Effects of the Oral Administration of Green Tea Polyphenol and Tannic Acid on Serum and Hepatic Lipid Contents and Fecal Steroid Excretion in Rats

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Green tea polyphenol (Polyphenon) or tannic acid was administered orally to rats at a dose of 0.01–1.0 or 0.1–1.0 g/kg for 23 days, and changes both in serum and hepatic lipid concentrations and in fecal steroid excretion were examined. The administration of 0.2–1.0 g/kg of Polyphenon caused a significant decrease in levels of serum HDL-cholesterol, whereas tannic acid had no significant effect on serum lipid concentrations. The hepatic triglyceride concentration was significantly higher than controls in rats given more than 0.5 g/kg of Polyphenon, whereas both hepatic triglyceride and phospholipid concentrations were significantly higher after tannic acid administration. Serum thiobarbituric acid reactive substances were significantly low in rats given either 1.0 g/kg of Polyphenon or more than 0.1 g/kg of tannic acid. Fecal neutral steroid excretion increased significantly in rats given a dose of 1.0 g/kg of either Polyphenon or tannic acid. The excretion of fecal bile acids increased significantly in rats given 0.2 g/kg of tannic acid, but then tended to decrease at higher doses; however, excretion of fecal bile acids did not change after Polyphenon administration. We found that alterations in the compositions of fecal neutral steroids and bile acids were independent of the tannic acid or Polyphenon dose: the ratio of coprostanol to cholesterol decreased significantly in rats given 0.05–0.2 g/kg of Polyphenon or 0.5 g/kg of tannic acid; and the ratio of cholic-acid-derived bile acids to chenodeoxycholic-acid-derived bile acids decreased significantly after administration of 0.05, 0.2 and 0.5 g/kg of Polyphenon or 0.1 and 0.5 g/kg of tannic acid. Primary bile acid excretion increased significantly only in rats given a dose of 0.1 g/kg of Polyphenon. This is the first report that documents the changes occurring in fecal steroid excretion induced by oral administration of green tea polyphenol or tannic acid.

Key words —— green tea polyphenol, tannic acid, fecal steroid excretion, rats, neutral steroids, bile acid

INTRODUCTION

Polyphenols are secondary metabolites of plants and are widely distributed in plant-derived foods, such as cereals, legumes, nuts, vegetables, fruits, and in beverages such as green or black tea, wine, fruit juice, beer and so on.[1–5] Polyphenols have been considered previously to be non- nutrients, or even to be toxins, as some tannins have toxic effects such as binding to proteins, reducing the absorption of proteins, minerals and some vitamins, suppressing body weight gain, and inducing hepatic necrosis and methohemoglobinemia.[1,2,5–11] However, polyphenols have been recently recognized as functionally active molecules, possessing antioxidant, anticancer, antimutagenic properties, as well as exerting protective effects against cardiovascular and other diseases.[1,4,12–21]

The dietary intake of polyphenols has been difficult to assess because of both the large number of unidentified polyphenols and the different analytical methods employed, which have yielded variable results.[1,22] The essential polyphenols in foods are flavonoids and condensed tannins. Recently, Scalbert and Williamson[23] reported the dietary intake of total polyphenols to be approximately 1 g/day. Although the use of polyphenols in the diet may increase in the future because of their known beneficial properties,[24] their metabolism, their interactions with nutrients, and their safe intake levels remain to be clarified.

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Green tea polyphenol is a typical condensed tannin and is commonly used as a health food in Japan (http://www.health-station.com/jhnfa). Tannic acid is a typical hydrolyzable tannin (a mixture of gallotannin) and is distributed widely in plants.[7,25]

Several reports on the effects of green tea polyphenol on lipid metabolism have been published. In human epidemiological studies, green tea consumption was inversely associated with serum levels of total cholesterol and LDL-cholesterol, but had no significant correlation with levels of HDL-cholesterol or triglycerides in healthy Japanese men.[26] Consumption of green or black tea did not influence plasma lipid levels and LDL oxidation in smokers.[27] In studies of animals fed high-fat or high-cholesterol diets, tea catechins reduced serum cholesterol and/or lipid levels in rats,[18–21,28–30] mice[20] and hamsters,[31] and increased fecal excretion of neutral steroids and bile acids in hamsters.[31] In rats fed a normal diet, a dose of 10 mg/kg of catechin produced the maximum reduction in plasma cholesterol and the maximum increase in excretion of fecal neutral steroids and bile acids.[28] Catechins are considered to lower cholesterol by a mechanism that suppresses cholesterol absorption in the intestine.[18,19,21,29,30,32] In contrast to catechins, there are few reports on the effects of tannic acid on lipid metabolism. Yugarani *et al*.[25,33] reported that tannic acid significantly reduced serum and hepatic lipid concentrations in rats fed high-fat diets; however, these authors administered low doses of tannic acid (less than 100 mg/kg) to humans and to animals fed high-fat diets. To date, there have been no reports of the effects of high doses of green tea polyphenol or tannic acid on the metabolism of lipids or on the composition of fecal steroids, which alters in some hepato-biliary[34] or colorectal diseases.[35]

In this study, we have examined the effects of a range of doses of green tea polyphenol and tannic acid on serum and hepatic lipid contents and on fecal steroid excretion, in particular on the composition of fecal steroids, in rats fed a normal diet.

