Effects of Grape Seed Polyphenols on Serum and Hepatic Lipid Contents and Fecal Steroid Excretion in Normal and Hypercholesterolemic Rats

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The effects of grape seed polyphenol (GSP) administered orally at doses of 0.01–1.0 g/kg per day to normal and hypercholesterolemic rats for 28 and 36 days, respectively, was evaluated by measuring changes both in the concentrations of serum and hepatic lipids and in fecal steroid excretion. Body weight gain decreased dose–dependently both in normal and hypercholesterolemic rats. Relative weight of the liver was significantly lower in normal rats given more than 0.2 g/kg GSP compared with control rats. Compared with control groups, relative weight of the liver was significantly lower in normal rats given more than 0.2 g/kg GSP, and in hypercholesterolemic rats given more than 0.1 g/kg GSP, respectively. In hypercholesterolemic rats, food or calorie intake was significantly lower in rats given more than 0.1 g/kg GSP than in control rats. Dose–dependency was observed in serum concentrations of serum triglycerides and thiobarbituric acid reactive substances and the content of hepatic phospholipids in normal rats, and in serum concentration and hepatic content of triglycerides in hypercholesterolemic rats. A significant increase in fecal excretions of neutral steroids and bile acids was found in normal rats given a dose of 1.0 g/kg GSP. Dose–dependent decrease of 12-keto lithocholic acid and increase of lithocholic acid were also observed. By contrast, there was no significant change in fecal excretions of neutral steroids and bile acids in hypercholesterolemic rats. Thus, GSP seems to affect lipid metabolism in rats by lowering the concentrations of serum and hepatic triglycerides rather than by altering the metabolism of cholesterol.

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INTRODUCTION

Grape seed polyphenol (GSP) is a typical condensed tannin. Its active constituents are the proanthocyanidins, which represent a variety of polymers of flavan-3-ol such as catechin and epicatechin.[1–4] GSP is an antioxidant that is more powerful than catechins in aqueous system *in vitro*.[5–7] GSP has various physiological effects *in vivo*, such as antioxidant effects,[8,9] protection against X-ray and ultraviolet rays,[10,11] chemoprevention,[12,13] anti-cancer or anti- tumor effects,[14–16] and inhibitory effects against atherosclerosis and hypercholesterolemia.[17–20] Recently, GSP has been considered as a potential health-food ingredient because of these beneficial properties.[21] No toxicity of GSP has been reported,[22–24] although GSP interacts with some kind of proteins.[25–27] To date, there have been no systematic reports of the effects of GSP on lipid metabolism such as the composition of fecal steroids, which alters in some hepatobiliary[28] or colorectal[29] diseases.

In this study, we have examined the effects of GSP on serum and hepatic lipid contents and on fecal steroid excretion, in particular on the composition of fecal steroids, in rats fed a normal and hypercholesterolemic diet.

MATERIALS AND METHODS

Materials —— Standards of neutral steroids, bile acids and derivative reagents for gas chromatography (GC) analysis, as described elsewhere,[30] were purchased from GL Sciences Inc. (Tokyo, Japan), Sigma Chemical Co. (St. Louis, MO, U.S.A.) and Steraloids, Inc. (Wilton, NH, U.S.A.). Ion-exchanged and redistilled water was used throughout the experiments. Other reagents and materials for chemical analysis were the same as reported previously.[30]

GSP was purchased from Tokiwa Shokubutsu Kagaku Kenkyusho (Chiba, Japan). Its proanthocyanidin content, measured by the vanillin-HCl method,[31] was more than 99%. The same lot of GSP was used throughout the study. GSP solutions 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 GSP 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 a flame ionization detector (FID), autosampler AOC-17A, and integrator C-R4A, was used to analyze fecal steroids. A Hitachi U-3210 spectrophotometer (Tokyo, Japan) was used for serum and hepatic lipid analysis. A Hitachi 650-60 fluorophotometer was used for the determination of serum thiobarbituric acid reactive substances (TBARS).

Diets —— A commercial non-purified F-2 diet (normal diet) was purchased from Funahashi Farm (Chiba, Japan). Lithogenic (C-CA diet) diet which containing 1.0% cholesterol and 0.5% cholic acid (CA), was obtained Clea Japan, Inc. (Tokyo, Japan). The compositions of these diets are shown in Table 1. No proanthocyanidins were detected in these diets by the vanillin-HCl method[31] ($n = 5$).

