Effects of Quercetin and Rutin on Serum and Hepatic Lipid Concentrations, Fecal Steroid Excretion and Serum Antioxidant Properties

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Effects of quercetin and rutin on serum and hepatic lipid concentrations, fecal steroid excretion and their antioxidant properties were investigated in rats by oral administration. No toxic symptom was observed even at the dose of 1.0 g/kg of quercetin or rutin. Serum and hepatic lipid concentrations and fecal steroid excretion was not influenced remarkably, but serum thiobarbituric acid reactive substances (TBARS) decreased dose-dependently with the administration of quercetin or rutin. The decrease of serum TBARS was significantly correlated with the increase of serum free flavonoids ($p < 0.05$–0.001). Serum flavonoid concentrations, especially free quercetin, were higher in rutin-administered rats than in quercetin-administered rats at doses of 1.0 g/kg for 10 d ($p < 0.05$–0.001). When 1.0 g/kg of quercetin or rutin was administered in a single dose, they remained in the blood as aglycone or their conjugates of quercetin and isorhamnetin, even three days after administration. Recovered flavonoids were only 0.13% and 0.89% in urine for 3 d and 0.03% and 0.13% in serum on day 3 by administration of quercetin and rutin, respectively. Thus, some part of the administered quercetin or rutin was metabolized and showed antioxidant property, but had no remarkable influence on serum or hepatic lipid concentrations or fecal steroid excretion in rats.

Key words —— quercetin, rutin, rat, flavonoid, thiobarbituric acid reactive substance, steroid excretion

INTRODUCTION

Flavonoid is the general name of compounds which have a diphenol structure and are found in vegetables, herbs, fruits and legumes. Nearly 5000 kinds of flavonoids have been reported. Flavonoids have several physiological properties: antioxidant, anti-bacteroidal, anti-virus, anti-inflammatory, estrogen or anti-estrogen, anti-mutagenic, anti-cancer, activation or inactivation of enzymes, and so on.[1–4] Epidemiological studies have shown a negative correlation between flavonoid intake and the occurrence of cardiovascular diseases.[5–7]

Flavonoids are abundant in foods. Hertog *et al*.[8] estimated the daily intake of flavonoids in Holland to be 23 mg in the form of 4-oxoflavonoid. Linseisen *et al*.[9] estimated the Bavarian daily intake of flavonoids to be: flavonol 12.0 mg, catechin 8.3 mg, flavanone 13.2 mg, anthocyanidin 2.7 mg, proanthocyanine 3.7 mg, and phloridin 0.7 mg. Kimira *et al*.[10] estimated the Japanese daily intake of flavonoids to be as follows: quercetin 8.28 mg, kaempferol 4.91 mg, rutin 1.50 mg, myricetin 0.57 mg, fisetin 0.39 mg, luteolin 0.28 mg, eriodictyol 0.27 mg, and myricitrin 0.01 mg.

Flavonoids are also popular components of health foods such as propolis[11–13] in Japan. Many dietary supplements containing flavonoids have been consumed in U.S.A.[14] Potential use of flavonoids as functional food materials might be expanded because of their useful physiological properties such as the prevention of cardiovascular diseases and cancer.

There have been some reports about the effect of flavonoids on serum and hepatic lipids and fecal steroid excretions that are very relevant to cardiovascular diseases and some cancer. Monforte *et al*.[15] reported that hesperidin significantly increased plasma levels of HDL-cholesterol and lowered total and LDL- cholesterol, total lipid and triglyceride in normolipidemic and hyperlipidemic rats. Igarashi and Ohmura[16] reported that isorhamnetin, rhamnetin or quercetin lowers the serum total cholesterol in rats fed a cholesterol-enriched diet and also lowers

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hepatic total cholesterol and thiobarbituric acid reactive substances (TBARS) in rats fed cholesterol-free diets. Basarkar and Nath[17] reported that epicatechin, hesperidin and quercetin reduced the elevation of serum and hepatic cholesterol significantly in cholesterol-fed rats. Valsa *et al.*[18] reported that catechin reduced serum and hepatic cholesterol and triglyceride levels, and increased fecal steroid excretion in normolipidemic rats. On the contrary, Hodgson *et al.*[19] reported that genistein and daidzein did not alter serum levels of total or HDL-cholesterol and triglyceride in humans. However, the relationship between lipid metabolism and the antioxidant properties of flavonoids has not yet been elucidated.

In the present study, we administered quercetin and its glycoside rutin, the flavonoids most abundantly consumed in foods, orally to rats at various doses. The changes in lipid content in serum and the liver, fecal steroid excretion and serum TBARS and flavonoid levels were investigated to consider the relationship between lipid metabolism and their serum antioxidant properties.

MATERIALS AND METHODS

Materials —— Cholesterol, 5α-cholestane, 5β-cholanic acid, lithocholic acid (LCA), deoxycholic acid (DCA), chenodeoxycholic acid (CDCA), hyodeoxycholic acid (HDCA) and cholic acid (CA) were purchased from GL Sciences Inc. (Tokyo, Japan). Coprostanone was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Coprostanol, nordeoxycholic acid (norDCA), 5-cholenic acid 3β-ol, isolithocholic acid (ILCA), isodeoxycholic acid (IDCA), murodeoxycholic acid (MDCA), 6-keto lithocholic acid (6KLCA), 12-keto lithocholic acid (12KLCA), α-muricholic acid (αMCA), β-muricholic acid (βMCA), ω-muricholic acid (ωMCA), 7-keto deoxycholic acid (7KDCA), and 12-keto chenodeoxycholic acid (12KCDCA) were purchased from Steraloids, Inc. (Wilton, NH, U.S.A.). Derivative reagents for GC analysis, hexafluoroisopropanol and trifluoroacetic acid anhydride, were purchased from GL Sciences Inc. Standards for HPLC, quercetin, rutin, isorhamnetin and tamarixetin were purchased from Extrasynthèse.

Choloylglycine hydrolase [EC 3.5.1.24 from *Clostridium perfringens* (welchii), 1500 units] and β-glucuronidase (EC 3.2.1.31 from *Helix pomatia*, 100000 units) were purchased from Sigma Chemical Co. Commercial kits for the determination of serum and hepatic lipids were purchased from Wako Pure Chemical Industries (Osaka, Japan). Sep-pak^R plus C_{18} cartridge column was purchased from Waters Corporation (Milford, MA). Fused silica capillary columns DB-210 (0.25 mm i.d. × 30 m, film thickness 0.25 μm or 0.5 μm) were purchased from J&W Scientific (Folsom, CA). HPLC column STR ODS II (4.6 mm i.d. × 250 mm) was purchased from Shinwa Chemical Industries (Kyoto, Japan).

Quercetin and rutin for administration to rats were purchased from Extrasynthèse (Geney, France). Their purities were more than 96% by HPLC. Carboxymethyl cellulose sodium salt (CMC-Na) and sodium cholate, analytical grade, were purchased from Wako Pure Chemical Industries. Formulations of quercetin and rutin were prepared before use. For each sample, 0.01–1.0 g was weighed, suspended in 1% CMC-Na and adjusted to 5 ml. Each formulation was administered to rats at the dose of 5 ml/kg body weight.

