

Green, oolong and black tea extracts modulate lipid metabolism in hyperlipidemia rats fed high-sucrose diet

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Abstract

The main goal of this study was to compare effects of ethanol-soluble fractions prepared from various types of teas on sucrose-induced hyperlipidemia in 5-week old male Sprague-Dawley rats. Rats ($n = 6-8$ per group) weighed approximately 200 g were randomly divided into control diet, sucrose-rich diet, green tea, oolong tea and black tea groups. Control-diet group was provided with modified AIN-93 diet while the others consumed sucrose-rich diet. Tea extracts (1% w/v) were supplied in the drink for green tea, oolong tea and black tea groups. Results indicated sucrose-rich diet induced hypertriglyceridemia and hypercholesterolemia. Food intake was reduced by oolong tea extract. Consuming oolong and black tea extracts also significantly decreased body weight gains and food efficiency. Hypertriglyceridemia was normalized by green and black tea drink on day 18 and by oolong tea extract on day 25, respectively. Hypercholesterolemia was normalized by green tea on day 18 and by oolong tea and black tea on day 25, respectively. Plasma HDL-cholesterol concentrations were not affected by any tea extract. The triglyceride content in the liver as well as the cholesterol content in the heart of rats fed sucrose-rich diet were elevated and were normalized by all types of tea drink tested. Although green and oolong tea extracts contained similar composition of catechin, our findings suggest green tea exerted greater antihyperlipidemic effect than oolong tea. Apparent fat absorption may be one of the mechanisms by which green tea reduced hyperlipidemia as well as fat storage in the liver and heart of rats consumed sucrose-rich diet. © 2001 Elsevier Science Inc. All rights reserved.

Keywords: Tea; Sucrose-rich diet; Lipid; Triglyceride; Cholesterol

1. Introduction

Tea (*Camellia sinensis*) is one of the most popular beverages consumed worldwide. Green, oolong and black teas are non-, partially- and fully-fermented/oxidized, respectively. Green tea contains considerable amount of catechin such as epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC) and epigallocatechin gallate (EGCG) [1,2]. The major components of black tea, in contrast, are theaflavins and thearubigins, the oxidation products of quinones and flavols. Oolong tea, widely consumed in the Chinese population, may retain a considerable amount of the original polyphenolic compound [1,2].

A number of studies have shown green and black teas exert anticholesterolemic effect [3–6]. Epidemiological

studies found consumption of green or black tea drink was inversely correlated with plasma cholesterol concentrations [3,4]. Maramatsu et al. [5] suggests that addition of 1–2% green tea extract to the lard/cholesterol diet reduced blood cholesterol levels in rats. When EGCG was added to the same diet, it exerted similar effects as green tea extract [6]. Therefore, catechins are probably the active ingredient of green tea. However, the biological effect of oolong tea remains unclear. Yen and Chen [7] compared the antimutagenic role of green, oolong and black teas and suggest oolong tea exerted the strongest effect. Therefore, it would be interesting to know how different types of tea modulate lipid metabolism.

A sucrose-rich diet (SRD) has been proven lipogenic [8,9]. It induces hypertriglyceridemia and elevates triglyceride (TG) content in the liver and the heart as soon as three weeks in rats [8,9]. Long-term feeding with SRD results in a steady state of hypertriglyceridemia and hyperglycemia [9]. Although green tea extract has been shown to suppress

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hyper-cholesterolemia induced by high fat/cholesterol diet, it is unclear how tea extracts prepared from green, oolong and black teas modulate TG metabolism in rats fed SRD. Therefore, the present study was undertaken to characterize the onset of modulatory effects of ethanol-soluble fractions of green, oolong and black teas on lipid metabolism and to examine the mechanisms by which tea may exert the anti-hyperlipidemic effects. In this study, catechin composition from green, oolong and black tea extracts that were extracted by ethanol, were determined. Effects of tea drink on plasma TG as well as total and HDL-cholesterol concentrations were determined on different time points (d 18 and 25). We also compared effects of tea extracts on TG and cholesterol contents in the liver and in the heart. Finally, we examined if tea extracts modulated energy retention by protein absorption, and affected lipid metabolism by apparent fat absorption and fecal bile acid excretion.