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 (four 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 weighing 147–167 g were used in the experiments. Each group consisted of five rats.

In experiment 1, GSP was administered orally at doses of 0.01, 0.05, 0.1, 0.2, 0.5 and 1.0 g/kg. Each rat received a daily dose between 13:00 and 15:00 every day for 28 days. Control rats received water at 10 ml/kg of body weight. Rats had free access to a normal diet and water. Feces were collected on days 18–20 by placing each rat in a metabolic cage. The animals were starved overnight following day 27. On day 28, they were anesthetized with diethylether, and blood was collected by heart puncture.

In experiment 2, GSP was administered orally at doses of 0.1, 0.2, 0.5 and 1.0 g/kg. Each rat received a daily dose between 13:00 and 15:00 every day for 36 days. Control rats received water at a dose of 10 ml/kg of body weight. Rats had free access to the C-CA diet and water. Feces were collected on days 25–27 by placing each rat in a metabolic cage. The animals were starved overnight following day 35. On day 36, they were anesthetized with diethylether, and blood was collected by heart puncture.

Table 1. Composition of the Nornmal Diet and the Lithogenic (C-CA) Diet

Composition [%]	Normal diet (Experiment 1)	Lithogenic (C-CA) diet (Experiment 2)
Moisture	7.67	8.80
Carbohydrates	58.27	49.42
Crude protein	20.22	25.02
Crude lipids	4.94	4.36
Crude fiber	3.66	4.07
Crude minerals	5.24	6.83
Cholesterol	—	1.00
Cholic acid	—	0.50

The liver was excised immediately after bleeding. Serum was obtained by centrifugation at 1500 rpm for 15 min at an ambient temperature. All samples were stored below –20°C before use.

Analytical Methods —— Serum concentrations of total and high density lipoprotein (HDL)-cholesterol), triglycerides, phospholipids and TBARS and contents of total cholesterol, triglycerides and phospholipids in the liver were analyzed as described previously.[32] Collected feces were dried overnight at 60°C and ground to a powder. Fecal neutral steroids and bile acids were determined by GC-FID as described previously.[30,32]

Statistical Analysis —— Data are expressed as means ± standard error of the 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). Dose–response was examined by linear-regression analysis after one-way ANOVA. Probability values of less than 0.05 were considered statistically significant.

RESULTS

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

Table 2 shows the effect of GSP administration on body weight gain, relative weights of the liver, kidney and spleen, food intake and dry weight of feces in rats.

In rats fed a normal diet, body weight gain decreased dose–dependently ($p < 0.005$). Body weight gain and relative weight of the liver were significantly lower in rats given GSP at a dose of more than 0.2 g/kg compared with control rats ($p < 0.05$)

Table 2. Effects of Oral Administration of GSP on Body Weight Gain, Relative Weight of the Visceral Organs, Food Intakes and the Dry Weight of Feces in Rats

Experiment 1	Control	Grape seed polyphenol (GSP)[g/kg]						Dose–response[c]
	(Normal)	0.01	0.05	0.1	0.2	0.5	1.0	(r^2)
Body weight gain [g/27 days]								
	172 ± 5	174 ± 8	159 ± 5	156 ± 3	137 ± 11**[b]	137 ± 4**	136 ± 8**	$p < 0.005$ (0.4709)
Relative weight of visceral organs (final) [% of body weight]								
Liver	3.33 ± 0.13	3.25 ± 0.10	2.95 ± 0.10	3.25 ± 0.12	2.82 ± 0.07**	2.87 ± 0.10*	2.94 ± 0.09*	N.S.[d]
Kidney	0.71 ± 0.03	0.71 ± 0.01	0.67 ± 0.02	0.73 ± 0.02	0.70 ± 0.01	0.71 ± 0.02	0.68 ± 0.02	
Spleen	0.25 ± 0.01	0.26 ± 0.01	0.25 ± 0.00	0.24 ± 0.01	0.23 ± 0.01	0.23 ± 0.01	0.22 ± 0.01*	
Food intake (days 18–20) [g/day]								
	19.3 ± 0.3	19.3 ± 0.8	16.0 ± 1.2	17.6 ± 0.7	16.6 ± 1.2	17.2 ± 0.6	17.2 ± 1.1	
Dry weight of feces (days 18–20) [g/day]								
	2.48 ± 0.18	2.61 ± 0.19	2.47 ± 0.10	2.34 ± 0.11	2.37 ± 0.14	2.50 ± 0.20	3.10 ± 0.35	