MATERIALS AND METHODS

Materials —— Standards of neutral steroids, bile acids and derivative reagents for GC analysis, as described elsewhere,[36] were purchased from GL Sciences Inc. (Tokyo, Japan), Sigma Chemical Co. (St. Louis, MO, U.S.A.) and Steraloids, Inc. (Wilton, NH, USA). (+)-Catechin (CT), (–)-catechin gallate (CG), (–)-gallocatechin (GC), (–)-gallocatechin gallate (GCG), (–) epicatechin (EC), (–)-epicatechin gallate (ECG), (–)-epigallocatechin (EGC) and (–) epigallocatechin gallate (EGCG) were from Extrasynthèse (Geney, France). Gallic acid (GA), pyrogallol (PG), ellagic acid (EA) and flavone were purchased from Wako Pure Chemical Industries (Osaka, Japan). Ion- exchanged and redistilled water was used throughout the experiments. HPLC grade acetonitrile, ethyl acetate and methanol were used for analyses. Other reagents were analytical grade.

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 lipids were from Wako Pure Chemical Industries. The Sep-pak^{R} C_{18} plus cartridge column was from Waters Corporation (Milford, MA, U.S.A.). Fused silica capillary columns DB-210 (0.25 mm i.d. × 30 m, film thickness 0.25 mm or 0.5 mm) were obtained from J&W Scientific (Folsom, CA, U.S.A.). The HPLC column STR ODSII (4.6 mm i.d. × 250 mm) was purchased from Shinwa Chemical Industries, Ltd. (Kyoto, Japan).

Polyphenon-100 (Polyphenon, green tea polyphenol, Lot No. 99A26) was purchased from Funakoshi (Tokyo, Japan). The content of catechin in Polyphenon was more than 80% by HPLC (EC 94 mg/g, EGC 134 mg/g, EGCG 539 mg/g, ECG 17 mg/g, GCG 29 mg/g and CG 0 mg/g). Tannic acid was obtained from Wako Pure Chemical Industries. The same lot of Polyphenon or tannic acid was used throughout the study. Polyphenol solutions of Polyphenon or tannic acid for oral administration were freshly prepared before use. For each sample, 0.01–1.0 g was weighed, dissolved in water and adjusted to 10 ml. Each polyphenol solution was administered to rats at a dose of 10 ml/kg body weight.

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

sis. A Hitachi 650-60 fluorophotometer was used for the determination of serum thiobarbituric acid reactive substances (TBARS).

Animal Experiments —— 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 Wistar rats (4 weeks old) were purchased from Clea Japan, Inc. (Tokyo, Japan) and kept in an air-conditioned room (23 ± 1°C, 50–60% humidity) illuminated for 12 hr a day (7:00 to 19:00). Rats had free access to a commercial non-purified diet (F-2, Funahashi Farm, Chiba, Japan) and water throughout the study. No catechins, tannic acid, nor its derivatives was detected in the commercial F-2 diet by HPLC ($n = 5$). Rats weighing 122–145 g were used in the experiments. Each group consisted of 5 rats.

Polyphenon was administered orally at doses of 0.01, 0.05, 0.1, 0.2, 0.5 and 1.0 g/kg, and tannic acid was administered orally at doses of 0.1, 0.2, 0.5 and 1.0 g/kg. Each rat received a daily dose between 11:00 and 13:00 every day for 23 days. Control rats received water at a dose of 10 ml/kg of body weight. Feces were collected on days 18–20 by placing each rat in a metabolic cage. The animals were starved overnight following day 22. On day 23, they were anesthetized with diethylether, and blood was collected by heart puncture. The liver was excised immediately after bleeding. All samples were stored below –20°C before use.

Analytical Methods —— Serum concentrations of total cholesterol and HDL-cholesterol, triglycerides, phospholipids and TBARS were determined with commercial kits. A portion of liver was homogenized with chloroform/methanol solution (2 : 1, v/v) and filtered. Aliquots of the filtrate (lipid extract) were mixed with sodium cholate solution and evaporated to dryness under a nitrogen stream. Total cholesterol, triglycerides and phospholipids in residues were determined with the kits.

Collected feces were dried overnight at 60°C and ground to a powder. Fecal neutral steroids and bile acids were determined by the methods of Grundy *et al*.[37] and Setchell *et al*.[38] with described modifications.[36] Briefly, 5α-cholestane (for neutral steroid determination) and nordeoxycholic acid (NDCA, for bile acid determination) were added as internal standards to a portion of the dried feces powder. Lipids were extracted by sonication and reflux for 1 hr with a chloroform/methanol mixture (2 : 1, v/v) first and then ethanol. The extract was evaporated to dryness for steroid analysis. Neutral steroids were extracted with *n*-hexane after saponification with methanolic potassium hydroxide solution, and determined by GC-FID using 5α-cholestane as an internal standard. The remaining aqueous fraction was neutralized with phosphoric acid after removing the methanol, and applied to a Sep-pak C_{18} plus cartridge column.[39] Bile acids were eluted with methanol. After evaporation to dryness, the residue was treated with choloylglycine hydrolase.[40] The bile acids were extracted with diethylether after acidification with 4 mol/l HCl, derivatized to hexafluoroisopropyl ester-trifluoroacetyl (HFIP-TFA) derivatives[41] and determined GC-FID using NDCA as an internal standard.

GC conditions were as follows: column, DB-210 (film thickness 0.25 μm for neutral steroid analysis, 0.50 μm for bile acid analysis); carrier gas, He 1.5 ml/min; column temperature programs, 60°C (2 min) → 10°C/min → 180°C (0 min) → 5°C/min → 230°C (12 min) for neutral steroids, 60°C (2 min) → 10°C/min → 180°C (0 min) → 5°C/min → 235°C (60 min) for bile acids; injection port temperature, 250°C; detector temperature, 250°C; detector, FID; injection method, splitless; injection volume, 2 μl.