Ion-exchanged and redistilled water or distilled water for HPLC was used throughout the experiments. Acetonitrile, methanol and ethyl acetate used for analysis were of HPLC grade. All other reagents used were of the highest purity commercially available.

Apparatus —— A Shimadzu Model GC-14A gas chromatograph (Kyoto, Japan), equipped with a flame ionization detector (FID), autosampler AOC-17A, and integrator C-R4A, was used for the determination of fecal steroids. A Hewlett Packard HP Series 1100 HPLC (Palo Alto, CA, U.S.A.), equipped with a degasser G1322A, binary pump G1312A, thermostatted column compartment G1330A, autosampler G1329A, diode array detector (DAD) G1315A, and ChemStation, was used for the flavonoid analysis. A Hitachi U-3210 spectrophotometer (Tokyo) was used for serum and hepatic lipid analysis and a Hitachi 650-60 fluorophotometer for serum TBARS.

Animal Treatment ——

Animals: All the procedures involving animals were conducted in compliance with Japanese law (Bulletin of Prime Minister's Office No. 6, March 1980) and guidelines established by the National Institute of Health Sciences. Male rats of the Wistar strain (4 weeks old) were purchased from Clea Japan, Inc. (Tokyo, Japan) and kept in an air-conditioned room (23 ± 1°C, 50–60% humidity) lighted for 12 h/d (07:00 to 19:00). Rats were given free access to a commercial nonpurified diet (F-2,

Funahashi Farm, Chiba, Japan) and water throughout the experiments. Animals weighing 121–144 g were used in Experiment 1 and those weighing 118–137 g were used in Experiments 2 and 3. Each group in Experiments 1–3 contained 5 rats.

Administration of Quercetin or Rutin for 22 d (Experiments 1 and 2): Quercetin or rutin was administered orally in doses of 0.01, 0.05, 0.1, 0.2, 0.5 and 1.0 g/kg for 22 d, respectively. Each sample was administered to individual rats between 09:00 and 11:00 every day. Rats of the control group were administered a 1% CMC-Na solution at 5 ml/kg. Feces were collected on d 18–19 by placing each rat in a metabolic cage. Rats were food-deprived overnight on day 21. On day 22, they were anesthetized with diethylether and blood was collected by heart puncture. The liver was excised immediately after bleeding.

Administration of Quercetin or Rutin (1.0 g/kg) for 10 d (Experiment 3): Quercetin or rutin was administered orally in doses of 1.0 g/kg for 10 days. Each sample was administered to individual rats between 09:00 and 11:00 every day. Rats were food-deprived overnight on day 9. On day 10, rats were anesthetized with diethylether and blood was collected by heart puncture.

Administration of Quercetin or Rutin in a Single Dose (1.0 g/kg) (Experiment 4): Quercetin or rutin was orally administered to rats in a single dose of 1.0 g/kg. Urine was collected on days 0–1, 1–2 and 2–3, by placing each rat in a metabolic cage. On day 3, rats were anesthetized with diethylether and blood was collected by heart puncture.

Determination of Lipids in Serum and Liver —— Serum concentrations of total cholesterol, HDL-cholesterol, triglycerides, phospholipids, free fatty acids and TBARS were determined using commercial kits. A portion of liver was homogenized with chloroform/methanol solution (2 : 1, v/v) and filtered. Adequate amounts of the filtrate (lipid extract) were mixed with sodium cholate solution and evaporated to dryness under a nitrogen stream, and total cholesterol, triglycerides and phospholipids in the residues were determined using the commercial kits.

Determination of Fecal Steroids —— Collected feces were dried at 60°C overnight and ground into a powder. Fecal neutral steroids and bile acids were analyzed by the methods of Grundy *et al.*[20] and Setchell *et al.*,[21] with some modifications.

To a portion of the ground dried feces, 5α-cholestane, 5β-cholanic acid and norDCA were added as internal standards. Lipids in the feces were extracted twice with ethanol and a chloroform/methanol mixture (2 : 1, v/v) respectively by sonication and reflux. Fractions of chloroform/methanol mixture and ethanol were collected and evaporated to dryness. Neutral lipids were extracted with *n*-hexane after the saponification with methanolic potassium hydroxide solution. Neutral steroids were determined by GC-FID using 5α-cholestane as an internal standard. After the removal of methanol by evaporation, the remaining fraction was neutralized with phosphoric acid, and applied to a Sep-pak^{R} plus C_{18} cartridge column[22] pre-washed with methanol and water. The column was again washed with water, and bile acids were eluted with methanol. The eluate was evaporated to dryness, redissolved with methanol and divided into two fractions. One portion of the divided sample was acidified with concentrated HCl and the free bile acids were extracted three times with diethylether. To the other portion of the fraction, methanol was evaporated and deconjugation was performed by choloylglycine hydrolase (EC 3.5.1.24).[23] Total bile acids were extracted with diethylether after the acidification with concentrated HCl. Free and total bile acids were derivatized to hexafluoroisopropyl ester-trifluoroacetyl (HFIP-TFA) derivatives[24] and determined by GC-FID using 5β-cholanic acid and norDCA as an internal standard.

GC conditions for steroid analysis were as follows: column, DB-210 (film thickness 0.25 μm for neutral steroids, 0.50 μm for bile acids); carrier gas, He 1.5 ml/min; column temperature, 60°C (2 min) $\rightarrow$ 10°C/min $\rightarrow$ 180°C $\rightarrow$ 5°C/min $\rightarrow$ 230°C for neutral steroids, 60°C (2 min) $\rightarrow$ 10°C/min $\rightarrow$ 235°C for bile acids; injection port and detector temperature, 250°C; detector, FID; injection method, splitless; injection volume, 2 μl.

Determination of Flavonoids in Serum and Urine —— Serum and urinary flavonoids were analyzed by the method of Manach *et al.*[25] and Franke *et al.*[26] with some modification.

Serum, measured to 0.5 ml was diluted with 2 ml of 0.1 M acetate buffer (pH 5.0) and 18.86 nmol of flavone was added as an internal standard. Urine was stocked for 1 d, diluted with water and adjusted to 25 ml. Five ml of the diluted urine was cleaned up with a Sep-pak^{R} plus C_{18} cartridge column, washed with water and eluted by methanol after 188.6 nmol of flavone was added as an internal standard. Eluate was evaporated to dryness, redissolved with 4 ml of 0.1 M acetate buffer (pH 5.0) and divided into two fractions.