Experiment 2	Control	C-CA + GSP [g/kg]				Dose–response
	(C-CA)	0.1	0.2	0.5	1.0	(r^2)
Body weight gain [g/35 days]						
	221 ± 11	174 ± 19	186 ± 15	178 ± 9	152 ± 15**	$p < 0.05$ (0.3931)
Relative liver weight [% of body weight] (day 36, fasted overnight)						
	5.48 ± 0.15	4.70 ± 0.05*	4.89 ± 0.15*	4.79 ± 0.25*	4.65 ± 0.16**	N.S.
Food intake [g/day] (days 0–35)						
	25.9 ± 0.5	23.3 ± 0.5**	23.5 ± 0.5*	22.3 ± 0.6***	21.2 ± 0.7***	N.S.
Calorie intake[a] [kcal/day] (days 0–35)						
	89.8 ± 1.8	80.5 ± 1.8**	81.4 ± 1.7*	77.3 ± 2.0***	73.4 ± 2.3***	N.S.
Calorie intake/body weight gain [kcal/g] (days 0–35)						
	14.2 ± 0.3	15.7 ± 0.4*	15.3 ± 0.3	15.2 ± 0.4	16.9 ± 0.5***	N.S.
Food intake [g/day] (days 25–27, in metabolic cage)						
	21.0 ± 0.5	22.0 ± 0.6	22.0 ± 1.6	21.1 ± 1.6	21.3 ± 1.4	
Dry weight of feces [g/day] (days 25–27, in metabolic cage)						
	5.64 ± 0.43	5.49 ± 0.21	5.33 ± 0.39	5.58 ± 0.41	5.82 ± 0.45	

Data are expressed as means ± S.E.M. ($n = 5$). *a*) Calorie intake was calculated using Atwater's coefficient (4 kcal/g for carbohydrates or proteins, and 9 kcal/g for lipids). *b*) 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). *c*) Dose–response was examined by linear-regression analysis. *d*) N.S., not significant.

(Table 2). Relative weight of the spleen was significantly lower only in rats administered 1.0 g/kg of GSP ($p < 0.05$). No significant differences were detected in the relative weight of the kidney, food intake at 18–20 days and dry weight of feces (Table 2).

In rats fed the C-CA diet, body weight gain decreased dose–dependently ($p < 0.05$). Body weight gain was significantly lower in rats administered 1.0 g/kg of GSP compared with control rats ($p < 0.01$). Relative weight of the liver and food or calorie intake during the experimental period were significantly lower in rats given more than 0.1 g/kg of GSP ($p < 0.05$) (Table 2). The ratio of calorie intake per body weight gain was significantly higher in rats administered 0.1 and 1.0 g/kg GSP than in control rats ($p < 0.05$). No significant differences were detected in food intake at 25–27 days and dry weight of feces (Table 2).

Effects on Hepatic and Serum Lipids and Serum TBARS

The effect of GSP administration on the concentrations of serum and hepatic lipids and serum TBARS of rats starved overnight (day 28 in rats fed a normal diet and day 36 in rats fed the C-CA diet) are shown in Table 3.

In rats fed a normal diet, dose–dependent decreases ($p < 0.05$) were detected in serum concentrations of triglycerides and TBARS, and hepatic phospholipid contents. GSP significantly lowered serum triglycerides in rats given more than 0.05 g/kg GSP as compared with control rats ($p < 0.05$),

Table 3. Effects of Oral Administration of GSP on Serum and Hepatic Lipids in Rats Fed a Normal Diet (Day 28, Food-deprived Overnight) or the C-CA Diet (Day 36, Food-deprived Overnight)