Serum polyphenols were analyzed by the method of Piskula and Terao[42] with some modifications. Serum (0.5 ml) was diluted with 2 ml of 0.1 mol/l acetate buffer (pH 5.0) and 18.86 nmol flavone was added as an internal standard. For determination of total polyphenols, β-glucuronidase (1,000 U) and sulfatase (55 U) were added to the serum preparation and incubated at 37°C for 2 hr. Enzymatic hydrolysis was stopped by addition of 1 ml of 0.5 mol/l HCl and 1 ml of methanol. Polyphenols were extracted twice with 4 ml of ethyl acetate. After evaporation to dryness, the residue was dissolved in 2 ml of methanol for determination by HPLC. Free polyphenols were analyzed without enzymatic hydrolysis.

HPLC conditions were as follows: column, STR ODS II; column oven temperature, 35°C; mobile phases, (A) water/phosphoric acid 1000 : 1 (v/v), (B) water/acetonitrile/phosphoric acid 200 : 800 : 1 (v/v/v); a gradient program, (B) 0% (0 min) → 15% (5 min) → 40% (30 min) → 100% (40–55 min) → 0 (56 min); detector, DAD; monitor wavelengths, 260 nm for EA, EGC, GC and PG, 280 nm for CG, CT, EC, ECG, EGCG, GA and GCG and flavone; injection volume, 10 μl.

Statistical Analysis —— Data are expressed as

means ± S.E.M. Statistical analysis was performed by one-way analysis of variances (ANOVA) followed by Dunnett's multiple comparison test using the 'Statlight' program (Yukms, Tokyo, Japan). Probability values of less than 0.05 were considered to be statistically significant.

RESULTS

Effects on Body Weight Gain, Liver Weight, Food Intakes and Dry Weight of Feces

Table 1 shows body weight gain, relative weight of the liver, food intake, dry weight of feces and volume of urine. In rats administered Polyphenon, body weight gain and relative weight of the liver were significantly lower than in the control group only in rats given a dose of 0.5 g/kg ($p < 0.05$) (Table 1). In contrast, administration of tannic acid suppressed body weight gain significantly compared with the control group ($p < 0.05$), even at a dose of 0.1 g/kg ($p < 0.05$) (Table 1). The relative weight of the liver was significantly lower in rats administered 0.5 g/kg of tannic acid compared with the controls ($p < 0.05$). Neither Polyphenon nor tannic acid significantly affected food intake, dry weight of feces or volume of urine.

Effects on Hepatic and Serum Lipids, Serum TBARS and Serum Polyphenols

The concentrations of serum and hepatic lipids and serum TBARS of rats starved overnight (day 23) are shown in Tables 2 and 3.

As shown in Table 2, rats given 0.2–1.0 g/kg of Polyphenon showed a significant decrease in serum HDL-cholesterol compared with control rats ($p < 0.05$). Hepatic triglyceride concentrations were significantly higher in animals administered 0.5 and 1.0 g/kg of Polyphenon compared with controls ($p < 0.05$). Serum TBARS was significantly lower than in controls only in rats administered 1.0 g/kg of Polyphenon ($p < 0.001$). No significant changes were detected otherwise. We did not detect catechins in serum at all (data not shown).

As shown in Table 3, significant elevations of hepatic triglyceride and phospholipid concentrations were observed in rats administered tannic acid at 0.1, 0.2 or 1.0 g/kg ($p < 0.05$) and 0.5 g/kg ($p < 0.05$), respectively. Serum TBARS were significantly reduced by tannic acid administration ($p < 0.05$), although no tannic-acid-derived phenolic compounds (EA, GA and PG) were detected in serum. Tannic acid had no effect on the concentrations of serum lipids or on the total hepatic cholesterol concentration.

Table 1. Effects of Polyphenon and Tannic Acid on Body Weight Gain, Liver Weight, Food Intakes, Dry Weight of Feces and Volume of Urine in Rats

Polyphenon (Green tea polyphenol)	Body weight gain [g/22 day]	Liver weight 23 days [g/100 g body weight]	Food Intakes 18–20 days [g/day]	Dry weight of feces 18–20 days [g/day]	Volume of urine 18–20 days [g/day]
Control	138.3 ± 3.5	3.38 ± 0.05	18.8 ± 0.8	2.62 ± 0.15	12.13 ± 1.08
Polyphenon 0.01 g/kg	139.6 ± 3.7	3.23 ± 0.14	17.6 ± 0.7	2.45 ± 0.24	10.15 ± 0.91
Polyphenon 0.05 g/kg	133.8 ± 4.4	3.22 ± 0.08	18.3 ± 0.8	2.55 ± 0.25	8.85 ± 0.31
Polyphenon 0.1 g/kg	142.8 ± 2.2	3.11 ± 0.07	17.3 ± 0.9	2.56 ± 0.22	11.20 ± 2.27
Polyphenon 0.2 g/kg	126.4 ± 4.9	3.12 ± 0.08	17.4 ± 1.2	2.28 ± 0.21	10.00 ± 1.19
Polyphenon 0.5 g/kg	116.8 ± 8.8*,a)	3.04 ± 0.12*	17.4 ± 1.0	2.47 ± 0.26	8.95 ± 0.53
Polyphenon 1.0 g/kg	124.0 ± 6.3	3.30 ± 0.09	17.9 ± 0.6	2.65 ± 0.24	15.85 ± 5.14
Tannic acid	Body weight gain [g/22 day]	Liver weight 23 days [g/100 g body weight]	Food Intakes 18–20 days [g/day]	Dry weight of feces 18–20 days [g/day]	Volume of urine 18–20 days [g/day]
Control	138.3 ± 3.5	3.38 ± 0.05	18.8 ± 0.8	2.62 ± 0.15	12.13 ± 1.08
Tannic acid 0.1 g/kg	118.2 ± 6.0*	3.27 ± 0.09	18.0 ± 0.9	2.62 ± 0.12	11.75 ± 0.49
Tannic acid 0.2 g/kg	119.2 ± 4.2*	3.11 ± 0.09	18.4 ± 1.6	2.70 ± 0.20	13.13 ± 1.87
Tannic acid 0.5 g/kg	110.7 ± 4.4***	2.96 ± 0.11**	16.5 ± 0.4	2.82 ± 0.16	10.70 ± 0.97
Tannic acid 1.0 g/kg	119.6 ± 6.7*	3.13 ± 0.10	19.2 ± 2.3	3.02 ± 0.21	10.80 ± 2.09