Table 1. Body Weight, Relative Weight of the Liver, Food Intake and Dry Weight of Feces in Rats

Experiment	Body weight gain (g/21 day)	Relative weight of the liver (final) (% of body weight)	Food intake Days 19–20 (g/day)	Dry weight of feces Days 19–20 (g)
Experiment 1 Quercetin				
Control	126.2±4.2	3.07±0.07	15.8±0.5	1.61±0.12
Quercetin 0.01 g/kg	135.5±1.9	3.24±0.09	16.3±0.3	1.53±0.13
Quercetin 0.05 g/kg	139.0±3.9	3.07±0.04	17.2±0.5	1.71±0.13
Quercetin 0.1 g/kg	129.6±2.0	3.08±0.08	17.6±1.0	1.81±0.16
Quercetin 0.2 g/kg	131.0±4.5	3.18±0.13	18.4±0.5	1.72±0.09
Quercetin 0.5 g/kg	133.4±4.6	3.05±0.06	17.6±1.2	1.99±0.11
Quercetin 1.0 g/kg	131.8±4.1	3.10±0.10	18.0±0.8	2.15±0.22
Experiment 2 Rutin				
Control	126.0±5.0	3.20±0.10	16.3±1.2	1.94±0.16
Rutin 0.01 g/kg	129.2±4.3	3.07±0.08	16.2±1.4	1.94±0.18
Rutin 0.05 g/kg	133.6±4.1	3.07±0.15	17.2±1.2	1.76±0.29
Rutin 0.1 g/kg	122.8±3.6	3.03±0.11	14.4±0.9	1.69±0.14
Rutin 0.2 g/kg	127.2±4.9	3.08±0.11	15.4±0.7	2.10±0.21
Rutin 0.5 g/kg	119.4±4.6	3.04±0.09	15.6±0.5	1.46±0.17
Rutin 1.0 g/kg	120.2±3.3	2.92±0.05	17.0±0.4	1.79±0.21

Data are represented as means S.E.M. ($n = 5$).

Total and free flavonoids were analyzed with and without an enzymatic hydrolysis. For total flavonoid determination, β-glucuronidase (1000 U) and sulfatase (55 U) were added to the above-mentioned serum preparation and one fraction of the urine preparation, then incubated at 37°C for 45 min. Enzymatic hydrolysis was terminated by the addition of 1 ml of 0.5 N HCl and 1 ml of methanol. Flavonoids were extracted twice with 4 ml of ethyl acetate. The organic layer was evaporated to dryness after centrifugation at 800 *g* for 15 min, and dissolved with 2 ml of methanol for the determination of flavonoids by HPLC. Free flavonoids were analyzed without the addition of β-glucuronidase and sulfatase to the serum preparations and another fraction of the urine preparation, as described above.

HPLC conditions were as follows: apparatus, HP 1100 series; column, STR ODS II (0.46 mmϕ × 250 mm); column oven temperature, 35°C; mobile phase, (A) water/phosphoric acid 1000 : 1 (v/v), (B) water/acetonitrile/phosphoric acid 200 : 800 : 1 (v/v/v); gradient program, B% 10–80 (50 min, linear gradient); detector, DAD; monitor wavelength, 370 nm for rutin, quercetin, isorhamnetin and tamarixetin, 280 nm for flavone; injection volume, 10 μl.

The percentage of distribution was calculated by assuming that all of the flavonoids in the blood were distributed in the serum and that the blood weight accounted for 7% of the whole body weight.

Statistical Methods —— Data are expressed as means SEM. Statistical analyses were performed by one-way analysis of variances (ANOVA) with subsequent Dunnett's multiple comparison test or Aspin-Welch *t* test, using the 'Statlight' program (Yukms, Tokyo). Dose-response was examined by linear regression analysis after 1-way ANOVA. Probability values lower than 0.05 were accepted for statistical significance.

RESULTS

Effects of Quercetin and Rutin on Body Weight Gain, Hepatic Weight, Food Intake, Dry Weight of Feces, Serum and Hepatic Lipids and Fecal Steroid Excretion (Experiments 1 and 2)

Table 1 shows the body weight gain, relative weight of the liver, food intake and dry weight of feces in rats administered 0.01–1.0 g/kg of quercetin or rutin for 22 d. Quercetin and rutin had no significant effect on any of these four factors.

Concentrations of serum and hepatic lipids on day 22 in rats food-deprived overnight are shown in Table 2. A significant decrease in serum HDL-cholesterol and triglyceride levels was observed in animals administered rutin at 0.5 g/kg and 1.0 g/kg, respectively, compared to the control rats. No sig-

Table 2. Hepatic and Serum Lipids of Rats Administered Quercetin or Rutin (Day 22, Food-Deprived Overnight)

	Control	Quercetin 0.01 g/kg	Quercetin 0.05 g/kg	Quercetin 0.1 g/kg	Quercetin 0.2 g/kg	Quercetin 0.5 g/kg	Quercetin 1.0 g/kg
Hepatic total cholesterol [mg/g]	2.803 ± 0.153	2.915 ± 0.143	2.734 ± 0.096	2.565 ± 0.286	2.448 ± 0.110	2.517 ± 0.157	2.710 ± 0.146
Hepatic triglycerides [mg/g]	15.84 ± 1.01	14.55 ± 0.88	13.10 ± 0.89	16.26 ± 1.17	12.93 ± 1.47	14.34 ± 1.18	14.05 ± 1.57
Hepatic phospholipids [mg/g]	25.27 ± 1.65	21.86 ± 1.13	22.24 ± 1.30	27.83 ± 0.69	23.07 ± 1.00	24.23 ± 1.05	24.21 ± 1.07
Serum total cholesterol [mg/dl]	67.98 ± 6.06	66.16 ± 2.87	65.49 ± 1.61	58.08 ± 1.52	59.27 ± 4.48	62.69 ± 2.23	63.33 ± 2.50
Serum HDL-cholesterol [mg/dl]	35.45 ± 3.09	34.78 ± 2.35	35.94 ± 2.56	28.78 ± 0.96	34.80 ± 3.11	30.28 ± 2.13	29.36 ± 0.92
Serum triglycerides [mg/dl]	61.43 ± 2.77	72.93 ± 3.89	71.28 ± 6.40	67.93 ± 5.83	55.34 ± 3.21	61.83 ± 4.28	52.03 ± 3.50
Serum phospholipids [mg/dl]	105.7 ± 4.3	109.4 ± 4.7	110.9 ± 1.9	102.7 ± 3.2	110.0 ± 4.2	106.4 ± 3.0	106.3 ± 5.3
Serum free fatty acids [meq/l]	0.927 ± 0.042	1.015 ± 0.065	0.911 ± 0.088	0.936 ± 0.024	1.017 ± 0.092	0.909 ± 0.061	0.993 ± 0.050

	Control	Rutin 0.01 g/kg	Rutin 0.05 g/kg	Rutin 0.1 g/kg	Rutin 0.2 g/kg	Rutin 0.5 g/kg	Rutin 1.0 g/kg
Hepatic total cholesterol [mg/g]	2.376 ± 0.106	2.376 ± 0.061	2.380 ± 0.086	2.341 ± 0.079	2.397 ± 0.044	2.450 ± 0.156	2.101 ± 0.067
Hepatic triglycerides [mg/g]	13.48 ± 1.19	13.67 ± 0.84	14.74 ± 1.04	12.42 ± 0.94	14.11 ± 1.62	11.68 ± 0.21	11.14 ± 0.51
Hepatic phospholipids [mg/g]	22.03 ± 0.56	20.63 ± 0.61	19.97 ± 0.35	19.54 ± 1.25	19.57 ± 0.83	20.12 ± 1.30	19.23 ± 0.68
Serum total cholesterol [mg/dl]	69.68 ± 2.47	63.83 ± 4.08	68.16 ± 3.51	67.14 ± 4.80	59.54 ± 4.29	62.22 ± 0.93	60.84 ± 1.67
Serum HDL-cholesterol [mg/dl]	42.34 ± 2.52	39.20 ± 1.73	43.16 ± 2.42	39.87 ± 3.08	37.06 ± 1.46	32.59 ± 1.72*a)	34.90 ± 2.70
Serum triglycerides [mg/dl]	63.14 ± 7.81	63.34 ± 5.83	48.99 ± 5.54	55.18 ± 11.84	58.83 ± 10.10	39.14 ± 6.14	32.08 ± 4.75*
Serum phospholipids [mg/dl]	108.6 ± 1.8	108.2 ± 7.4	110.6 ± 7.0	111.8 ± 5.8	105.2 ± 3.6	98.05 ± 2.16	101.0 ± 3.4
Serum free fatty acids [meq/l]	0.977 ± 0.060	1.058 ± 0.085	1.186 ± 0.081	1.052 ± 0.099	1.003 ± 0.083	0.934 ± 0.067	0.872 ± 0.042