2. Materials and methods

2.1. Materials

The green tea, oolong tea and black tea made in Taiwan were purchased in bulk and stored in dry atmosphere. Casein, corn oil, AIN-93G mineral mix, and AIN-93 vitamin mix were purchased from ICN Biomedicals Inc. (Costa Mesa, CA, USA). Corn starch and sucrose were obtained from Thailand (Benkok) and Taiwan Sugar Co. (Taiwan), respectively. Cellulose, cysteine, choline bitartrate, TBHQ, authentic standard EGC, EC, EGCG, ECG, and (+)-catechin for HPLC analysis, plasma triglyceride kit, cholesterol kit, and HDL-precipitation kits were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Animals and experimental design

Male Sprague-Dawley rats (National Laboratory Animal Breeding and Research Center of the National Science Council, Taipei, Taiwan) weighed about 200 g were housed in individual stainless steel cages with free access to chow diet and water in a room maintained at $24 \pm 2^\circ\text{C}$ on a 800–2000 light-dark cycle. Animals were adapted to the animal facility for five days before the experiment. Rats were randomly divided into groups of control diet (CD), sucrose-rich diet (SRD), green tea (G), oolong tea (O), and black tea (B) groups with 6–8 animals per group. Rats were provided free access to experimental diet and fluid for 25 days. The composition of diet is shown in Table 1 [10]. Control diet and SRD groups drank distilled water. Ethanol-soluble tea extracts were supplied in tea drink (1% w/v). Tea drink was freshly prepared as the intake was measured every day. Care and treatment of rats were in compliance with the Guide for the Care and Use of Laboratory Animals [11] and local institutional guidelines. Body weight and food intake were measured twice a week. Venous blood of

Table 1
Composition of experimental diets^a

Ingredient	Control diet (CD)	Sucrose-rich diet (SRD)
	g/kg diet	
Casein	200	200
Corn starch	530	0
Sucrose	50	580
Corn oil	70	70
Cellulose	100	100
AIN-93G mineral mix	35	35
AIN-93 vitamin mix	10	10
L-Cystine	3	3
Choline bitartrate	2.5	2.5
TBHQ	0.014	0.014

^a Modified from the AIN-93 diet [10].

animals fasted for 20 h were taken from the tail on d 18 and 25 into heparinized tubes and immediately centrifuged at 3000 rpm at 4°C for 10 min. Feces individually collected from d 21–23 were lyophilized, flushed with nitrogen and stored at room temperature until analyses for fat, nitrogen and bile acid contents. After the fasting blood was drawn as described, animals were anesthetized with ether before organs were removed on the last day. The liver, heart, stomach, kidney, lumbar fat pad, small intestine and large intestine were weighed after proper cleaning and rinsing with saline. The plasma, liver and heart were stored at -20°C until further analysis.

2.3. Preparation of tea extract

Green tea, oolong tea and black tea were purchased in bulk. Tea leaves were grounded in a miller as temperature of the container was maintained at less than 50°C . Tea powder was extracted with 95% ethanol (1:10, w/v) for 2 h with constant stir. Suspension was filtered through Whatman No. 1 filter paper to retain the clear solution. The residue was extracted again. The pooled tea solution was vacuum evaporated below 50°C . The dried extracts were stored at 4°C .

2.4. Analysis of catechin

Catechin content of each extract was analyzed by the method of Terada et al. [12] using LDC-HPLC system (LDC Thermo pump 3500, LDC-Milton Roy detector, LDC 4100 integrator, Florida, USA). The column was Nova-Pak C18 (3.9 x 150 mm, 4 μ , Waters, USA) and the mobile phase was constituted of solvent A (0.1% acetonitrile, 0.1% phosphoric acid and 5% dimethylformamide) and solvent B (acetonitrile) with gradient elution, i.e. solvent B was increased from 1% to 15% within 30 min and then dramatically increased to 90% within 10 min. The flow rate was 1 ml/min and detection was made at 230 nm.