Data are presented as means ± S.E.M. ($n = 5$). *a*) Significantly different from the control group in each experiment. (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, by 1-way ANOVA followed by Dunnett's multiple comparison test)

Table 2. Effect of Polyphenon on Serum and Hepatic Lipids in Rats

	Control	Polyphenon [g/kg]					
		0.01	0.05	0.1	0.2	0.5	1.0
Serum lipids [mg/100 ml] and TBARS[a)] [nmol of generated malondialdehyde/ml]							
Total cholesterol	67.29 ± 3.37	65.63 ± 3.78	69.11 ± 3.50	78.55 ± 5.22	69.70 ± 5.96	67.33 ± 5.15	56.42 ± 1.59
HDL-cholesterol	50.94 ± 1.97	44.58 ± 2.18	45.87 ± 1.53	49.14 ± 3.00	41.99 ± 0.92*[,b)]	40.15 ± 2.65*	42.48 ± 2.44*
Triglycerides	56.54 ± 4.97	47.54 ± 0.51	46.91 ± 5.42	42.96 ± 5.14	53.71 ± 6.36	60.88 ± 7.45	60.15 ± 6.89
Phospholipids	106.73 ± 3.21	94.14 ± 6.58	89.04 ± 2.51	102.23 ± 2.68	101.82 ± 3.45	101.50 ± 4.51	103.35 ± 4.20
TBARS	2.289 ± 0.070	2.208 ± 0.087	2.140 ± 0.090	2.224 ± 0.103	2.067 ± 0.085	2.130 ± 0.093	1.548 ± 0.022***
Hepatic lipids [mg/g fresh weight]							
Total cholesterol	1.992 ± 0.047	1.901 ± 0.064	2.131 ± 0.136	2.291 ± 0.156	2.030 ± 0.088	2.180 ± 0.136	2.337 ± 0.163
Triglycerides	9.95 ± 0.67	9.40 ± 0.78	11.92 ± 2.11	10.05 ± 1.45	13.53 ± 1.00	14.38 ± 0.95*	14.62 ± 0.65*
Phospholipids	17.00 ± 1.17	15.09 ± 0.54	18.87 ± 1.99	16.11 ± 1.99	21.70 ± 1.62	21.60 ± 1.41	21.89 ± 1.23

Data are expressed as means ± S.E.M. ($n = 5$). *a*) TBARS, thiobarbituric acid reactive substances. *b*) Significantly different from the control group. (*$p < 0.05$, ***$p < 0.001$, by Dunnett's multiple comparison test)

Table 3. Effect of Tannic Acid on Serum and Hepatic Lipids in Rats

	Control	Tannic acid [g/kg]			
		0.1	0.2	0.5	1.0
Serum lipids [mg/100 ml] and TBARS[a)] [nmol of generated malondialdehyde/mL]					
Total cholesterol	67.29 ± 3.37	63.65 ± 4.95	60.43 ± 1.87	66.00 ± 4.64	57.89 ± 2.87
HDL-cholesterol	50.94 ± 1.97	51.71 ± 2.74	47.82 ± 1.48	44.24 ± 2.64	45.19 ± 2.08
Triglycerides	56.54 ± 4.97	63.38 ± 2.78	46.68 ± 4.45	41.31 ± 4.03	49.90 ± 5.71
Phospholipids	106.73 ± 3.21	117.93 ± 5.00	107.90 ± 0.81	97.28 ± 5.69	109.00 ± 3.43
TBARS	2.289 ± 0.070	1.994 ± 0.084*[,b)]	1.710 ± 0.086***	1.822 ± 0.072***	1.366 ± 0.057***
Hepatic lipids [mg/g fresh weight]					
Total cholesterol	1.992 ± 0.047	2.039 ± 0.103	2.193 ± 0.108	1.583 ± 0.228	2.191 ± 0.084
Triglycerides	9.95 ± 0.67	13.34 ± 0.94*	13.70 ± 0.29**	9.81 ± 0.94	14.62 ± 0.65***
Phospholipids	17.00 ± 1.17	19.19 ± 0.71	20.13 ± 1.00	22.65 ± 2.51*	21.89 ± 1.23

Data are expressed as means ± S.E.M. ($n = 5$). *a*) TBARS, thiobarbituric acid reactive substances. *b*) Significantly different from the control group. (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, by Dunnett's multiple comparison test)

Effects on Fecal Steroid Excretion

The fecal steroid excretion of the rats at days 18–20 is summarized in Tables 4 and 5.

