sodium taurocholate
10	20% (w/w) soybean oil and 3% (w/v) sucrose fatty acid ester
11	20% (w/w) soybean oil and 3% (w/v) polyglycerol fatty acid ester
12	20% (w/w) soybean oil and 3% (w/v) sodium taurocholate
13	10% (v/v) ethanol
14	20% (v/v) ethanol
15	30% (v/v) ethanol
16	50% (v/v) ethanol

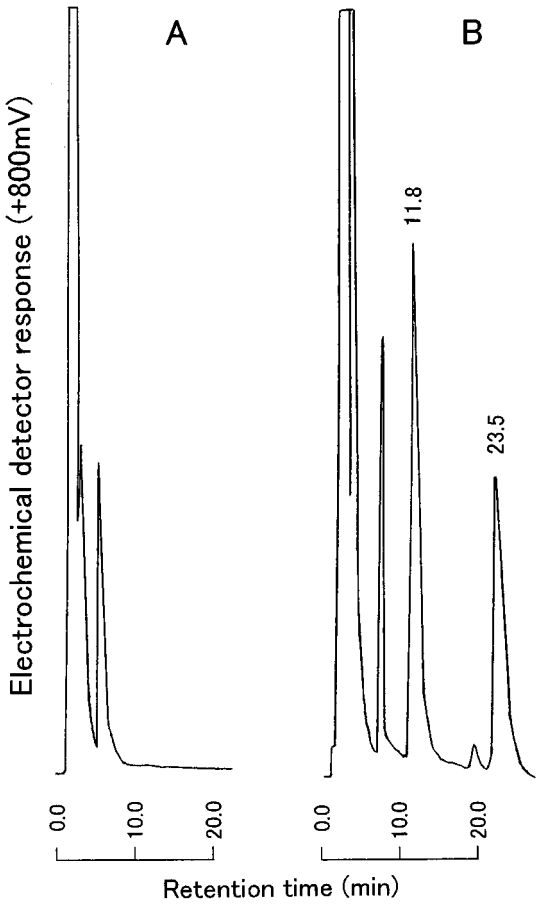

Figure 1. Representative HPLC chromatograms of rat plasma extracts 2 h after oral administration of 150 μmol/kg quercetin: (A) no plasma enzymatic hydrolysis; (B) plasma hydrolyzed with sulfatse/β-glucuronidase. Peaks with retention times of 11.8 and 23.6 min are quercetin and isorhamnetin, respectively.

to its aglycone, we used quercetin aglycone but not its glycoside in this study. After quercetin was administered orally in water along with lipids, emulsifiers, or ethanol, plasma concentrations of quercetin and its metabolites were measured as a function of time by high-performance liquid chromatography (HPLC) with electrochemical detection.