Table 2
Catechin contents (%) of the extracts prepared from green tea, oolong tea and black tea^a

Tea	EGC	(+)-catechin	EC	EGCG	ECG	Total catechins
Green	13.51	0.48	3.97	20.16	7.88	45.98
Oolong	17.83	1.24	3.19	19.06	5.77	47.08
Black	1.72	0.32	2.15	1.35	0.67	6.12

^a Catechin content was determined by the method described in Material and Methods and indicated as weight percent of the extract.

^b All values are means of three replicates.

2.5. Analyses of plasma TG, true TG, total cholesterol, and HDL-cholesterol concentrations

Plasma total TG and true TG, and total and HDL-cholesterol concentrations were determined according to the protocol provided by Sigma Chemical Co. (Sigma diagnostics, procedure #337-B, and #352, respectively, St. Louis, MO, USA). High-density lipoprotein was separated from plasma by precipitating VLDL and LDL with dextran sulfate (Sigma Chem. Co., St. Louis, MO, USA).

2.6. Analyses of TG and cholesterol contents in the liver and heart

Lipids in the liver and heart were extracted with chloroform-methanol mixture (2:1, v/v) as described by Folch et al [13]. The contents of total TG and total cholesterol in the liver and heart were measured according to the methods described by Soloni [14] and Mann [15], respectively.

2.7. Apparent absorption of fat and protein

Feces were lyophilized and grounded in a miller. Fats from aliquots of dry feces and diet was continuously extracted with ether using Soxhlet apparatus for 6 h. Apparent fat absorption is calculated as follows:

Apparent absorption of fat (%) = (fat consumed–fat in feces)/fat consumed × 100%

Curde nitrogen contents of dry feces and diet were determined using the semi-micro kjeldahl method.

2.8. Determination of fecal bile acid content

Aliquots of dry fecal samples were extracted with mixture of chloroform and methanol (1:1 v/v) at 65°C. The supernatant was dried under a stream of nitrogen. Samples were dissolved in a bovine serum albumin solution (2.5 mg/ml) and fecal bile acid was determined according to the method provided by Sigma Chem. Co. (Sigma diagnostics, procedure #450, St. Louis, MO.).

2.9. Statistics

All data are presented as means ± SE and were analyzed with SPSS for Window, v.6.0 (SPSS Inc., Chicago, USA). Plasma total TG, true TG, total cholesterol, and HDL-

cholesterol concentrations were analyzed using one-way ANOVA followed by LSD test at each time point. The remaining data were analyzed with one-way ANOVA followed by post-hop LSD test. Effects were considered significant at $p < 0.05$.

3. Results

3.1. Composition of tea extracts

Catechins are a major component of the green tea and oolong tea extracts. Almost half of these tea extracts were mixtures of catechins (Table 2). In contrast, monomeric catechins only constitute less than 10% of the black tea extract. The sum of EGC and EGCG weighed more than 70% of the catechin mixture in the green and oolong tea extracts, and only half of the black tea extract (Table 2).

3.2. Effects of tea extracts on energy metabolism

Table 3 shows food intake, fluid intake, body weight gain and food efficiency of rats fed test diets and drinks. Cumulative food intake was significantly suppressed in oolong tea group, whereas fluid intake was not significantly different between groups. The average of food intake (mean ± SE) in CD, SRD, G, O and B groups were 23.93 ± 0.77 , 22.33 ± 0.78 , 20.1 ± 1.04 , 19.75 ± 0.54 and 21.5 ± 0.94 g/day, respectively. Average fluid intake (mean ± SE) was 38.51 ± 1.85 , 36.34 ± 1.58 , 38.98 ± 0.95 , 40.96 ± 1.42 , and 37.80 ± 2.23 g/day, for CD, SRD, G, O and B groups, respectively. Therefore, the amounts of tea extracts consumed were similar between groups. Body weight gains for rats in C and SRD groups were not different. Consumption of oolong and black tea extracts, however, effectively reduced the weight gain for nearly 35% and 29%, respectively, as compared to the SRD group. Both oolong tea and black tea drink reduced the food efficiency of SRD by 27%. Most of the relative organ weights were not affected by the supplement of tea extract as shown in Table 4. However, black tea decreased the weight of the fat pad and green tea increased the weight of the small intestine.