As shown in Table 4, there was no significant increase in fecal neutral steroid and bile acid outputs, but the levels of fecal neutral steroids of rats given 1.0 g/kg of Polyphenon were higher than those of the control group ($p < 0.001$). In rats administered 0.05, 0.1 or 0.2 g/kg of Polyphenon, the cholesterol/coprostanol ratio (Cp/Ch ratio) decreased significantly compared with the control group ($p < 0.01$), reflecting an increase in cholesterol excretion and a decrease in coprostanol excretion ($p < 0.01$). In rats administered 1.0 g/kg of Polyphenon, coprostanone excretion increased significantly ($p < 0.05$), but there was no significant change in the Cp/Ch ratio compared with the controls. Compared with controls, 0.05, 0.2 or 0.5 g/kg doses of Polyphenon significantly reduced the cholic-acid-derived bile acids/chenodeoxycholic acid- derived bile acids ratio (CA/CDCA ratio) ($p < 0.01$), reflecting a significant decrease in isodeoxycholic acid (IDCA) and 12-keto lithocholic acid (12KLCA) ($p < 0.05$). A significant increase in 12-keto chenodeoxycholic acid (12KCDCA) was detected at a dose of 1.0 g/kg ($p < 0.05$), although compared with controls the total keto bile acids did not change. A significant increase in primary bile acids caused by β-muricholic acid (βMCA) was observed only in rats given 0.1 g/kg of Polyphenon ($p < 0.001$). Keto bile acids decreased significantly only in rats administered 0.5 g/kg of Polyphenon ($p < 0.05$). There were no other significant changes following Polyphenon administration.

As shown in Table 5, the fecal excretion of neutral steroids increased significantly over control val-

Table 4. Effect of Polyphenon on Fecal Steroid Excretion in Rats

	Control	Polyphenon [g/kg]					
		0.01	0.05	0.1	0.2	0.5	1.0
Fecal excretion of neutral steroids [μmol/day]							
	18.15 ± 0.89	17.27 ± 1.30	19.54 ± 1.56	18.80 ± 1.63	17.35 ± 1.61	20.77 ± 2.30	27.03 ± 2.26***
Composition of fecal neutral steroids [%]							
Cholesterol (Ch)	35.20 ± 1.38	37.85 ± 2.29	49.93 ± 3.25**f)	51.31 ± 6.77**	72.91 ± 2.04***	40.47 ± 2.57	33.83 ± 0.57
Coprostanol (Cp)	58.96 ± 1.51	56.94 ± 2.23	45.15 ± 3.27**	43.45 ± 6.74**	21.61 ± 2.05***	54.70 ± 2.48	57.55 ± 1.69
Coprostanone	6.12 ± 0.61	5.21 ± 0.08	4.93 ± 0.12	5.24 ± 0.25	5.48 ± 0.05	4.83 ± 0.10	8.61 ± 1.26*
Cp/Ch ratio	1.718 ± 0.125	1.543 ± 0.159	0.934 ± 0.117**	0.970 ± 0.234**	0.300 ± 0.035***	1.386 ± 0.138	1.706 ± 0.076
Fecal excretion of bile acids [μmol/day]							
	15.54 ± 0.59	15.41 ± 1.79	15.59 ± 1.22	16.72 ± 1.75	15.03 ± 0.92	14.50 ± 1.40	16.23 ± 1.60
Composition of fecal bile acids [%]							
Cholic acid derived bile acids							
CA$^{a)}$	0.67 ± 0.49	0.40 ± 0.14	0.14 ± 0.04	0.28 ± 0.11	0.12 ± 0.03	0.25 ± 0.06	0.23 ± 0.07
DCA	12.78 ± 1.30	14.45 ± 1.70	9.80 ± 0.44	15.14 ± 1.34	9.36 ± 1.39	12.08 ± 1.04	8.41 ± 0.57
IDCA	3.44 ± 0.35	3.01 ± 0.52	1.97 ± 0.17*	2.48 ± 0.27	1.69 ± 0.19**	2.15 ± 0.27*	3.23 ± 0.39
12KLCA	9.38 ± 0.59	8.07 ± 0.81	6.39 ± 0.54*	8.32 ± 0.87	4.92 ± 0.66***	4.61 ± 0.60***	8.38 ± 0.93
12KCDCA	2.92 ± 0.47	2.34 ± 0.37	2.98 ± 0.29	1.49 ± 0.20	2.77 ± 0.38	1.73 ± 0.57	4.93 ± 0.69*
Chenodeoxycholic acid derived bile acids							
βMCA	5.63 ± 1.35	7.10 ± 0.96	7.98 ± 1.24	15.07 ± 2.25***	4.80 ± 0.35	7.26 ± 1.21	2.44 ± 0.52
LCA	7.81 ± 0.80	8.07 ± 1.21	9.12 ± 0.78	7.47 ± 0.85	6.67 ± 0.85	8.43 ± 0.74	8.01 ± 0.38
ILCA	1.73 ± 0.22	1.71 ± 0.10	1.69 ± 0.19	1.16 ± 0.30	1.43 ± 0.14	1.81 ± 0.10	2.17 ± 0.13
HDCA	26.04 ± 3.40	30.39 ± 4.06	30.10 ± 4.03	14.95 ± 3.55	43.94 ± 1.74*	32.69 ± 6.39	31.83 ± 3.18
MDCA	3.09 ± 0.33	2.99 ± 0.34	3.05 ± 0.30	2.91 ± 0.46**	3.81 ± 0.71	4.72 ± 0.77*	2.16 ± 0.21
αMCA	2.05 ± 0.38	1.85 ± 0.30	2.25 ± 0.26	2.81 ± 0.71	1.68 ± 0.35	2.50 ± 0.54	2.53 ± 0.20
ωMCA	16.42 ± 3.56	11.48 ± 3.61	15.48 ± 1.65	20.10 ± 3.09	7.08 ± 1.34	15.73 ± 3.15	13.94 ± 4.18
6KLCA	8.52 ± 1.53	8.55 ± 1.61	8.77 ± 0.57	4.16 ± 0.62	10.17 ± 1.43	5.92 ± 1.38	12.66 ± 1.33
Other bile acids							
5-cholenic 3β-ol	ND$^{e)}$	0.04 ± 0.02	0.02 ± 0.01	0.03 ± 0.02	ND	0.09 ± 0.03	ND
CA/CDCA ratio$^{b)}$	0.415 ± 0.032	0.396 ± 0.026	0.271 ± 0.011**	0.385 ± 0.022	0.234 ± 0.021***	0.267 ± 0.031**	0.338 ± 0.025
Primary bile acids$^{c)}$ [%]	6.30 ± 1.76	7.02 ± 0.79	8.12 ± 1.22	15.37 ± 2.19***	4.91 ± 0.34	7.52 ± 1.21	2.67 ± 0.48
Keto bile acids$^{d)}$ [%]	20.82 ± 1.87	18.97 ± 2.41	18.14 ± 1.25	13.97 ± 1.27	17.85 ± 2.34	12.27 ± 2.42*	25.96 ± 1.89