Data are expressed as means S.E.M. ($n = 5$). *a*) Different significantly from the control group. ($*p < 0.05$ *vs*. Control, 1-way ANOVA followed by Dunnett's multiple comparison test).

nificant changes were detected in serum or hepatic lipids otherwise.

Fecal steroid excretion in rats administered quercetin at days 19–20 is shown in Table 3. Quercetin did not influence the amounts or composition of fecal neutral steroids at all. A significant increase in the percentage of MDCA was observed in rats administered 0.2 and 0.5 g/kg of quercetin, and a significant increase of the percentages of DCA, IDCA, αMCA. A decrease of 12KLCA was observed in rats administered 0.5 g/kg of quercetin, compared to the control rats (Table 3). Otherwise, there were no significant changes in the amounts nor in the composition of fecal bile acids (Table 3).

Fecal steroid excretion in rats administered rutin at days 19–20 is shown in Table 4. The fecal coprostanol/cholesterol ratio decreased significantly in those administered 0.01, 0.5 and 1.0 g/kg compared to the control rats. Excreted amounts of neutral steroid did not change significantly. Fecal bile acid excretion increased significantly only in rats administered 0.01 g/kg of rutin, compared to the control rats. There were no significant changes in cholic acid derived bile acids/chenodeoxycholic acid derived bile acids (CAs/CDCAs) ratio nor in the ratio of primary bile acids, although the percentage of αMCA decreased significantly in rats administered 0.1 and 0.2 g/kg.

Correlation of Serum TBARS and Free Flavonoid Concentrations (Experiments 1 and 2)

Table 5 shows the concentrations of serum TBARS and free flavonoids in rats on day 22, when food-deprived overnight. Serum was a pale lemon-yellow color only in animals administered 1.0 g/kg of rutin. Administration of quercetin and rutin reduced serum TBARS significantly in a dose-dependent manner ($p < 0.001$, r^2 0.4619 in the rutin-administered group and $p < 0.001$, r^2 0.7774 in the quercetin-administered group). Quercetin and isorhamnetin (one of the metabolites of quercetin) were detected in the serum (Fig. 1). Both the free quercetin and isorhamnetin increased dose-dependently in rats administered quercetin ($p < 0.001$, r^2 0.5963 and 0.6681) and rutin ($p < 0.01$, r^2 0.1978 and 0.2255).

Concentration and Composition of Serum Flavonoids in Rats Administered 1.0 g/kg of Quercetin or Rutin for 10 d (Experiment 3)

Table 6 shows the concentration and composition of serum flavonoids in rats administered 1.0 g/kg of quercetin or rutin for 10 d. Serum was a pale lemon-yellow color only in rats administered rutin.

Detected serum free flavonoids were quercetin and isorhamnetin. Concentrations of flavonoids in both the total and free types were significantly higher in rats administered rutin, than in those administered

Table 3. Fecal Excretion of Bile Acids in Rats Administered Quercetin (Days 18–19)

	Control	Quercetin 0.01 g/kg	Quercetin 0.05 g/kg	Quercetin 0.1 g/kg	Quercetin 0.2 g/kg	Quercetin 0.5 g/kg	Quercetin 1.0 g/kg
Fecal neutral steroid excretion							
Coprostanol (Cp) [μ mol/day]	8.55 ±0.72	7.25 ±0.76	7.05 ±0.86	7.95 ±0.80	8.20 ±0.58	8.75 ±0.73	9.46 ±0.82
Cholesterol (Ch) [μmol/day]	3.95 ±0.23	4.02 ±0.69	4.89 ±0.95	3.92 ±0.45	4.03 ±0.39	4.54 ±0.28	5.15 ±0.42
Coprostanone [μmol/day]	0.95 ±0.13	0.88 ±0.15	0.90 ±0.15	0.96 ±0.06	0.78 ±0.11	1.07 ±0.11	1.32 ±0.20
Total [μmol/day]	13.45 ±0.92	12.15 ±1.11	12.84 ±1.16	12.83 ±1.25	13.01 ±0.81	14.35 ±1.07	15.93 ±1.29
Cp/Ch ratio	2.14 ±0.09	1.98 ±0.25	1.63 ±0.26	2.05 ±0.09	2.12 ±0.29	1.92 ±0.09	1.86 ±0.13
Fecal bile acid excretion							
Total bile acids [μmol/day]	10.30 ±0.73	8.87 ±1.07	10.32 ±0.99	9.92 ±0.95	11.13 ±1.47	12.51 ±0.73	11.22 ±1.02
Compositions of fecal bile acids [%]							
Cholic acid derived bile acids (CAs)							
Cholic acid (CA)	0.55 ±0.16	1.01 ±0.29	0.63 ±0.36	0.46 ±0.13	0.90 ±0.33	0.88 ±0.24	0.67 ±0.24
Deoxycholic acid (DCA)	12.48 ±0.92	13.89 ±1.03	14.64 ±1.69	14.98 ±0.87	14.90 ±1.12	17.57 ±1.27*a)	13.35 ±1.81
Isodeoxycholic acid (IDCA)	3.00 ±0.29	3.25 ±0.25	3.21 ±0.49	3.22 ±0.22	3.06 ±0.17	5.29 ±0.53**	3.73 ±0.43
12-Ketolithocholic acid (12KLCA)	7.51 ±0.71	5.03 ±0.70	7.32 ±0.36	7.60 ±0.96	5.98 ±1.08	3.62 ±0.40**	5.79 ±0.65
7-Ketodeoxycholic acid (7KDCA)	0.39 ±0.27	0.19 ±0.19	0.55 ±0.34	0.20 ±0.12	0.56 ±0.24	0.12 ±0.12	0.25 ±0.17
12-Ketochenodeoxycholic acid (12KCDCA)	1.44 ±0.55	1.13 ±0.24	0.82 ±0.19	0.65 ±0.18	0.36 ±0.19	0.37 ±0.07	1.32 ±0.56
Chenodeoxycholic acid derived bile acids (CDCAs)							
β-Muricholic acid (βMCA)	9.61 ±2.75	5.80 ±1.06	9.66 ±2.49	7.56 ±2.13	9.48 ±1.39	4.58 ±1.16	5.56 ±1.97
Lithocholic acid (LCA)	5.50 ±0.53	5.45 ±0.57	5.22 ±0.58	4.90 ±0.34	4.91 ±0.38	5.34 ±0.66	5.52 ±0.37
Isolithocholic acid (ILCA)	1.16 ±0.10	0.67 ±0.24	1.02 ±0.25	1.02 ±0.09	0.95 ±0.08	1.12 ±0.21	1.13 ±0.10
Hyodeoxycholic acid (HDCA))	31.31 ±5.90	45.51 ±2.14	32.13 ±3.60	32.25 ±9.40	21.56 ±8.56	35.40 ±4.39	36.62 ±2.79
Murodeoxycholic acid (MDCA)	2.25 ±0.59	2.47 ±0.22	3.27 ±0.83	2.07 ±0.45	5.01 ±0.22**	4.98 ±0.17**	3.72 ±0.82
α-Muricholic acid (αMCA)	2.01 ±0.50	1.28 ±0.24	2.53 ±0.71	1.69 ±0.38	4.33 ±0.54	3.25 ±0.35*	3.15 ±0.65
ω-Muricholic acid (ωMCA)	17.18 ±3.09	8.73 ±0.89	15.53 ±3.54	19.16 ±6.60	24.90 ±6.26	14.28 ±3.45	14.43 ±2.48
6-Ketolithocholic acid (6KLCA)	5.26 ±1.35	5.01 ±0.48	4.22 ±0.79	3.91 ±1.07	2.82 ±0.32	2.71 ±0.21	5.25 ±1.64
Others							
3β-Hydroxy 5-cholenic acid	0.35 ±0.10	0.60 ±0.22	0.73 ±0.26	0.33 ±0.09	0.29 ±0.11	0.50 ±0.18	0.48 ±0.16
CAs/CDCAs ratio	0.341±0.027	0.328±0.022	0.379±0.031	0.378±0.034	0.350±0.026	0.392±0.038	0.320±0.026
Ratio of primary bile acids[b)] [%]	10.16 ±2.75	6.06 ±1.54	10.29 ±2.58	8.02 ±2.16	10.09 ±0.88	5.45 ±1.34	6.23 ±1.94