3.3. Effects of tea extracts on lipid metabolism

In this study, feeding SRD caused the elevations of plasma total TG, true TG and total cholesterol concentra-

Table 3
Body weight gain, food intake, fluid intake, and food efficiency of rats fed drinks and diets as indicated^{a,b}

Group	Diet and drink ^c	Body weight gain	g/25d		Food efficiency Kcal/g
			Food take	Fluid intake	
CD	CD + distilled water	137.2 ± 11.0	574.3 ± 18.4	885.8 ± 42.6	6.10 ± 0.44
SRD	SRD + distilled water	139.7 ± 11.4	552.9 ± 19.3	835.9 ± 21.1	6.77 ± 0.47
G	SRD + green tea drink	129.0 ± 6.9	510.2 ± 26.8	896.5 ± 21.9	6.57 ± 0.56
O	SRD + Oolong tea drink	90.8 ± 8.2*	489.2 ± 13.4*	942.0 ± 32.7	4.76 ± 0.45*
B	SRD + black tea drink	99.4 ± 10.2*	551.4 ± 16.6	869.4 ± 51.3	4.59 ± 0.23*

^a Data are expressed as mean ± SE, n = 6–8.

^b Asterisk indicates significant difference at P < 0.05 as compared to the SRD group using LSD test.

^c Tea drinks were 1% (w/v) green, oolong and black tea extract solutions.

tions as early as on d 18 (Fig. 1). Consuming green and black tea extracts for 18 days almost normalized the plasma total TG concentrations (Figure 1A). Green tea drink further dramatically reduced plasma total TG concentrations to 50% of the levels in the CD group at the end of the study. Consuming black tea also further reduced plasma TG concentration to 76% of the level in the CD group on d 25. In contrast, oolong tea extract did not normalize plasma TG concentration until d 25. Plasma true TG concentration was significantly decreased from d 18 by green tea extract (Fig. 1B). Suppressed effect of black tea on plasma true TG concentration occurred on d 25. Oolong tea, in contrast, did not modulate plasma true TG concentration.

Plasma total cholesterol concentrations in SRD group were elevated for 25% on day 18 and further increased to 35% on d 25 as compared to CD group (Fig. 1C). Again, green tea extract was most effective in reducing plasma cholesterol level. Hypercholesterolemia was first normalized on day 18 by green tea drink, and later by oolong tea and black tea drink on day 25. None of the tea extracts effectively altered plasma HDL-cholesterol concentrations although oolong tea extract consistently tended to increase this fraction (Fig. 1D).

Sucrose-rich diet significantly elevated hepatic TG contents by approximately 3-folds, as compared to that in the CD group (Table 5). This increase in TG content was significantly reduced with green tea drink and was fully normalized with oolong tea and black tea drink. Sucrose-

rich diet increased hepatic cholesterol content for nearly 60%. None of the tea drink supplied in this study could reverse the elevated hepatic cholesterol content caused by consuming SRD. On the other hand, consuming SRD did not elevate TG content in the heart but elevated cholesterol content for nearly 30%. Consuming green tea and oolong tea drink effectively normalized heart cholesterol content.

The apparent protein absorption of all groups were close to 94% (Table 6). In contrast, the apparent fat absorption of SRD diet was significantly decreased for 7% by green tea extract. The concentration and daily excretion of fecal bile acid were not statistically different among groups.