Data are expressed as means ± S.E.M. ($n = 5$). a) Abbreviations are: CA, cholic acid; DCA, deoxycholic acid; IDCA. Isodeoxycholic acid; 12KLCA, 12-keto deoxycholic acid; 12KCDCA, 12-keto chenodeoxycholic acid; βMCA, β-muricholic acid; LCA, lithocholic acid, ILCA, isolithocholic acid; HDCA, hyodeoxycholic acid; MDCA, murodeoxycholic acid; αMCA, α-muricholic acid; ωMCA, ω-muricholic acid; 6KLCA, 6-keto lithocholic acid; 5-cholenic 3β-ol, 3β-hydroxy 5-cholenic acid. b) CA/CDCA ratio: The ratio of fecal cholic acid-derived bile acids/chenodeoxycholic acid-derived bile acids. c) Primary bile acids: Total of CA and βMCA. d) Keto bile acids: Total of 12KLCA, 12KCDCA and 6KLCA. e) ND: Not detected. f) Significantly different from the control group. (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, by 1-way-ANOVA followed by Dunnett's multiple comparison test)

ues in rats given 1.0 g/kg of tannic acid ($p < 0.01$). At doses of less than 0.5 g/kg, tannic acid administration markedly affected the composition of fecal neutral steroids. The percentage of cholesterol increased and that of coprostanol decreased significantly in rats given 0.5 or 1.0 g/kg of tannic acid ($p < 0.01$). The Cp/Ch ratio also decreased significantly in rats given 0.5 g/kg of tannic acid ($p < 0.01$), although no significant changes were detected at a dose of 1.0 g/kg of tannic acid. By contrast, fecal bile acid excretion increased significantly over control values in rats administered 0.2 g/kg of tannic acid ($p < 0.05$). Changes in bile acid composition were observed in rats administered 0.1, 0.5 or 1.0 g/kg of tannic acid. The CA/CDCA ratio was lower significantly in rats administered 0.1 or 0.5 g/kg of tannic acid ($p < 0.01$), reflecting significant decreases in IDCA and 12KLCA. Rats given 1.0 g/kg of tannic acid showed a significant decrease in IDCA and a significant increase in lithocholic acid (LCA) ($p < 0.05$), but their CA/CDCA ratio did not change significantly (Table 5). The percentage of total keto bile acids decreased significantly in rats given 0.5 g/kg of tannic acid; however, there were no significant changes in the percentage of primary bile acids at all (Table 5).

DISCUSSION

Studies on the metabolism of catechins[42–44] and tannic acid[45–48] have been reported by several researchers. Suganuma *et al.*[44] found that in mice [3H](–)-EGCG was absorbed easily from the digestive tract and distributed widely into various organs, and then excreted in the urine (6.4–6.6%) or feces (37.7–33.1%) within 24 hr. Piskula and Terao[42] have proposed that the formation of the glucuronide of EC occurs at the intestinal mucosa, and that EC enters the blood circulation exclusively in the glucuronized form and is then sulfated on the liver and methylated in the liver and kidney. Following tannic acid administration in animals, GA, 4-O-methyl gallic acid (4OMG) and PG were found to be excreted in urine in chickens[45] and PG was detected in serum and urine of calves.[41] In sheep given tannic acid orally, GA and PG were present in ruminal fluid and serum, while GA, PG and 4OMG were found in urine.[47,48]