Experiment 1	Control	GSP [g/kg]						Dose–response[c]
	(Normal)	0.01	0.05	0.1	0.2	0.5	1.0	(r^2)
Serum lipids [mg/100 ml] and TBARS[a] [nmol of generated malondialdehyde/ml]								
Serum total cholesterol	52.5 ± 2.7	53.4 ± 3.3	51.5 ± 2.5	54.7 ± 5.5	46.7 ± 2.2	48.6 ± 3.3	52.2 ± 3.6	
Serum HDL-cholesterol	38.5 ± 0.8	37.3 ± 2.4	38.1 ± 1.4	37.7 ± 3.9	32.8 ± 1.8	35.7 ± 2.3	35.3 ± 3.0	
Serum triglycerides	80.9 ± 5.1	74.3 ± 6.3	63.4 ± 5.9*[b]	63.2 ± 3.4*	44.4 ± 2.7***	43.2 ± 4.0***	37.6 ± 3.2***	$p < 0.001$ (0.6928)
Serum phospholipids	87.7 ± 3.6	82.5 ± 4.2	81.9 ± 5.0	85.5 ± 4.9	76.7 ± 3.3	78.0 ± 3.7	86.5 ± 3.2	
Serum TBARS	3.01 ± 0.24	3.06 ± 0.30	2.93 ± 0.10	3.03 ± 0.17	2.42 ± 0.11	2.53 ± 0.11	2.04 ± 0.11**	$p < 0.05$ (0.3282)
Hepatic lipid concentrations [mg/g fresh liver]								
Cholesterol	2.40 ± 0.20	2.37 ± 0.04	2.40 ± 0.26	2.24 ± 0.17	2.57 ± 0.25	2.37 ± 0.19	2.33 ± 0.23	
Triglycerides	11.9 ± 0.9	11.0 ± 0.7	11.5 ± 0.9	8.84 ± 0.67	12.1 ± 0.9	10.9 ± 1.2	11.7 ± 1.7	
Phospholipids	22.1 ± 1.8	23.0 ± 0.8	20.1 ± 1.5	17.2 ± 1.0	20.0 ± 1.8	16.5 ± 1.2	19.0 ± 2.1	
Hepatic lipids [mg/liver]								
Cholesterol	25.1 ± 2.3	24.6 ± 0.3	22.1 ± 2.2	22.7 ± 0.8	19.8 ± 2.1	18.8 ± 1.3	18.9 ± 1.6	
Triglycerides	125 ± 12	115 ± 6	105 ± 6	92.8 ± 12.5	94.3 ± 9.6	95.3 ± 15.2	94.3 ± 11.2	
Phospholipids	220 ± 15	232 ± 9	184 ± 7	185 ± 10	155 ± 18**	131 ± 11***	149 ± 9**	$p < 0.01$ (0.5566)

Experiment 2	Control	C-CA + GSP [g/kg]				Dose–response
	(C-CA)	0.1	0.2	0.5	1.0	(r^2)
Serum lipids [mg/100 ml] and TBARS[a] [nmol of generated malondialdehyde/ml]						
Total cholesterol	229 ± 22	196 ± 29	211 ± 16	176 ± 5	219 ± 27	
HDL-cholesterol	14.8 ± 1.1	15.0 ± 1.1	14.0 ± 0.8	16.7 ± 1.7	15.4 ± 1.1	
Triglycerides	55.8 ± 4.9	46.5 ± 5.5	27.8 ± 4.1***[b]	32.0 ± 4.0**	29.4 ± 4.1**	$p < 0.05$ (0.5064)
Phospholipids	142 ± 10	121 ± 12	124 ± 7	113 ± 8	119 ± 11	
Hepatic lipid concentrations [mg/g fresh weight]						
Cholesterol	72.0 ± 3.8	77.7 ± 3.7	79.5 ± 2.0	68.2 ± 4.2	82.4 ± 4.4	
Triglycerides	60.4 ± 3.1	62.2 ± 2.4	49.4 ± 6.0	51.9 ± 5.4	50.6 ± 2.2	
Phospholipids	30.0 ± 1.8	32.3 ± 2.7	29.8 ± 2.5	28.9 ± 3.1	35.0 ± 0.4	
Hepatic lipids [mg/liver]						
Cholesterol	1408 ± 92	1103 ± 81	1231 ± 100	1037 ± 96*	1093 ± 93	
Triglycerides	1174 ± 48	892 ± 95	773 ± 114**	768 ± 49**	675 ± 61***	$p < 0.005$ (0.5909)
Phospholipids	586 ± 40	482 ± 34	456 ± 40	427 ± 29*	466 ± 37	N.S.[d]

Data are expressed as means S.E.M. ($n = 5$). *a*) TBARS, Thiobarbituric acid reactive substances. *b*) Significantly different from the control group. (**$p < 0.01$, ***$p < 0.001$, *vs.* Control, by Dunnett's multiple comparison test). *c*) Dose–response was analyzed by linear-regression analysis. *d*) N.S., not significant.

although other serum lipids did not change significantly. Serum TBARS was significantly low only in rats given 1.0 g/kg GSP. Hepatic phospholipids content was significantly low in rats given more than 0.2 g/kg GSP ($p < 0.05$). No significant differences were observed in the hepatic concentrations of cholesterol, triglycerides and phosholipids, and in the hepatic contents of cholesterol and triglycerides within each group (Table 3).