4. Discussion

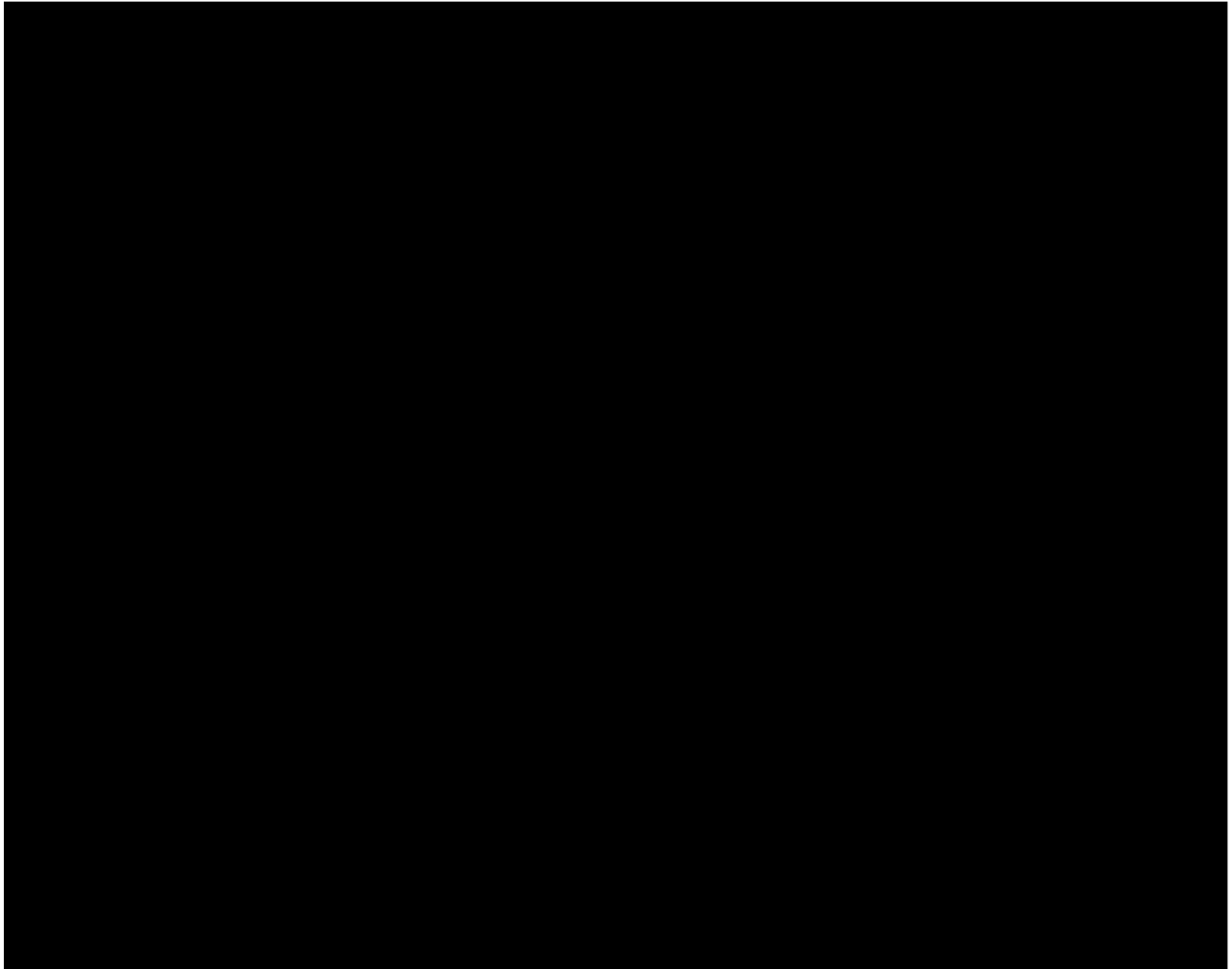
The purpose of this study was to 1) compare the onset of the antihyperlipidemic effect of green, oolong and black tea supplement, 2) examine the changes in the lipid content of the liver and heart, and 3) illustrate the mechanisms by which tea extract modulated lipid and energy metabolisms in rats fed high sucrose diet. Based on previous studies, sucrose-induced hyperlipidemia started on d 15 and persisted until day 35 [8,9]. Therefore, we investigate effects of tea extract on d 18 for onset of tea effect. Consistent with previous studies, SRD exerted lipogenic effects on both d 18 and d 25 (Fig. 1 and Table 5). All tea extracts prevented hypertriglyceridemia as well as hypercholesterolemia in-