Data are expressed as means S.E.M. ($n = 5$). *a*) Different significantly from the control group. (*$p < 0.05$, **$p < 0.01$ *vs.* Control, 1-way ANOVA followed by Dunnett's multiple comparison test). *b*) Primary bile acids: Total of CA, CDCA and βMCA.

quercetin ($p < 0.05$–0.001). The percentages of serum total quercetin and aglycone were significantly higher and that of the total isorhamnetin was significantly lower in rats administered rutin, compared to those administered quercetin ($p < 0.05$–0.001).

Serum and Urinary Flavonoids in Rats Administered 1.0 g/kg of Rutin or Quercetin in a Single Dose (Experiment 4)

Table 7 shows the concentration and composition of serum flavonoids in rats on day 3 after administration of 1.0 g/kg of quercetin or rutin. Detected flavonoids were quercetin and isorhamnetin. Both the total and free flavonoid concentrations tended to be higher in rutin-administered rats than in quercetin-administered rats. The concentrations of total quercetin and free flavonoids, especially isorhamnetin, were significantly higher in rats administered rutin compared to those administered quercetin ($p < 0.01$–0.05). There were no significant differences in the composition of total flavonoids or the percentage of aglycone at all.

Table 7 also shows the amounts and composition of urinary flavonoids excreted 3 d after oral administration of quercetin or rutin at a dose of 1.0 g/kg. Detected flavonoids were quercetin, isorhamnetin and tamarixetin, the chemical structures of metabolites of quercetin are shown in Fig. 1. Both the concentration and composition of urinary flavonoids varied individually and statistical significance was not detected at all.

Table 8 shows the percentage of urinary flavonoid excretion during the three days and serum flavonoid distribution on day 3 in rats administered 1.0 g/kg of quercetin or rutin in a single dose. The percentage of urinary flavonoid excretion was varied because of the various concentrations given (Table 7). There was no significance in the percentage of urinary flavonoids between rutin-administered and quercetin-administered rats. On the contrary, distribution of flavonoids in serum on day 3 was significantly higher in animals administered rutin than

Table 4. Fecal Steroid Excretion in Rats Administered Rutin (Days 18–19)

	Control	Rutin 0.01 g/kg	Rutin 0.05 g/kg	Rutin 0.1 g/kg	Rutin 0.2 g/kg	Rutin 0.5 g/kg	Rutin 1.0 g/kg
Fecal neutral steroid excretion							
Coprostanol (Cp) [μ mol/day]	9.51 ±0.47	7.36 ± 0.49	7.96 ±0.63	8.17 ±0.68	8.81 ±0.93	6.75 ± 1.05	6.59 ±0.85
Cholesterol (Ch) [μmol/day]	3.94 ±0.27	3.37 ± 0.29	5.41 ±0.63	4.17 ±0.56	3.94 ±0.39	4.86 ± 0.41	3.85 ±0.32
Coprostanone [μmol/day]	0.50 ±0.07	0.54 ± 0.03	0.48 ±0.08	0.56 ±0.10	0.54 ±0.08	0.51 ± 0.18	0.42 ±0.05
Total [μmol/day]	13.96 ±0.36	13.31 ± 0.69	12.60 ±1.17	12.07 ±1.11	14.20 ±1.37	11.11 ± 1.54	12.47 ±1.75
Cp/Ch ratio	2.46 ±0.24	1.46 ± 0.23**[a]	1.97 ±0.14	2.12 ±0.16	1.80 ±0.07	1.72 ± 0.12*	1.29 ±0.21**
Fecal bile acid excretion							
Total bile acids [μmol/day]	12.14 ±0.41	17.14 ± 0.60*	10.99 ±0.54	11.25 ±1.11	11.38 ±1.26	9.69 ± 1.78	9.96 ±1.21
Compositions of fecal bile acids [%]							
Cholic acid derived bile acids (CAs)							
Cholic acid (CA)	0.74 ±0.13	0.86 ± 0.13	0.67 ±0.24	1.18 ±0.11	1.02 ±0.17	1.17 ± 0.27	1.49 ±0.26
Deoxycholic acid (DCA)	10.58 ±0.64	12.60 ± 0.58	11.28 ±2.49	10.96 ±1.15	14.89 ±0.51	13.50 ± 1.38	13.07 ±1.52
Isodeoxycholic acid (IDCA)	2.71 ±0.12	3.18 ± 0.56	2.57 ±0.16	2.02 ±0.24	2.52 ±0.10	3.15 ± 0.54	2.92 ±0.34
12-Ketolithocholic acid (12KLCA)	4.80 ±0.63	4.37 ± 0.63	6.19 ±1.17	4.33 ±0.41	6.15 ±0.41	7.40 ± 0.91	5.93 ±1.01
7-Ketodeoxycholic acid (7KDCA)	0.20 ±0.20	0.51 ± 0.35	n.d.[b]	n.d.	0.12 ±0.12	0.45 ± 0.45	0.19 ±0.19
12-Ketochenodeoxycholic acid (12KCDCA)	0.54 ±0.54	0.46 ± 0.27	1.01 ±0.60	0.34 ±0.34	0.20 ±0.20	0.40 ± 0.20	1.11 ±0.71
Chenodeoxycholic acid derived bile acids (CDCAs)							
β-Muricholic acid (βMCA)	11.56 ±0.58	11.09 ± 2.32	8.61 ±1.63	6.06 ±0.50	8.77 ±1.12	8.99 ± 2.94	9.23 ±1.74
Lithocholic acid (LCA)	5.77 ±2.63	5.52 ± 0.69	6.96 ±0.59	6.13 ±0.54	5.90 ±0.53	6.66 ± 0.40	5.73 ±0.74
Isolithocholic acid (ILCA)	1.05 ±0.06	0.87 ± 0.04	1.42 ±0.15	1.32 ±0.14	0.94 ±0.28	1.26 ± 0.09	1.01 ±0.16
Hyodeoxycholic acid (HDCA))	25.00 ±6.83	34.32 ±10.37	33.90 ±4.89	49.56 ±1.92	41.16 ±2.88	32.36 ±14.53	32.01 ±5.39
Murodeoxycholic acid (MDCA)	2.66 ±0.92	1.50 ± 0.37	1.90 ±0.45	1.90 ±0.22	1.75 ±0.15	1.72 ± 0.51	2.05 ±0.86
α-Muricholic acid (αMCA)	2.36 ±0.11	1.96 ± 0.64	1.49 ±0.16	1.04 ±0.13*	1.13 ±0.11*	1.69 ± 0.50	1.88 ±0.22
ω-Muricholic acid (ωMCA)	25.25 ±6.71	17.38 ± 6.88	16.50 ±4.32	8.16 ±0.44	10.68 ±2.79	19.36 ± 9.46	17.87 ±3.80
6-Ketolithocholic acid (6KLCA)	3.32 ±1.30	1.92 ± 0.97	3.78 ±1.19	2.14 ±0.50	1.97 ±0.53	1.72 ± 1.03	3.32 ±1.73
Others							
3β-Hydroxy 5-cholenic acid	0.38 ±0.05	0.20 ± 0.07	0.50 ±0.06	0.73 ±0.18	0.81 ±0.21	0.73 ± 0.24	1.42 ±0.76
CAs/CDCAs ratio	0.289±0.007	0.343± 0.040	0.342±0.055	0.304±0.033	0.372±0.014	0.391± 0.046	0.349±0.017
Ratio of primary bile acids[c] [%]	12.30 ±0.56	11.95 ± 2.39	9.28 ±1.64	7.24 ±0.39	9.80 ±1.14	11.61 ± 2.79	10.52 ±1.58