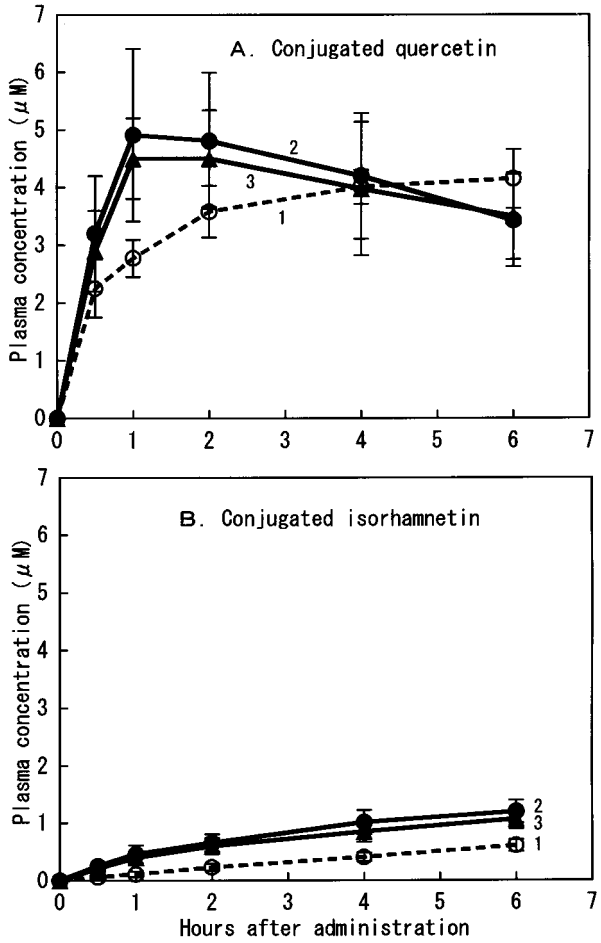

Figure 2. Effect of co-administration of lipids on the quercetin metabolite concentration in rat plasma after administration of 150 μmol/kg quercetin. Panels represent rat plasma concentration of conjugated quercetin (A) and conjugated isorhamnetin (B): ○, water group (control group); ●, lecithin group; ▲, soybean oil group. Numerals indicate the number of rat groups shown in **Table 1**. Values are means ± SD (n = 5).

MATERIALS AND METHODS

Chemicals. Quercetin, soybean lecithin, and soybean oil were purchased from Wako Pure Chemicals (Osaka, Japan), and isorhamnetin was purchased from Extrasynthese SA (Geney, France). Sulfatase type H-5 (from *Helix pomatia*) containing β-glucuronidase and sulfatase was purchased from Sigma Chemical Co. (St. Louis, MO). Two emulsifiers, a hydrophilic sucrose fatty acid ester "Ryoto sugar ester S1670" and a hydrophilic polyglycerol fatty acid ester "Ryoto polyglycerol ester SWA-15D", were kindly supplied by Mitsubishi-Kagaku Foods Corp. (Tokyo, Japan). Other chemicals were of analytical or HPLC grade.

Animals and Diets. Nine week old Wistar male rats weighing 190−200 g were supplied by Japan SLC, Inc. (Hamamatsu, Japan). The animals were kept in an environmentally controlled animal facility operating on a 12 h dark/light cycle at 23 ± 1 °C and 55% humidity for 5−6 days before experiments, with free access to tap water and standard MF diet (Oriental Yeast Co. Ltd., Tokyo). All rats were fasted for 15 h prior to quercetin administration. The first group of five rats (control group) was orally administered 150 μmol/kg of quercetin in 2 mL of water by direct stomach intubation. Fifteen groups of rats (five rats per group) were given 150 μmol/kg of quercetin in 2 mL of vehicles composed of water and constituents listed in **Table 1**. Quercetin was vortexed for 1 min with each vehicle and sonicated for 1 min at an amplitude of 30 using a high-intensity ultrasonic processor. Blood samples (~200 μL) were collected from the tail vein before and at 0.5, 1, 2, 4, and 6 h after administration, and blood plasma was immediately prepared by centrifugation for 15 min at 4 °C and 1000*g*.