In rats fed the C-CA diet, dose–dependent decreases ($p < 0.05$) were detected in serum concentration and hepatic contents of triglycerides. The serum concentration of triglycerides was significantly lower in rats given more than 0.2 g/kg GSP than in control rats ($p < 0.05$), although other serum lipids did not change significantly. The hepatic content of triglycerides was significantly low in rats given more than 0.2 g/kg GSP ($p < 0.05$). Other hepatic lipid factors did not change significantly with GSP administration. The serum triglyceride concentration and hepatic triglyceride content decreased dose–dependently with GSP administration (Table 3).

Table 4. Effects of Oral Administration of GSP on Fecal Excretion of Neutral Steroids and Bile Acids in Rats Fed a Normal Diet (Experiment 1, Day 18–20)

	Control	GSP [g/kg]						Dose–response[e]
	(Normal)	0.01	0.05	0.1	0.2	0.5	1.0	(r^2)
Fecal excretion of neutral steroids [μmol/day]								
	15.1 ± 1.6	15.5 ± 1.1	18.0 ± 0.7	16.8 ± 0.5	18.3 ± 1.1	16.5 ± 0.8	21.6 ± 1.7**	N.S.[f]
Compositions of fecal neutral steroids [%]								
Cholesterol (Ch)	36.8 ± 3.0	38.8 ± 3.0	34.8 ± 1.2	33.3 ± 2.4	39.0 ± 4.7	37.5 ± 2.3	45.3 ± 1.9	
Coprostanol (Cp)	58.2 ± 3.2	56.5 ± 2.9	60.7 ± 1.3	62.1 ± 2.5	56.8 ± 4.4	58.1 ± 2.3	50.3 ± 2.0	
Coprostanone	5.1 ± 0.4	4.7 ± 0.2	4.5 ± 0.4	4.4 ± 0.2	4.2 ± 0.3	4.4 ± 0.2	4.4 ± 0.2	
Cp/Ch	1.64 ± 0.18	1.52 ± 0.21	1.76 ± 0.09	1.94 ± 0.25	1.58 ± 0.26	1.59 ± 0.15	1.13 ± 0.09	
Fecal excretion of bile acids [μmol/day]								
	11.2 ± 1.4	13.1 ± 2.6	10.8 ± 1.2	11.9 ± 1.2	13.6 ± 1.1	13.3 ± 1.7	19.3 ± 3.2*	N.S.
Compositions of fecal bile acids [%]								
Cholic acid derived bile acids (CAs)								
CA[a]	0.4 ± 0.1	0.9 ± 0.2*[d]	0.6 ± 0.1	0.8 ± 0.1	0.5 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	
DCA	13.0 ± 0.7	14.6 ± 0.6	10.6 ± 0.6	9.6 ± 0.6	12.6 ± 1.3	10.3 ± 0.6	13.0 ± 1.7	
IDCA	4.6 ± 1.0	5.2 ± 0.6	3.9 ± 0.3	3.7 ± 0.2	3.5 ± 0.3	2.6 ± 0.3	2.4 ± 0.6*	$p < 0.05$ (0.3275)
12KLCA	10.2 ± 0.7	9.4 ± 1.3	10.9 ± 1.1	7.8 ± 0.9	9.1 ± 0.7	9.3 ± 0.9	10.6 ± 0.8	
7KDCA	0.7 ± 0.7	ND	ND	1.0 ± 1.0	0.1 ± 0.1	0.3 ± 0.3	1.0 ± 0.4	
12KCDCA	1.1 ± 0.8	1.5 ± 0.4	1.6 ± 0.5	3.0 ± 0.3	1.1 ± 0.6	1.2 ± 0.6	1.6 ± 0.5	
Chenodeoxycholic acid derived bile acids (CDCAs)								
βMCA	9.7 ± 1.6	7.0 ± 0.7	9.0 ± 1.4	4.9 ± 0.6	8.2 ± 0.9	7.4 ± 1.2	8.1 ± 1.3	
LCA	3.8 ± 0.2	3.9 ± 0.2	5.0 ± 0.4	5.3 ± 0.3*	4.1 ± 0.3	5.2 ± 0.2	6.3 ± 0.7***	$p < 0.05$ (0.3405)
ILCA	1.1 ± 0.2	1.0 ± 0.1	1.4 ± 0.1	2.1 ± 0.1***	1.0 ± 0.1	1.4 ± 0.2	1.5 ± 0.1	
HDCA	13.2 ± 7.3	35.0 ± 1.5	24.1 ± 7.4	32.8 ± 2.4	24.9 ± 8.0	24.1 ± 8.5	27.2 ± 4.3	
MDCA	1.5 ± 0.4	2.9 ± 0.3	2.1 ± 0.4	3.9 ± 0.7**	2.7 ± 0.5	3.5 ± 0.6*	2.3 ± 0.2	N.S.
αMCA	1.8 ± 0.4	0.9 ± 0.2	1.8 ± 0.4	1.2 ± 0.4	1.2 ± 0.3	1.4 ± 0.1	1.5 ± 0.3	
ωMCA	36.6 ± 7.2	15.1 ± 1.4	26.2 ± 7.1	14.7 ± 3.7	26.8 ± 8.8	27.4 ± 9.8	16.8 ± 4.6	
6KLCA	2.9 ± 1.2	3.7 ± 0.7	3.5 ± 0.8	7.5 ± 1.6	3.7 ± 1.3	4.0 ± 1.6	6.7 ± 1.6	
CA/CDCA ratio[b]	0.440 ± 0.030	0.472 ± 0.056	0.387 ± 0.009	0.365 ± 0.020	0.375 ± 0.037	0.330 ± 0.026	0.409 ± 0.036	
Primary bile acids[c]	10.1 ± 1.5	7.9 ± 0.7	9.5 ± 1.3	5.8 ± 0.7*	8.8 ± 1.0	8.1 ± 1.1	8.8 ± 1.3	