Table 4
Relative weights of liver, heart, kidney, lumbar fat pad, small intestine and large intestine in rats fed control diet (C), sucrose-rich diet (SRD), SRD with green tea extract (G), oolong tea extract (O), and black tea extract (B)^{a,b}

Organ Group	Liver	Heart	Kidney	Lumbar fat pad	Small intestine	Large intestine
	g/100g body weight					
C	2.97 ± 0.16	0.37 ± 0.01	0.72 ± 0.02	1.15 ± 0.15	1.36 ± 0.09	0.34 ± 0.06
SRD	3.20 ± 0.18	0.33 ± 0.01	0.74 ± 0.03	1.12 ± 0.19	1.36 ± 0.07	0.26 ± 0.03
G	3.45 ± 0.28	0.34 ± 0.02	0.83 ± 0.05	1.17 ± 0.11	1.66 ± 0.12*	0.33 ± 0.03
O	3.04 ± 0.05	0.34 ± 0.01	0.78 ± 0.04	1.25 ± 0.16	1.51 ± 0.09	0.37 ± 0.01
B	2.88 ± 0.10	0.32 ± 0.01	0.77 ± 0.01	0.77 ± 0.07	1.49 ± 0.06	0.33 ± 0.02

^a Data are expressed as means ± SE, n = 6–8.

^b Asterisk indicates significant difference at P < 0.05 as compared to the SRD group with Dunnett's test.



Plasma total triglyceride (A), true triglyceride (B), total cholesterol (C), and HDL-cholesterol (D) of rats fed experimental diets for 18 and 25 days. Data are expressed as mean \pm SE. Asterisk indicates significant difference as compared to SRD. The groups of rats presented here are the same as those in Table 3.

duced by SRD (Fig. 1). However, the onsets of these effects were different among treatments. Green tea extract consistently exerted the greatest potency in normalizing hyperlipidemia without altering food intake, fluid intake, growth rate and food efficiency (Fig. 1 and Table 3). Hepatic TG and cholesterol contents of rats fed SRD also increased as ex-

pected (Table 5). All the tea treatments normalized the hepatic TG content. Feeding SRD for 25 days caused elevated heart cholesterol, but not TG contents. Green tea and oolong tea, but not black tea, effectively normalized heart cholesterol content. Based on the composition of tea extract and apparent fat absorption, we suggest that green tea and black tea may exert their effects via different mechanisms. The effect of green tea extract on antihyperlipidemia was probably associated with decreasing fat absorption. On the other hand, black tea extract may reduce hyperlipidemia by reduced food efficiency.

Ethanol was used to extract catechin in the present study since it has been shown an ideal solvent for high recovery of tea catechins [2]. Massive oral doses of tannic acid have been reported to depress growth rate and increase fecal protein in rats [17,18]. Therefore, we fed rats the tea extract with drink instead of with diet in order to prevent the possible adverse effect. Unfortunately, both oolong tea and black tea extracts exerted antihyperlipidemic effect as well as weight-reducing effects in the present study. Oolong tea

Table 5
Effects of tea extracts on total lipid, triglyceride and cholesterol in the liver and heart (mg/g)^{a,b}

	Liver		Heart	
	TG	Cholesterol	TG	Cholesterol
CD	6.97 \pm 0.98*	1.34 \pm 0.16*	4.27 \pm 0.54	2.11 \pm 0.20*
SRD	27.71 \pm 7.87	2.12 \pm 0.20	4.44 \pm 0.59	2.73 \pm 0.32
G	8.35 \pm 1.50*	1.69 \pm 0.12	4.13 \pm 0.96	1.84 \pm 0.12*
O	6.71 \pm 1.01*	2.69 \pm 0.30	4.70 \pm 1.32	2.09 \pm 0.19*
B	6.23 \pm 1.38*	2.66 \pm 0.25	4.27 \pm 0.95	2.14 \pm 0.14

^a Data were expressed as mean \pm SE with n = 6–8 for each group.