We found that feces were colored black in rats

Table 5. Effect of Tannic Acid on Fecal Steroid Excretion in Rats

	Control	Tannic acid [g/kg]			
		0.1	0.2	0.5	1.0
Fecal excretion of neutral steroids [μmol/day]					
	18.15 ± 0.89	18.93 ± 1.04	21.19 ± 3.28	23.29 ± 3.96	27.96 ± 1.69**
Composition of fecal neutral steroids [%]					
Cholesterol (Ch)	35.20 ± 1.38	40.22 ± 4.66	35.41 ± 1.87	64.03 ± 4.32***	55.53 ± 9.92**
Coprostanol (Cp)	58.96 ± 1.51	53.26 ± 4.52	57.50 ± 2.32	31.07 ± 4.37***	37.84 ± 9.98**
Coprostanone	6.12 ± 0.61	6.52 ± 0.39	7.09 ± 0.96	4.89 ± 0.30	6.63 ± 0.30
Cp/Ch ratio	1.718 ± 0.125	1.438 ± 0.254	1.654 ± 0.146	0.515 ± 0.110**	0.974 ± 0.411
Fecal excretion of bile acids [μmol/day]					
	15.54 ± 0.59	18.58 ± 0.89	20.57 ± 2.41*	15.50 ± 1.51	12.52 ± 1.02
Composition of fecal bile acids [%]					
Cholic acid derived bile acids					
CA[a)]	0.67 ± 0.49	0.27 ± 0.21	0.21 ± 0.06	0.29 ± 0.10	0.32 ± 0.03
DCA	12.78 ± 1.31	9.74 ± 0.86	11.58 ± 2.51	11.08 ± 0.60	11.65 ± 1.19
IDCA	3.44 ± 0.36	2.23 ± 0.21*[f)]	2.56 ± 0.27	2.25 ± 0.14*	1.84 ± 0.29**
12KLCA	9.38 ± 0.59	6.49 ± 0.47*	9.72 ± 0.87	5.64 ± 0.87**	7.66 ± 0.87
7KDCA	ND[e)]	0.16 ± 0.16	0.10 ± 0.10	ND	ND
12KCDCA	2.92 ± 0.47	2.79 ± 0.34	2.16 ± 0.61	2.20 ± 0.61	2.54 ± 0.15
Chenodeoxycholic acid derived bile acids					
βMCA	5.63 ± 1.35	5.00 ± 0.42	2.74 ± 0.83	8.46 ± 3.37	3.13 ± 0.44
LCA	7.81 ± 0.80	7.97 ± 0.38	7.20 ± 0.36	8.03 ± 0.96	10.94 ± 0.92*
ILCA	1.73 ± 0.22	1.93 ± 0.16	1.73 ± 0.13	2.48 ± 0.62	2.55 ± 0.29
HDCA	26.04 ± 3.40	39.19 ± 2.91	34.58 ± 6.62	35.15 ± 5.94	30.96 ± 2.28
MDCA	3.09 ± 0.33	2.72 ± 0.18	3.61 ± 0.66	3.40 ± 0.46	3.08 ± 0.46
αMCA	2.05 ± 0.39	2.51 ± 0.27	2.43 ± 0.60	2.70 ± 0.81	3.00 ± 0.29
ωMCA	16.42 ± 3.56	9.65 ± 1.62	13.94 ± 5.15	11.88 ± 4.42	12.56 ± 2.43
6KLCA	8.52 ± 1.53	9.00 ± 1.04	6.82 ± 1.30	6.44 ± 1.29	9.77 ± 0.91
Other bile acids					
5-cholenic 3β-ol	ND	ND	0.05 ± 0.04	ND	ND
CA/CDCA ratio[b)]	0.415 ± 0.032	0.278 ± 0.019**	0.360 ± 0.030	0.274 ± 0.018**	0.319 ± 0.032
Primary bile acids[c)] [%]	6.30 ± 1.75	3.27 ± 0.59	2.95 ± 0.86	8.75 ± 3.37	3.45 ± 0.47
Keto bile acids[d)] [%]	20.82 ± 1.87	18.44 ± 1.12	18.80 ± 2.53	14.27 ± 1.22*	19.97 ± 0.72

Data are expressed as means ± S.E.M. ($n = 5$). *a*) Abbreviations are: 7KDCA, 7-keto deoxycholic acid; and others are the same as described in Table 4. *b*) CA/CDCA ratio: The ratio of fecal cholic acid-derived bile acids/chenodeoxycholic acid-derived bile acids. *c*) Primary bile acids: Total of CA and βMCA. *d*) Keto bile acids: Total of 12KLCA, 7KDCA, 12KCDCA and 6KLCA. *e*) ND: Not detected. *f*) Significantly different from the control group. (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ $vs.$ Control, by 1-way-ANOVA followed by Dunnett's multiple comparison test)

administered 0.5 or 1.0 g/kg of Polyphenon, and in those administered 0.1, 0.2, 0.5 or 1.0 g/kg of tannic acid. Urine was colored yellow or lemon-yellow in rats administered 0.5 or 1.0 g/kg of Polyphenon and in those administered tannic acid at doses 0.2, 0.5 or 1.0 g/kg compared with the rats of other groups. A portion of the administered Polyphenon or tannic acid might be absorbed from the intestine, metabolized, and excreted into urine. Although a portion might also be excreted in the feces without absorption, this remains to be clarified as we did not measure levels of Polyphenon and tannic acid, or their metabolites, in urine and feces. We did not detect any catechins or metabolites of tannic acid in rat serum in this study; these compounds may have been rapidly metabolized or eliminated.

Polyphenon significantly suppressed body weight gain and significantly reduced the relative weight of the liver at a non-physiological dose of 0.5 g/kg, but there was no significant effect at all on food intakes, dry weight of feces or volume of urine (Table 1). High doses (more than 0.5 g/kg) of green tea polyphenol might be toxic to rats, but the mechanism for this remains to be elucidated. Similar to previously reported results,[6)] tannic acid significantly suppressed body weight gain at doses of more than 0.1 g/kg, but it did not have a significant effect at all on food intake, dry weight of feces or volume of

urine (Table 1). The suppressed body weight gain might be caused by the toxicity of tannic acid metabolites, as Zhu *et al*.[48] have reported that methemoglobinemia induced by oral tannic acid administration in sheep was caused by PG. However, we do not know why 0.5 g/kg, and not 1.0 g/kg, of Polyphenon and tannic acid had the most suppressive effect on the growth of rats.