Data are expressed as means SEM ($n = 5$). *a*) Abbreviations are: CA, cholic acid; DCA, deoxycholic acid; IDCA, Isodeoxycholic acid; 12KLCA, 12-keto deoxycholic acid; 7KDCA, 7-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. *b*) Primary bile acids: Total of CA and βMCA. *c*) CA/CDCA ratio: The ratio of fecal cholic acid-derived bile acids/chenodeoxycholic acid-derived bile acids. *d*) 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). *e*) Dose–response was analyzed by linear-regression analysis. *f*) N.S., not significant.

Effects on Fecal Steroid Excretion

The effect of GSP administration on fecal steroid excretion at days 18–20 for rats fed a normal diet and at days 25–27 for those fed the C-CA diet are summarized in Tables 4 and 5.

When rats were fed a normal diet, fecal excretions of neutral steroids (cholesterol and its metabolites) and bile acids increased significantly only in rats administered 1.0 g/kg GSP as compared with control rats ($p < 0.05$, Table 4). The composition of neutral steroids did not change significantly with GSP administration. Dose–dependent decrease in isodeoxycholic acid (IDCA) and increase in lithocholic acid (LCA) ($p < 0.05$) was detected, although no significant change in cholic acid-derived bile acids/chenodeoxycholic acid-derived bile acids (CA/CDCA) ratio was observed with GSP administration (Table 4). Significant increases in the percentage of fecal bile acids as compared with the control group were observed as follows: CA at a dose of 0.01 g/kg GSP, LCA at doses of 0.1 and 1.0 g/kg GSP, isolithocholic acid (ILCA) at a dose of 0.1 g/kg GSP, and murodeoxycholic acid (MDCA) at doses of 0.1 and 0.5 g/kg GSP ($p < 0.05$). The percentages

Table 5. Effects of Oral Administration of GSP on Fecal Excretion of Neutral Steroids and Bile Acids in Rats Fed the C-CA Diet (Experiment 2, days 25–27)