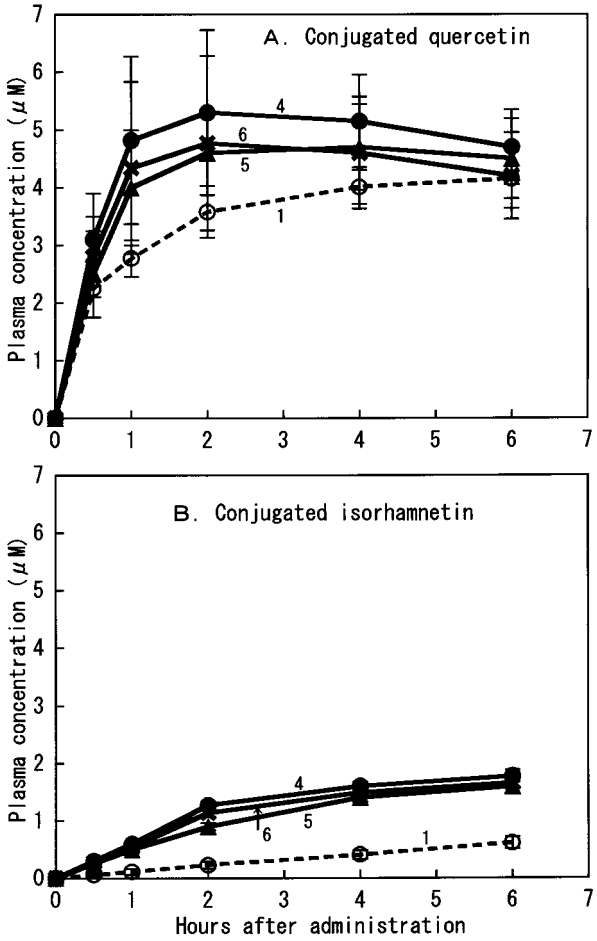

Figure 3. Effect of co-administration of emulsifiers on the quercetin metabolite concentration in rat plasma after administration of 150 μmol/kg quercetin. Panels represent rat plasma concentration of conjugated quercetin (A) and conjugated isorhamnetin (B): ○, water group (control group); ●, sucrose fatty acid ester group; ▲, polyglycerol ester group; ×, sodium taurocholate group. Numerals indicate the number of rat groups shown in **Table 1**. Values are means ± SD (n = 5).

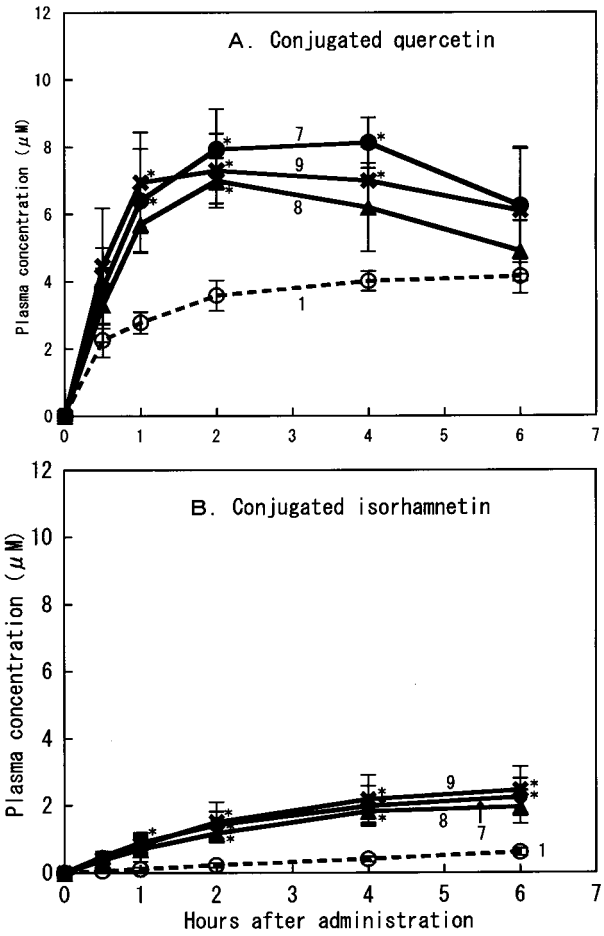

Figure 4. Effect of co-administration of both lecithin and emulsifiers on the quercetin metabolite concentration in rat plasma after administration of 150 μmol/kg quercetin. Panels represent rat plasma concentration of conjugated quercetin (A) and conjugated isorhamnetin (B): ○, water group (control group); ●, lecithin and sucrose fatty acid ester group; ▲, lecithin and polyglycerol ester group; ×, lecithin and sodium taurocholate group. Numerals indicate the number of rat groups shown in **Table 1**. Values are means ± SD (n = 5). *Significantly different from the control group at the same time point (P < 0.05).