^b Asterisk indicates significant difference at P < 0.05 as compared to the SRD group with LSD test.

Table 6
Effects of tea extracts on protein and fat absorption, fecal bile acid concentration, and fecal bile acid excretion^{a,b}

Group	Protein absorption	Fat absorption	Bile acid concentration	Daily bile acid excretion
	(%)	(%)	($\mu\text{mol/g}$)	(μmol)
CD	94.4 \pm 0.1	58.9 \pm 2.2	13.65 \pm 1.38	33.26 \pm 2.62
SRD	94.6 \pm 0.4	55.7 \pm 3.3	11.90 \pm 2.99	27.52 \pm 2.19
G	94.1 \pm 0.4	48.3 \pm 2.0*	11.63 \pm 1.96	27.53 \pm 2.46
O	93.8 \pm 0.3	59.9 \pm 1.6	13.22 \pm 1.24	31.34 \pm 2.70
B	93.8 \pm 0.2	56.1 \pm 1.4	14.13 \pm 1.46	30.90 \pm 3.62

^a Data were expressed as mean \pm SE with n = 6–8 for each group.

^b Asterisk indicates significant difference at P < 0.05 as compared to the SRD group with LSD test.

suppressed appetite and food efficiency while black tea only suppressed food efficiency. Ha and Huang [19] compared the protein precipitating ability of various types of teas and found effects of oolong and black teas are stronger than the unfermented green tea. Therefore, decreased protein absorption may explain the adverse effect of tea extract on growth rate in this study. We therefore further determined apparent protein absorption and found oolong tea and black tea extracts tended to decrease apparent protein absorption, although this effect was not statistically significant. Therefore, the mechanism by which oolong tea and black tea extracts suppressed growth remained further investigation.

Catechins extracted from green tea have been shown to normalize plasma cholesterol concentration without affecting HDL-cholesterol concentration, growth rate and food efficiency in rats [5,6]. In this study, we confirm the role of green tea on blood cholesterol, and further suggest that green tea also prevent hypertriglyceridemia in rats. The onsets of anti-hyperlipidemia effects were different for different types of tea. Green tea extract consistently normalized plasma TG and cholesterol concentrations on d 18 and further decreased plasma TG concentrations below levels obtained by the control diet on d 25. Oolong tea, in contrast, did not suppress hyperlipidemia until d 25. Black tea extract suppressed hypertriglyceridemia on d 18, but did not suppress hypercholesterolemia until d 25. The onset and magnitude of effects suggest that green tea extract was more potent than oolong tea extract in reducing hyperlipidemia induced by SRD.

Hepatic TG content was, in agreement to previous studies, increased by SRD [8,9]. It has been shown that SRD induces net production of very-low-density lipoproteins (VLDL) in the liver and expression of apolipoproteins A and E, reduction of plasma postheparin lipase activities and hepatic triglyceride lipase activities [19,20]. Therefore, tea extract may modulate lipid metabolism by apparent fat absorption and the other mechanisms. Besides, oolong and black tea extracts decreased food efficiency, which may lead to less fat deposition in the liver. Hepatic cholesterol content also significantly increased in the SRD group, but tea extracts failed to normalize it. Lipid metabolism in the heart has rarely been explored. In our study, we found SRD also induced greater cholesterol content in the heart, which was reversed by both green and oolong tea extracts. However, heart

TG failed to increase as shown in previous studies, possibly due to greater fiber content (100 g per kg) in our diet [8,21].

In conclusion, green tea extract not only did exert anti-hyperlipidemic effects with the shortest onset and greatest magnitude, but also suppressed the lipotropic effects of SRD both in the liver and the heart. Consumption of oolong tea, the semi-fermented tea, for 25 days was also effective in modulating plasma lipid, hepatic TG and heart cholesterol contents in rats fed SRD. Although oolong tea extract contained greater amount of catechin monomers than green tea extract did, the effects of green tea on lipid metabolism were stronger than the others. Therefore, we suggest that ingredients of tea besides catechin monomer, i.e., dietary fiber, may also play a role in modulating lipid and energy metabolisms in this study.

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