	Control	GSP [g/kg]				Dose–response[f]
	(Normal)	0.1	0.2	0.5	1.0	(r^2)
Fecal excretion of neutral steroids [μmol/day]						
	269 ± 7	301 ± 28	270 ± 20	276 ± 26	275 ± 22	
Composition of fecal neutral steroids [%]						
Cholesterol (Ch)	72.1 ± 5.2	72.2 ± 4.3	71.2 ± 3.3	71.2 ± 2.7	67.9 ± 2.1	
Coprostanol (Cp)	24.1 ± 4.6	24.4 ± 3.9	24.7 ± 2.6	25.2 ± 2.5	27.3 ± 2.0	
Coprostanone	3.8 ± 0.6	3.4 ± 0.4	4.2 ± 0.8	3.6 ± 0.3	4.9 ± 0.1	
Cp/Ch ratio	0.352 ± 0.088	0.357 ± 0.080	0.356 ± 0.050	0.362 ± 0.048	0.407 ± 0.042	
Fecal excretion of bile acids [μmol/day]						
	208 ± 10	231 ± 27	209 ± 15	212 ± 15	207 ± 13	
Composition of fecal bile acids [%]						
Cholic acid derived bile acids						
CA[a]	14.0 ± 1.5	13.1 ± 1.6	11.7 ± 2.7	9.2 ± 0.9	9.0 ± 0.9	
DCA	24.4 ± 3.0	26.1 ± 2.5	27.1 ± 2.3	28.9 ± 1.5	31.2 ± 1.4	
IDCA	3.8 ± 0.9	3.7 ± 1.0	4.5 ± 1.1	3.2 ± 0.5	4.1 ± 0.4	
12KLCA	8.4 ± 0.5	8.8 ± 1.1	7.4 ± 0.2	12.4 ± 0.8**[e]	12.5 ± 0.7**	N.S.[g]
7KLCA	1.3 ± 0.2	1.8 ± 0.2	1.1 ± 0.3	1.4 ± 0.2	1.6 ± 0.2	
7KDCA	5.3 ± 1.2	5.5 ± 0.9	5.8 ± 0.8	3.8 ± 0.7	3.5 ± 0.2	
12KCDCA	1.7 ± 0.7	1.9 ± 0.8	1.5 ± 0.5	0.3 ± 0.3	ND	
Chenodeoxycholic acid derived bile acids						
βMCA	13.6 ± 3.4	13.2 ± 1.3	17.0 ± 1.8	14.6 ± 1.0	12.9 ± 0.2	
LCA	2.0 ± 0.4	2.7 ± 0.4	2.6 ± 0.5	3.4 ± 0.3	4.2 ± 0.3**	N.S.
ILCA	0.2 ± 0.2	ND[d]	ND	ND	0.1 ± 0.1	
HDCA	0.9 ± 0.4	1.4 ± 0.5	1.0 ± 0.1	1.1 ± 0.2	1.9 ± 0.6	
MDCA	0.2 ± 0.2	1.4 ± 0.6	0.8 ± 0.8	ND	ND	
αMCA	8.4 ± 0.3	7.6 ± 0.6	8.1 ± 0.5	7.0 ± 0.4	6.0 ± 0.8*	N.S.
ωMCA	11.2 ± 1.3	10.2 ± 1.7	7.6 ± 1.1	10.8 ± 0.8	8.5 ± 1.4	
6KLCA	4.6 ± 0.5	2.8 ± 0.8	4.1 ± 0.5	4.0 ± 0.5	4.5 ± 1.4	
CA/CDCA ratio[b]	1.49 ± 0.22	1.58 ± 0.14	1.46 ± 0.11	1.47 ± 0.12	1.65 ± 0.14	
Primary bile acids[c] [%]	30.5 ± 5.0	26.3 ± 2.4	28.7 ± 3.5	23.8 ± 1.5	21.8 ± 0.9	

Data are expressed as means SEM ($n = 5$). *a*) Abbreviations are: 7KLCA, 7-keto lithocholic acid. See the legends of Table 4 for others. *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*) ND: Not detected. *e*) Significantly different from the control group. ($*p < 0.05$, $**p < 0.01$, by 1-way-ANOVA followed by Dunnett's multiple comparison test) *f*) Dose–response was analyzed by linear-regression analysis. *g*) N.S., not significant.

of IDCA, β–muricholic acid (βMCA) and the primary bile acids were significantly lower in rats given 1.0, 0.1 and 0.1 g/kg GSP, respectively, than in control rats ($p < 0.05$).

In rats fed the C-CA diet, the percentages of 12-keto deoxycholic acid (12KLCA) and LCA were significantly higher in rats given 0.5 and 1.0 g/kg GSP, 1.0 g/kg GSP, respectively, than in control rats ($p < 0.01$). The percentage of α–muricholic acid (αMCA) was significantly lower in rats given 1.0 g/kg GSP, respectively, compared with control rats ($p < 0.05$).