Enzymatic Hydrolysis of Quercetin and Methylated Quercetin Conjugates in Plasma and Determination of Quercetin and Methylated Quercetin. Plasma (50 μL) was mixed with 50 μL of sulfatase type H-5 solution in 0.1 M sodium acetate buffer, pH 5.0. This enzyme preparation contained 500 units of β-glucuronidase and 25 units of sulfatase. The mixture was incubated at 37 °C for 50 min. Released quercetin and methylated quercetin were extracted by adding 900 μL of methanol/acetic acid (100:5, v/v) to the reaction mixture, vortexing for 30 s, sonicating for 30 s, again vortexing for 30 s, and centrifuging for 5 min at 4 °C and 5000g. The supernatant was diluted with water (1:1, v/v), and 20 μL was injected onto an HPLC column (TSK gel ODS-80Ts, 5 μm, 150 mm ×4.6 mm, TOSOH, Tokyo, Japan). The mobile phase was composed of water/methanol/acetic acid (53:45:2, v/v/v) and 50 mM lithium acetate. The flow rate was 0.9 mL/min. Elution was monitored with an electrochemical detector (Coulochem II, ESA, Bedford, MA) with the first electrode potential of +100 mV and the second electrode potential of +800 mV. Quercetin and isorhamnetin were quantitatively determined by an external standard method. The detection limits for quercetin and isorhamnetin were 5 and 10 nM, respectively, with a linear detector response up to 20 μM.

Quercetin's Solubility Test. The amount of quercetin solubilized in each of the sixteen vehicles used for administration was measured. Quercetin was vortexed with each vehicle, sonicated as described above for administration, and centrifuged for 5 min at 4 °C and 5000g, followed by filtration through a 0.2 μm syringe filter. The quercetin present in the filtrates was regarded as solubilized, and its content was measured by the HPLC analysis as described above.

Data Analysis. Reported values represent means ± standard deviation (SD). Statistical analysis was evaluated by the Dunnett (for quercetin metabolite plasma concentration) and Bonferroni/Dunn (for quercetin's solubility) post-hoc multiple comparison test to identify significantly different means using StatView for Windows version 5.0 (SAS Institute Inc., Cary, NC). The level of significance was set at P < 0.05.

RESULTS

Typical HPLC chromatograms of rat plasma extracts after oral administration of quercetin are shown in **Figure 1**. The treatment with sulfatase type H-5 released two compounds that eluted at 11.8 and 23.5 min. The first and second compounds have the same chromatographic properties as the qercetin and isorhamnetin (3′-methoxyquercetin) standards, respectively, in HPLC analysis. These compounds had been identified by comparison with authentic standards in HPLC and liquid chromatography/mass spectroscopy analysis in a previous study (*7*). Free, nonconjugated quercetin and nonconjugated isorhamnetin were not detected in the plasma of every rat group.

Figure 2 shows the effects of co-administration of lecithin and soybean oil on the plasma concentration of conjugated forms of quercetin and those of isorhamnetin. These lipids were likely