Data are expressed as means ± S.E.M. ($n = 5$). *a*) Different significantly from the control group. (*$p < 0.05$, $p < 0.01$ *vs.* Control, 1-way ANOVA followed by Dunnett's multiple comparison test), *b*) n.d., Not detected, *c*) Primary bile acids: Total of CA, CDCA and βMCA.

in those administered quercetin ($p < 0.05$, Table 8). In the urinary flavonoids during the three days, 94.1% and 85.6% were excreted within one day after administration of quercetin or rutin, respectively. In the case of quercetin administration, the recovered flavonoids during the three days were only 0.13% in urine and 0.03% in serum. Similarly, recovered flavonoids during the three days were only 0.89% in urine and 0.13% in serum when rutin was administered.

DISCUSSION

As to the adverse effect of rutin and quercetin, it has been reported that LD_{50} is 160 mg/kg (*p.o.*, mice) for quercetin and 950 mg (i.v., mice, propylene glycol solution), respectively.[27] The toxicological data show that quercetin has mutagenicity[28,29] or no mutagenicity,[30] but that rutin has no mutagenicity.[28] There have been reports that quercetin has carcinogenicity[31,32] or no carcinogenicity.[33] Both flavonoids reported to have anti-carcinogenic properties.[34,35]

The metabolism of quercetin and rutin has been investigated by several researchers. Ueno *et al.*[36] reported that when [4-^{14}C]quercetin was administered orally, about 20% was absorbed from the digestive tract, more than 30% was decomposed to yield $^{14}CO_2$, and around 30% was excreted unchanged in the feces. The absorbed [^{14}C] quercetin was rapidly excreted into the bile and urine within 48 h as glucuronides and sulfate conjugates of quercetin, 3'-*O*-monomethyl [^{14}C] quercetin ([^{14}C] isorhamnetin) and 4'-*O*-monomethyl [^{14}C] quercetin ([^{14}C] tamarixetin).[36] Rutin is hydrolyzed by intestinal glycosidase to yield quercetin, an aglycone of rutin, and absorbed from the intestine.[2] The part of quercetin absorbed in the small intestine is conjugated and further secreted in the intestinal lumen.[37] Absorption of quercetin after its oral administration ranged from 0 to over 50% of the dose in humans.[38] Hollman *et al.*[39] indicated that conjugation of quer-

Table 5. Correlation of Serum TBARS and Flavonoids

	Control	Quercetin 0.01 g/kg	Quercetin 0.05 g/kg	Quercetin 0.1 g/kg	Quercetin 0.2 g/kg	Quercetin 0.5 g/kg	Quercetin 1.0 g/kg	Dose-response Significance (r^2)
Serum TBARS[b]	[generated MDA nmol/ml]							
	4.474±0.148	4.742±0.254	5.328±0.179[*a]	4.375±0.358	2.701±0.184***	2.020±0.109***	2.214±0.205***	p <0.001 (0.4619)
Serum free flavonoids [nmol/ml]								
Quercetin	n.d. [c]	n.d.	n.d.	0.419±0.171	0.407±0.166	0.903±0.052	1.110±0.093	p <0.001 (0.5963)
Isorhamnetin	n.d.	0.166±0.055	0.119±0.073	0.594±0.157	0.527±0.135	1.062±0.055	1.326±0.077	p <0.001 (0.6681)
Correlation of serum free flavonoids and TBARS								
Serum free quercetin *vs*. TBARS			p <0.001 (r^2 0.4951)					
Serum free isorhamnetin *vs*. TBARS			p <0.001 (r^2 0.5318)					

	Control	Rutin 0.01 g/kg	Rutin 0.05 g/kg	Rutin 0.1 g/kg	Rutin 0.2 g/kg	Rutin 0.5 g/kg	Rutin 1.0 g/kg	Dose-response Significance (r^2)
Serum TBARS[b]	[generated MDA nmol/ml]							
	3.749±0.255	3.082±0.083*	2.974±0.107**	2.753±0.181***	1.834±0.144***	1.535±0.099***	1.680±0.110***	p <0.001 (0.7774)
Serum free flavonoids [nmol/ml]								
Quercetin	n.d.[c]	n.d.	n.d.	n.d.	0.208±0.093	0.830±0.164	10.23 ±3.35	p <0.01 (0.1978)
Isorhamnetin	n.d.	n.d.	n.d.	n.d.	n.d.	0.407±0.176	3.225±0.905	p <0.01 (0.2255)
Correlation of serum free flavonoids and TBARS								
Serum free quercetin *vs*. TBARS			p <0.05 (r^2 0.1185)					
Serum free isorhamnetin *vs*. TBARS			p <0.05 (r^2 0.1382)					

Data are expressed as means S.E.M. ($n = 5$). *a*) Different significantly from the control group. (* p <0.05 *vs*. Control, ** p <0.01, *** p <0.001 *vs*. Control, 1-way ANOVA followed by Dunnett's multiple comparison test). *b*) TBARS: thiobarbituric acid reactive compounds, indicated as malondialdehyde generated. *c*) n.d., not detected (less than 0.05 nmol/ml).

cetin with glucose enhanced absorption from the small intestine and theorized that repeated consumption of quercetin-containing foods would cause the quercetin accumulation in blood because of its long half-life of elimination.