No other significant changes were observed in fecal steroid excretion (Tables 4 and 5).

DISCUSSION

A few studies have reported the effects of GSP on lipid metabolism. Yamakoshi *et al.*[17] reported that proanthocyanidins, the major polyphenols in red wine, might trap reactive oxygen species in aqueous components such as plasma and intestinal fluid of the arterial wall, thereby inhibiting oxidation of LDL and showing an antiatherosclerotic activity. Tebib *et al.*[19] showed that polymeric grape seed tannins exert a hypocholesterolemic effect in rats fed a high- cholesterol diet. They also reported that dietary grape seed tannins have a pronounced antihypercholesterolemic effect in rats by enhancing reverse cholesterol transport and also reducing in-

testinal cholesterol absorption and increasing bile acid excretion.[20] These phenomena manifested 9 weeks after the experimental period,[20] and it is unknown whether these cholesterol-lowering effects are apparent in the shorter term. To date, a study on the precise changes in fecal steroid composition that occur after GSP administration has not been reported. We therefore investigated the effect of GSP administration on fecal steroid excretion as well as the effect on serum and hepatic lipid contents in rats fed a normal or lithogenic diet for 28 or 36 days, respectively.

The relative weight of the liver in rats fed the C-CA diet was about 2-fold higher than in rats fed a normal diet because of the fatty liver (Table 2). Food intake during the experimental period was measured only in rats fed the C-CA diet. Food intake during the experimental period (35 days) tended to decrease as the dose of GSP increased, although this decrease was not found to be significant at days 25–27 (Table 2). It is possible that rats given a high dose of GSP ate less during the early period as compared with those on lower doses of GSP, which would reflect the lower relative liver weight of the higher-dose GSP animals. The ratio of calorie intake (calculated using Atwater's coefficient) to body weight gain had a tendency to increase with GSP administration (Table 2). By contrast, Vallet *et al*.[25] reported that GSP reduces nitrogen digestibility significantly but not starch and fat digestibility. It is possible that the administration of an excessive dose of GSP may reduce food intake, which in turn suppresses body weight gain.

In rats fed the C-CA diet, the serum concentrations of total cholesterol and phospholipids and the hepatic concentrations or contents of cholesterol, phospholipids and triglycerides increased between 1.4- and 62.2-fold, but the serum HDL-cholesterol concentration decreased by about half, as compared with those of rats fed a normal diet (Table 3). Administration of GSP lowered the concentration of serum triglycerides in a dose–dependent manner in rats fed both types of diet, although no significant changes were observed in the concentrations of serum and hepatic cholesterol (Table 3). Thus, GSP has an effect of lowering serum triglycerides, however, the underlying mechanism is unknown.

The fecal excretion of neutral steroids and bile acids, the CA/CDCA ratio and the percentages of primary bile acids were higher, whereas the fecal coprostanol/cholesterol (cp/ch) ratio was lower in rats fed the C-CA diet than in rats fed a normal diet. The fecal percentage of LCA tended to increase with GSP administration in rats fed both types of diet (Tables 4 and 5). Significant increases in the fecal excretions of neutral steroids and bile acids were observed only in rats given 1.0 g/kg GSP and fed a normal diet (Table 4). Thus, GSP may possibly have a weak effect of stimulating the excretions of fecal neutral steroids and bile acids. Contrary to the report of Tebib *et al*.,[19,20] a significant increase in fecal excretion of neutral steroids and/or bile acids was not found in rats fed the C-CA diet (Table 5). This discrepancy might be caused by differences in the composition of the diets and in the method of GSP administration. In our study, both cholesterol and CA was added to the diets to stimulate cholesterol absorption from the intestine, and GSP was administered by Magen-sonde, rather than by gavage.

In conclusion, an excess quantity of GSP may have the effect of reducing food intake and therefore should not be taken in one dose. In addition, administration of GSP seems to affect the lipid metabolism of rats by lowering the concentrations or contents of serum and hepatic triglycerides rather than by altering cholesterol metabolism. Scalbert and Williamson[33] have reported that the daily intake of polyphenol in man was about 1.0 g/kg. This is equivalent to a dose of 0.02 g/kg for a body weight of 50 kg. In this study, we observed significant effects of GSP administration in rats at doses of 0.05 g/kg. So far, there have been no reports about the metabolism of GSP; therefore, the metabolism of GSP should be clarified to elucidate the mechanism of its various physiological functions.

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