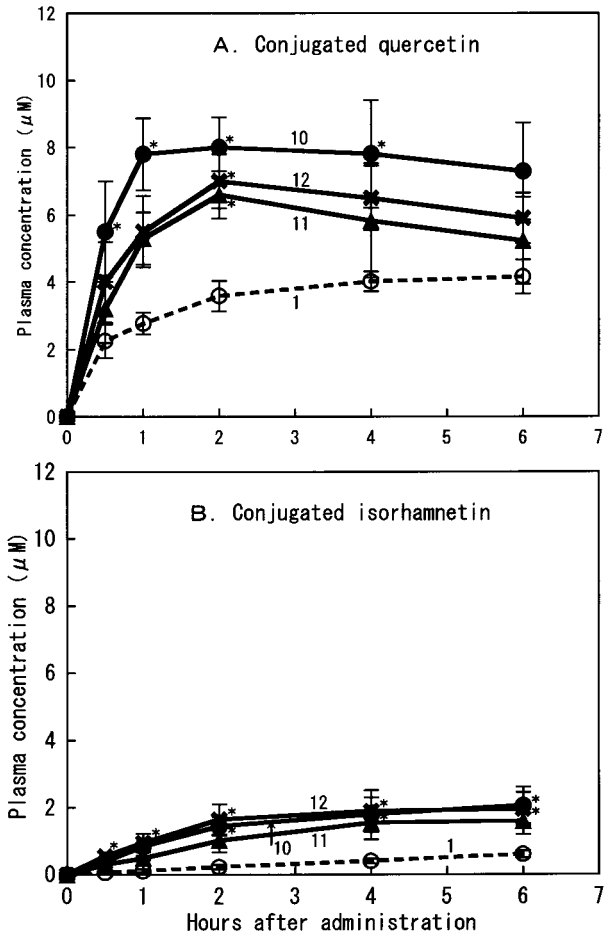

Figure 5. Effect of co-administration of both soybean oil and emulsifiers on the quercetin metabolite concentration in rat plasma after administration of 150 μmol/kg quercetin. Panels represent rat plasma concentration of conjugated quercetin (A) and conjugated isorhamnetin (B): ○, water group (control group); ●, soybean oil and sucrose fatty acid ester group; ▲, soybean oil and polyglycerol ester group; ×, soybean oil and sodium taurocholate group. Numerals indicate the number of rat groups shown in **Table 1**. Values are means ± SD (n = 5). *Significantly different from the control group at the same time point (P < 0.05).

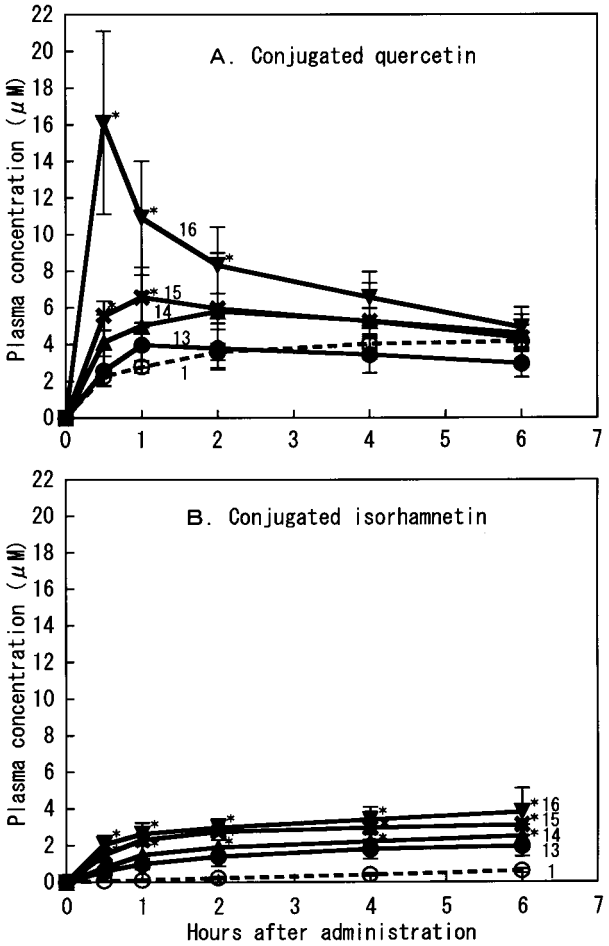

Figure 6. Effect of ethanol concentration in a vehicle on the quercetin metabolite concentration in rat plasma after administration of 150 μmol/kg quercetin. Panels represent rat plasma concentration of conjugated quercetin (A) and conjugated isorhamnetin (B): ○, 0% ethanol group (control group); ●, 10% ethanol group; ▲, 20% ethanol group; ×, 30% ethanol group; ▼, 50% ethanol group. Numerals indicate the number of rat groups shown in **Table 1**. Values are means ± SD (n = 5). *Significantly different from the control group at the same time point (P < 0.05).

to increase the total conjugated quercetin concentration during the first 2 h after administration (**Figure 2A**) and total conjugated isorhamnetin concentration throughout the experimental period (**Figure 2B**). However, statistically significant differences were not observed between these groups and the water group (control group).

Figure 3 represents quercetin absorption profiles in rat groups co-administered with emulsifiers, sucrose fatty acid ester, polyglycerol fatty acid ester, or sodium taurocholate. There were no statistically significant differences in conjugated quercetin metabolites plasma concentration between these groups and the control group, although these emulsifiers were likely to raise their accumulation in the plasma.