Compared with controls, a significant decrease in levels of serum HDL-cholesterol was observed in rats given 0.2–1.0 g/kg of Polyphenon (Table 2). The hepatic triglyceride concentration was significantly higher in rats administered 0.5 and 1.0 g/kg Polyphenon than in control animals (Table 2). These results confirm that green tea polyphenol affects lipid metabolism in rats, as reported previously.[18,19,21,27–29] The concentration of serum TBARS in rats given 1.0 g/kg of Polyphenon was significantly lower than in the controls, although polyphenol was not detected in serum (Table 2). This suggests that antioxidative substances that were not detectable by HPLC may have been present. Unlike Polyphenon, tannic acid had no significant effects on serum lipid concentrations, but a dose as low as 0.1 g/kg of tannic acid was sufficient to significantly increase hepatic concentrations of triglycerides or phospholipids (Table 3). Tannic acid was more potent than Polyphenon in reducing serum TBARS, which were significantly reduced compared with control levels at doses of more than 0.1 g/kg (Table 3). We did not detect any metabolites of tannic acid, however, so antioxidant substances that reduce serum TBARS might be present in serum but undetectable by HPLC.

Cholesterol is metabolized to bile acids such as cholic acid (CA), chenodeoxycholic acid (CDCA) and murine-specific β-muricholic acid (βMCA) (primary bile acids) in the liver, and these primary bile acids are then conjugated with taurine or glycine and excreted into the duodenum.[49] Some of the conjugated bile acids are deconjugated and further metabolized by 7-α-dehydroxylation or epimerization to secondary bile acids, such as deoxycholic acid (DCA), LCA and murine-specific a- or w-muricholic acid (αMCA, ωMCA), and so on; and some are even oxidized to keto-bile acids by intestinal flora.[49] A high percentage of bile acids are reabsorbed efficiently from the intestine by active or passive transport, and the remains are excreted into feces. Hydrophilic bile acids tend to be absorbed more efficiently than hydrophobic bile acids. The elimination of fecal bile acids is balanced by new biosynthesis from cholesterol.[50] A proportion of cholesterol is also metabolized by intestinal flora to coprostanol, coprostanone, and so on. Alteration in bile acid composition may occur in some hepato-cystic disease such as hepatolithiasis,[34] and Kamano *et al*.[35] have reported that a high DCA concentration and a high DCA/CA ratio in feces may indicate colorectal cancer in humans. Thus, the composition of fecal steroids might be important in evaluating effects on lipid metabolism.

There are no reports on the effect of green tea polyphenol on fecal steroid excretion except those of Valsa *et al*.[28] and Chan *et al*.[31] Ikeda *et al*.[29] reported that the ability of catechins to decrease micellar solubility and increase intestinal absorption of cholesterol in rats followed the order: EGCG > ECG > EGC, EC. Unlike Valsa *et al*.,[28] we found here that fecal steroid excretion did not increase significantly at dose of 0.01 g/kg of Polyphenon (Table 4). As Polyphenon is a mixture of catechins, this discrepancy in results may be caused by differences in the catechin composition of the green tea polyphenols used in the two studies. The changes that we observed in fecal steroid excretion may be attributed to EGCG, the most abundant component of Polyphenon. Fecal levels of neutral steroids increased significantly compared with control rats only in rats administered 1.0 g/kg of Polyphenon (Table 4). In contrast, changes in the compositions of fecal neutral steroids and/or bile acids was dose-independent and rather biphasic. Compared with control animals, the Cp/Ch ratio decreased significantly only in rats administered 0.05, 0.1 or 0.2 g/kg of Polyphenon; the CA/CDCA ratio decreased significantly only in rats administered 0.05, 0.2 or 0.5 g/kg Polyphenon; and the percentage of primary bile acids increased significantly only in those administered 0.2 g/kg of Polyphenon (Table 3). These results suggest that green tea polyphenol dose–dependently affects intestinal flora and alters the metabolism of neutral steroids and bile acids, and show for the first time that green tea polyphenol changes the composition of fecal neutral steroids and bile acids. At present, we do not know whether the increase of fecal primary bile acids in rats given 0.1 g/kg of Polyphenon is related to the anticarcinogenic property[14,15] of green tea polyphenol.

We have also shown for the first time that tannic acid changes the composition of fecal neutral steroids and bile acids in rats. Similar to the administration of Polyphenon, changes in the amounts and compositions of fecal neutral steroids and bile acids

were dependent on the dose of tannic acid. Excretion of neutral steroids and bile acids increased significantly in rats administered tannic acid at 1.0 and 0.2 g/kg, respectively, as compared with controls (Table 5); however, why fecal bile acid excretion tended to increase at dosages below 0.2 g/kg and then decreased at more than 0.2 g/kg is unknown. Chung *et al*.[51] have reported that tannic acid has an inhibitory effect on the growth of intestinal bacteria, and this property may influence the fecal compositions of neutral steroids or bile acids, such as the significant decrease in both the Cp/Ch ratio and the keto bile acid ratio at dose of 0.5 g/kg (Table 5). Unlike Polyphenon, tannic acid did not alter the primary bile acid ratio. Tannic acid and green tea polyphenol might differ in their effects on fecal steroid excretion, even though at high dose (1.0 g/kg) both agents increase fecal neutral steroid excretion. The reason for the dose-independent alteration in fecal steroid excretion remains to be determined.

In conclusion, green tea polyphenol and tannic acid, independent of dose, might affect intestinal flora and alter the excretion and the composition of fecal steroids; however, the mechanisms by which they cause these alterations may be different. Further study is needed to elucidate the interactions of polyphenols with the cholesterol metabolism pathway.

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