Dietary quercetin is recovered in rat plasma as conjugated derivatives of isorhamnetin and quercetin.[40] Most of the plasma metabolites of quercetin are glucurono-sulfo conjugates of isorhamnetin and a minor part are glucuronides of quercetin and its methoxylated forms,[41] although tamarixetin appeared only in the early stage of administration of quercetin and rutin.[25] These metabolites also exhibit antioxidant properties.[41] Contrarily, tamarixetin is recovered from the bile and urine of rats fed quercetin.[40]

Although the dose of 1.0 g/kg is near to the reported LD_{50},[27] quercetin and rutin revealed no toxicological symptoms at doses as high as 1.0 g/kg by chronic oral administration for 22 d. There were no effects on body weight gain, relative weight of the liver, food intake or dry weight of feces (Table 1). There also seemed no remarkable influence on serum and hepatic lipid concentrations (Table 2) or fecal steroid excretion (Tables 3 and 4). On the contrary, serum TBARS decreased in a dose-dependent manner from administration of quercetin and rutin (Table 5), which concurs with previous reports.[25,40,41] The decrease of serum TBARS was significantly correlated with the increase of serum free flavonoids ($p < 0.001$ by quercetin administration and $p < 0.05$ by rutin administration, Table 5).

There have been no reports on the metabolism of quercetin or rutin administered in non-physiological high doses. To evaluate the relationship between lipid metabolism and the serum antioxidant properties of these substances, their metabolism at high doses was investigated. First, 1.0 g/kg of quercetin or rutin was orally administered to rats each day for 10 d and the serum flavonoids were determined (Experiment 3). Next, 1.0 g/kg of quercetin or rutin was administered to rats in a single dose and the flavonoid concentrations excreted in urine for 3 d and in serum at day 3 after administration were determined (Experiment 4).

Although no free quercetin or isorhamnetin in plasma has been reported to date, relatively high levels were detected in the serum of rats administered 1.0 g/kg of rutin or quercetin (Tables 5 and 6). With repeated administrations (Experiment 3), serum concentrations of total and free flavonoids and percentages of total quercetin and aglycone were higher in rutin-administered rats than in quercetin-administered rats ($p < 0.05$–0.001, Table 6). In the single administration (Experiment 4), the administered quercetin and rutin remained in the blood as agly-

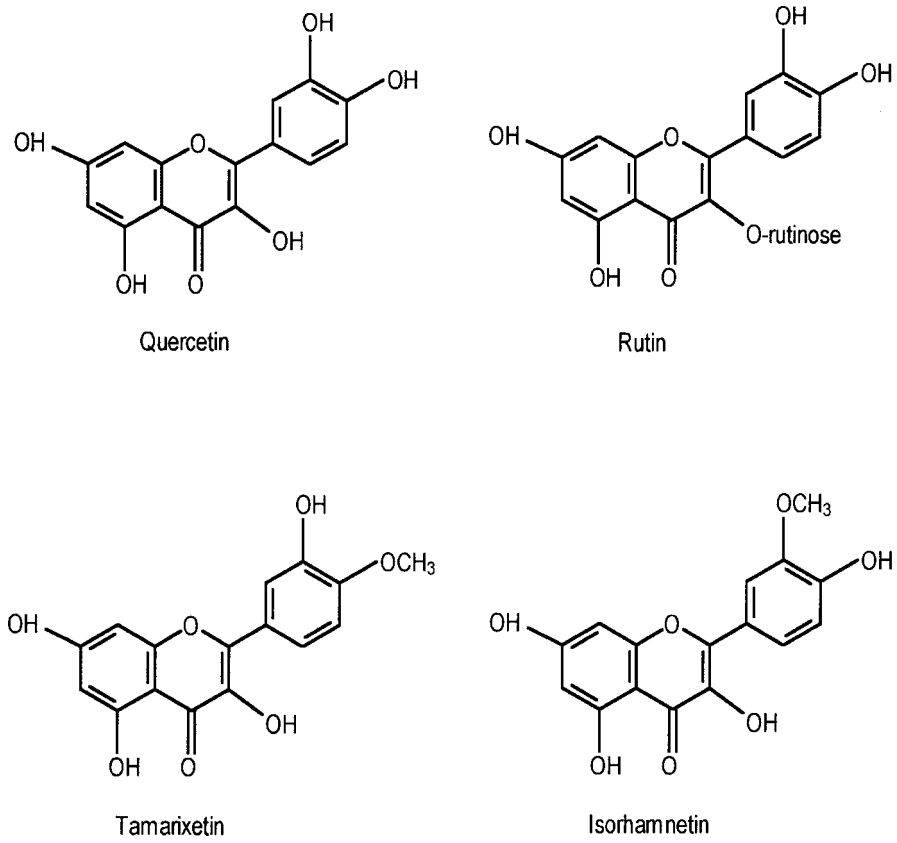

Fig. 1. Chemical Structures of Quercetin, Rutin and Their Metabolites

Table 6. Concentration and Composition of Serum Flavonoids (Repeated Administration, Day 10)

	Quercetin 1.0 g/kg	Rutin 1.0 g/kg	Significance[a)]
Total flavonoids [nmol/ml]			
Quercetin	9.73±0.87	34.32±5.42	$p < 0.05$
Isorhamnetin	31.57±2.76	69.67±2.12	$p < 0.001$
Total	41.30±3.22	103.99±6.71	$p < 0.001$
Free flavonoids [nmol/ml]			
Quercetin	0.56±0.14	6.37±1.37	$p < 0.05$
Isorhamnetin	1.20±0.16	4.38±0.38	$p < 0.001$
Total	1.76±0.27	10.75±1.71	$p < 0.01$
Composition of flavonoids [%]			
Total quercetin	23.70±1.89	32.36±2.90	$p < 0.05$
Total isorhamnetin	76.30±1.89	67.64±2.90	$p < 0.05$
Aglycone[b)]	4.25±0.56	10.16±1.09	$p < 0.01$

Data are expressed as means S.E.M. ($n = 5$). *a*) Significance was compared between quercetin-fed group and rutin-fed group. Different significantly from quercetin-fed group. (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ *vs.* Control, by Aspin-Welch *t* test). *b*) Sum of the percentage of free quercetin and isorhamnetin.