When quercetin in emulsions containing lecithin and any of the three emulsifiers was administered to the rats (rat group nos. 7−9), quercetin absorption profiles were as shown in **Figure 4**. All of the three combinations statistically and significantly increased the plasma concentrations of conjugated quercetin (**Figure 4A**) and conjugated isorhamnetin (**Figure 4B**) as compared with the control group. The combination of soybean oil with any of three emulsifiers (rat group nos. 10−12) also enhanced the absorption of quercetin significantly (**Figure 5**). Among the three tested groups, the combination of soybean oil with sucrose fatty acid ester was the most effective for enhancing quercetin absorption.

Figure 6 presents quercetin absorption profiles in five groups of rats administered with quercetin in 0−50% (v/v) ethanol. Ethanol was likely to enhance quercetin absorption depending on the ethanol concentration. Thirty and fifty percent of ethanol statistically and significantly raised the conjugated quercetin plasma concentration within the first 1 or 2 h after administration. In particular, the effect of 50% ethanol was remarkable; conjugated quercetin in rat plasma reached its maximum concentration of 16.1 ± 5.0 μM as soon as 0.5 h after administration, and then, its plasma level started to decrease. Statistically significant differences in conjugated isorhamnetin plasma concentration were observed until 6 h after administration between the 20−50% ethanol groups and the 0% ethanol group (control group). However, the 20% ethanol group was not significantly different in total quercetin metabolites plasma concentration from the control group.

Quercetin's solubility in tested solutions is shown in **Figure 7**. The concentration of quercetin solubilized in water was as low as 0.55 μM. The addition of lecithin or emulsifiers to the

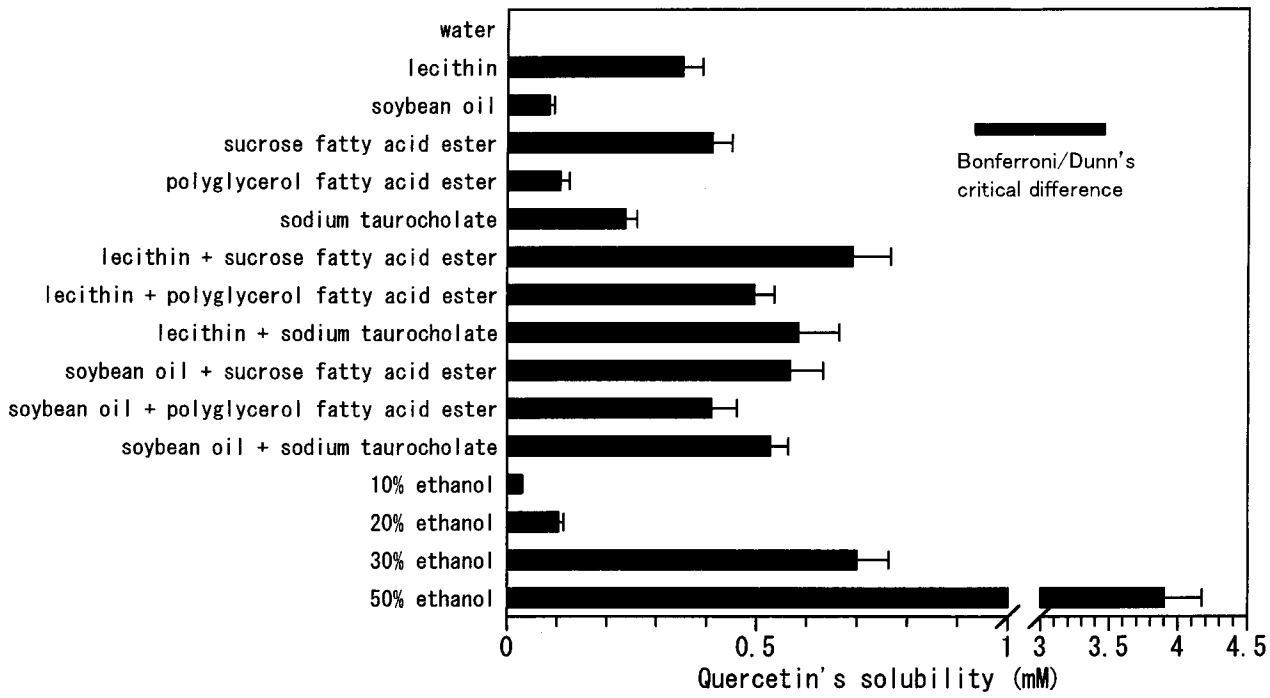

Figure 7. Quercetin's solubility in vehicles used for administration. Values are means ± SD ($n = 3$). Bar indicates Bonferroni/Dunn's critical difference ($P < 0.05$).

water suspension of quercetin increased its solubility. The coexistence of both lipids and emulsifiers was more effective for the solubilization of quercetin; if quercetin's solubility in water is taken as 1, the relative quercetin solubility in a vehicle containing 10% lecithin and 3% of a sucrose fatty acid ester was 1.25×10^3. Ethanol increased quercetin's solubility depending on its concentration, and 50% ethanol gave the highest solubility among the solutions tested in this study.

DISCUSSION

Flavonoids are present mainly as glycosides in plant foods such as vegetables and fruits. The mechanism of absorption of quercetin glucosides into intestinal epithelial cells has been controversial. However, it was recently suggested that hydrolysis to quercetin aglycone is required for the effective absorption of quercetin glucosides (*18*). Moreover, quercetin glucosides were shown to be efficiently hydrolyzed in the small intestine by β-glucosidases to its aglycone, most of which is then absorbed (*29*).

The efficiency of quercetin absorption has been reported to be affected by its solubility (*24*). In this study, when quercetin in a water suspension was administered to rats, low concentrations of less than 5 μM of conjugated quercetin metabolites were found in rat plasma because of quercetin's low solubility. It seemed that solubilization of quercetin by the addition of food constituents such as emulsifiers and ethanol to water could enhance quercetin absorption. Therefore, the present study focused on investigating how the co-administration of lipids, emulsifiers, and ethanol affects the efficiency of quercetin absorption.

When rat plasma extracts from 0.5 to 6 h after oral administration of quercetin were treated with sulfatase type H-5, two major compounds, quercetin and isorhamnetin, were released from their glucuronide and/or sulfate conjugates; this agreed with Manach et al. (*13*) and Piskula and Terao (*24*). As shown in **Figure 1**, a very small peak ($t_r = 19.4$ min) was also observed in an HPLC profile of the plasma. This compound, considered to be a methylated metabolite of quercetin other than isorhamnetin, has not been determined in this study because of a minor metabolite of quercetin.

The accumulation of conjugated isorhamnetin in the rat plasma was significantly smaller than that of conjugated quercetin in all rat groups, although the ratio of methylated/nonmethylated quercetin increased as the time after administration increased (**Figures 2−6**). However, it has been reported for (−)-epicatechin, another flavonoid with a catechol-like structure in the B ring, that ~40% of its plasma metabolites was methylated during the first 30 min and continued to increase in proportion to reach 75% after 8 h (*14*). These findings suggest that methylation, recognized as a metabolic path of flavonoids following glucuronidation and sulfation, proceeds less rapidly in the metabolism of quercetin as compared with that of (−)-epicatechin.

The maximum plasma concentration of total quercetin and isorhamnetin conjugates in each group was compared, 5.5 and 5.1 μM in the lecithin group and the soybean group, respectively, as compared with 4.8 μM in the water group. Their maximum plasma concentrations were 6.8, 6.1, and 6.1 μM in the sucrose fatty acid ester, polyglycerol fatty acid ester, sodium taurocholate groups, respectively; 10.1, 8.2, and 9.2 μM, respectively, in their combinations with lecithin; and 9.6, 7.6, and 8.7 μM, respectively, in their combinations with soybean oil. The concentrations were 5.2, 7.7, 8.9, and 18.2 μM in the 10, 20, 30, and 50% ethanol groups, respectively. Of all of the groups, the 50% ethanol group gave the highest maximum plasma concentration; it was approximately twice as high as those in the combinations of emulsifiers with lecithin.