cone or the conjugates of quercetin and isorhamnetin even three days after oral administration (Tables 7 and 8), which suggests the accumulation of flavonoids in the blood as Hollman *et al.*[39] speculated. Large variations (S.E.M.) in the amount and composition of urinary flavonoids (Table 7) suggest individual variation in the metabolism of quercetin or rutin in rats as in humans.[38] The molecular weight of rutin (610.53) is more than twice that of quercetin (302.24), an aglycone of rutin, *i.e.,* the molar

Table 7. Concentration and Composition of Serum and Urinary Flavonoids (Single Dose, Days 0–3)

	Quercetin 1.0 g/kg	Rutin 1.0 g/kg	Significance[a)]
Serum flavonoids (day 3)			
Total flavonoids [nmol/ml]			
Quercetin	3.04± 0.36	7.58± 1.44	$p < 0.05$
Isorhamnetin	8.77± 1.11	20.30± 4.02	N.S.[b)]
Total	11.81± 1.40	27.88± 5.41	N.S.
Free flavonoids [nmol/ml]			
Quercetin	Not detected[c)]	0.45± 0.23	N.S.
Isorhamnetin	0.35± 0.10	0.99± 0.09	$p < 0.01$
Total	0.35± 0.10	1.44± 0.28	$p < 0.05$
Composition of serum flavonoids [%]			
Total quercetin	25.97± 1.40	27.42± 1.21	N.S.
Total isorhamnetin	74.02± 1.40	72.58± 1.21	N.S.
Aglycone	3.61± 1.64	6.03± 1.42	N.S.
Urinary flavonoids (days 0–3)			
Total flavonoids excreted [nmol/3 days]			
Quercetin	308.3 ±172.1	1012.7 ± 587.6	N.S.
Isorhamnetin	189.1 ±106.5	666.5 ± 349.7	N.S.
Tamarixetin	231.9 ±110.6	714.3 ± 264.6	N.S.
Total	729.4 ±388.9	2393.5 ±1188.7	N.S.
Free flavonoids excreted [nmol/3 days]			
Quercetin	91.4 ± 35.2	571.4 ± 426.8	N.S.
Isorhamnetin	68.8 ± 33.3	385.2 ± 270.9	N.S.
Tamarixetin	82.3 ± 29.8	426.6 ± 201.5	N.S.
Total	242.5 ± 96.3	1383.1 ± 892.4	N.S.
Composition of urinary flavonoids [%]			
Total quercetin	40.84± 6.63	37.46± 3.00	N.S.
Total isorhamnetin	24.85± 1.41	27.29± 3.31	N.S.
Total tamarixetin	34.30± 6.33	35.25± 5.52	N.S.
Aglycone[d)]	36.71± 9.65	58.12± 11.47	N.S.

Data are expressed as means S.E.M. ($n = 5$). *a*) Different significantly from quercetin-fed group. ($*p < 0.05$, $**p < 0.01$, $***p < 0.001$ *vs*. Control, by Aspin-Welch *t* test). *b*) Not significant ($p > 0.05$ by Aspin-Welch *t* test). *c*) Not detected (less than 0.1 nmol/ml). *d*) Sum of the percentage of free quercetin, isorhamnetin and tamarixetin.

amount of administered quercetin is nearly twice that of rutin. It is suggested that the metabolism of both flavonoids might be somewhat different in rats administered large oral doses.

The amount of recovered flavonoids after the administration of quercetin and rutin in a single dose was only 0.13% and 0.89% in the urine for 3 d, and 0.03% and 0.13% in the serum on day 3, respectively (Table 9). In this study we did not determine fecal flavonoids because it was difficult to isolate flavonoids from the interactive substances. Most of the administered quercetin and rutin might be excreted into the feces with or without degradation in the intestine, degraded to other compounds such as phenols[38)] in the liver and excreted into the urine or decomposed to yield CO_2[36)] and exhaled.

Thus, some part of the orally administered quercetin or rutin was absorbed from the intestine, metabolized and appeared in serum as conjugates or free isorhamnetin and quercetin. These serum flavonoids might reveal the antioxidant properties as suggested by the dose-dependent decrease in serum TBARS (Table 5). On the contrary, no remarkable changes were observed in the concentrations of serum and hepatic lipids (Table 2) or in fecal steroid excretions (Tables 3 and 4) in normal rats administered various doses of quercetin or rutin. From these results, it was considered that the antioxidant properties of orally administered quercetin and rutin might not influence the serum and hepatic lipid levels or fecal steroid excretion in rats fed a normal diet.

Table 8. Percentage of Urinary Excretion and Serum Distribution of Flavonoids (Single Dose, Days 0–3)

	Quercetin 1.0 g/kg	Rutin 1.0 g/kg	Significance[a)]
Percentage of urinary excretion [%] (molar ratio)			
Day 0–1			
Quercetin	0.0524±0.0333	0.3261±0.1777	N.S.[b)]
Isorhamnetin	0.0336±0.0201	0.2092±0.1016	N.S.
Tamarixetin	0.0408±0.0212	0.2320±0.0774	N.S.
Total	0.1269±0.0745	0.7637±0.3515	N.S.
Day 1–2			
Quercetin	0.0064±0.0016	0.0456±0.0345	N.S.
Isorhamnetin	0.0028±0.0009	0.0336±0.0240	N.S.
Tamarixetin	0.0036±0.0009	0.0307±0.0194	N.S.
Total	0.0128±0.0030	0.1106±0.0776	N.S.
Day 2–3			
Quercetin	0.0005±0.0002	0.0044±0.0020	N.S.
Isorhamnetin	0.0000±0.0000	0.0061±0.0044	N.S.
Tamarixetin	0.0000±0.0000	0.0043±0.0026	N.S.
Total	0.0006±0.0002	0.0148±0.0089	N.S.
Days 0–3			
Quercetin	0.0580±0.0339	0.3761±0.2134	N.S.
Isorhamnetin	0.0355±0.0209	0.2490±0.1281	N.S.
Tamarixetin	0.0436±0.0219	0.2670±0.0968	N.S.
Total	0.1349±0.0769	0.8921±0.4329	N.S.
Percentage of distribution in blood[c)] [%] (molar ratio)			
Quercetin	0.0072±0.0008	0.0361±0.0069	$p < 0.05$
Isorhamnetin	0.0207±0.0026	0.0965±0.0189	$p < 0.05$
Total	0.0279±0.0033	0.1326±0.0572	$p < 0.05$

Data are expressed as means S.E.M. ($n = 5$). *a*) Different significantly from quercetin-fed group by Aspin-Welch *t* test. *b*) Not significant ($p > 0.05$ by Aspin-Welch *t* test). *c*) Percentage of distribution was calculated by assuming that all flavonoids in blood distributed in serum and that blood weight accounted for 7% of total body weight.

In conclusion, 1) no toxicological symptoms were observed in rats administered quercetin or rutin in a dose as high as 1.0 g/kg by gavage for 22 d; 2) oral administrations of these substances showed dose-dependent antioxidant properties but did not remarkably influence serum or hepatic lipid concentrations or fecal steroid excretions in rats fed a normal diet.

The interest in non-nutrients such as polyphenols including flavonoids is increasing today as reflected in the expansion of interest in the health of individuals. It would be useful to elucidate the metabolism and toxicity of these compounds more precisely.

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