The addition of 10% lecithin to a water suspension of quercetin could emulsify the suspension to some extent, while the emulsifying effect of 20% soybean oil was not expected. The oral administration of quercetin with the vehicles containing these lipids to rats was found to not significantly affect quercetin absorption, although it rather increased the plasma concentration of conjugated quercetin metabolites (**Figure 2**). Such weak

absorption-enhancing effects might be attributed to solubilization of only a part of quercetin in the vehicles, as suggested from quercetin's solubility shown in **Figure 7**. Previously, catechin absorption was shown to be enhanced when catechin was administered along with phospholipids (Pietta, P. G.; Simonetti, P. Presented at the International Symposium on Antioxidant Food Supplements in Human Health, October 16−18, 1997, Kaminoyama, Japan, unpublished data). It is suggested that phospholipids such as lecithin are more effective for the efficient flavonoid absorption among lipids.

Emulsifiers such as sucrose fatty acid esters and polyglycerol fatty acid esters are often added to a variety of processed foods including confectionaries, bread, ice cream, whipped cream, and margarine. Many brands of sucrose fatty acid esters and polyglycerol fatty acid esters, possessing different fatty acid compositions and the resulting different hydrophilicity, are available. In this study, we used a hydrophilic sucrose fatty acid ester and a hydrophilic polyglycerol fatty acid ester, which were suitable for emulsifying oil in water, and sodium taurocholate, a bile constituent, as emulsifiers. From the results presented in **Figures 3** and **7**, it is suggested that these emulsifiers increase quercetin's solubility resulting in higher quercetin absorption, similar to lipids. Previous research demonstrated that bile enhanced absorption of 3-palmitoyl-(+)-catechin in rats (*30*). Bile constituents such as sodium taurocholate might play an important role in flavonoid absorption from the alimentary tract, and an alternative for its effective absorption would be the co-administration of some emulsifiers.

The effects of lipids or emulsifiers were not significant, while a combination of emulsifiers with lecithin or soybean oil gave statistically significant absorption-enhancing effects except for that of a polyglycerol fatty acid ester with soybean oil (**Figures 4** and **5**). Quercetin's solubility in vehicles obtained by these combinations was mostly higher than that in the presence of either the lipids or the emulsifiers (**Figure 7**). This suggests that quercetin's solubility in lipid micelles is an important factor for its higher absorption from the alimentary tract. However, it is also possible that the presence of both lipids and emulsifiers might have additional significance for enhancing quercetin absorption, because statistically significant differences in quercetin's solubility were not always observed between lipid or emulsifier groups and their combination groups (**Figure 7**).

When foods are digested, phospholipids, one of the components of bile, enter the digestive tract together with bile acids. That is, both phospholipids and emulsifiers are always present in food digestion. This seems to be reasonable for effective flavonoid absorption. The results presented in this study also suggest that the ingestion of both lipids and emulsifiers from the diet could complement the role of bile for enhancing quercetin absorption.

The quercetin absorption-enhancing effect of ethanol was found to be concentration-dependent, and the 30 and 50% (v/v) ethanol group showed statistically significant differences in conjugated quercetin metabolite plasma concentration as compared with the control group (**Figure 6**). From the results that quercetin's solubility in 0−50% ethanol solutions increased with the increase in ethanol concentration (**Figure 7**), the quercetin absorption-enhancing function of ethanol could be in part attributed to the solubilization of quercetin. It is supposed that solubilization of quercetin by lipids, emulsifiers, ethanol, or their combination allows quercetin to easily solubilize in intestinal epithelial cells, resulting in the enhancement of its absorption.

The results presented here indicate that a combination of lipids and emulsifiers or more than 30% ethanol significantly enhances the intestinal absorption of quercetin and that the effect is related to quercetin's solubility in the vehicle. However, an ethanol concentration of more than 30%, which showed a significant quercetin absorption-enhancing effect, is too high to be used in the diet. In conclusion, a combination of lipids and emulsifiers is useful for better quercetin absorption. It is expected that the co-ingestion of these constituents along with quercetin-rich fruits and vegetables could enhance the intestinal absorption of queretin.

Although this study has focused on quercetin aglycone, plant foods are rich in quercetin glycosides, in general. Thus, further study will be required for the absorption efficiency of quercetin glycosides when various kinds of foods such as lipid foods and emulsified foods are ingested at the same time.

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