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Theflavins and Theasinensin A Derived from Fermented Tea Have Antihyperglycemic and Hypotriacylglycerolemic Effects in KK-A^y Mice and Sprague–Dawley Rats

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ABSTRACT: Although tea polyphenols are reported to improve serum glucose and lipid levels by inhibiting amylase activity and reducing lipid absorption, in vivo data are lacking. We evaluated in vivo the antihyperglycemic and hypotriacylglycerolemic effects of theaflavins (TFs) and theasinensin A (TSA) refined from fermented tea to purities of 12 and 59%, respectively. Feeding male KK-A^y mice diets with 0.1% TFs or TSA for 6 weeks reduced serum glucose levels by >30% compared to a control diet. Rats fed diets containing 0.2% TFs or TSA for 4 weeks had higher fecal fat excretion and 33% lower hepatic triacylglycerol; hepatic fatty acid synthase activity was not affected. Oral administration of TFs or TSA reduced the increase in serum triacylglycerol after an oral bolus of a fat emulsion. These results indicate TFs and TSA induce antihyperglycemic responses in diabetic mice and are hypotriacylglycerolemic in rats by suppressing intestinal fat absorption.

KEYWORDS: *theaflavins, theasinensin A, black tea polyphenols, antihyperglycemic effect, hypotriacylglycerolemic effect*

INTRODUCTION

Tea (*Camellia sinensis* L.) is one of the most popular beverages consumed around the world. The processed teas are classified into three types according to the degree of fermentation. Unfermented, semifermented, and fully fermented teas are called green tea, oolong tea, and black tea, respectively. In the manufacture of green tea, harvested fresh leaves are immediately steamed to inactivate enzymes, especially polyphenol oxidase. Consequently, green tea contains a relatively high content of monomeric catechins. Green tea consists of four major catechins: (−)-epicatechin (EC), (−)-epicatechin-3-gallate (ECG), (−)-epigallocatechin (EGC), and (−)-epigallocatechin-3-gallate (EGCG). Tea catechins have been reported to have antiobese, antidiabetic, hypocholesterolemic, hypotriacylglycerolemic, antioxidative, and anticarcinogenic properties.[1−8] During the fermentation processing of black tea, a large proportion of the catechins undergo oxidative polymerization by polyphenol oxidase, generating black tea polyphenols such as theaflavins (TFs), theasinensins (TSs), and other high molecular components (e.g., thearubigins). The TFs characteristically occur as dimers of EGC and EC or their galloyl esters and are categorized into the following components; theaflavin (TF), theaflavin-3-*O*-gallate (TF-3-*O*-Gal), theaflavin-3′-*O*-gallate (TF-3′-*O*-Gal), and theaflavin-3,3′-di-*O*-gallate (TF-3,3′-di-*O*-Gal). Theasinensin A (TSA) consists of two galloyl groups and is a major component of TFs.

The TFs associated with black tea have been shown to provide several health benefits, such as antidiabetic, antiobese, hypocholesterolemic, and anticarcinogenic properties.[9−15] Nakai et al.[16] reported that TFs and TSA were more potent inhibitors of pancreatic lipase than EGCG. The study by Abe et al.[17] revealed TSA to be a potent inhibitor of squalene epoxidase, a rate-limiting enzyme in cholesterol synthesis. Moreover, we recently determined the mechanism underlying the cholesterol-lowering activity of TFs and TSA is the stimulation of fecal steroid excretion.[18] Collectively, these studies confirm the hypocholesterolemic action of black tea polyphenols. Using human HepG2 cells, Lin et al.[9] also revealed that TFs reduce hepatic fat accumulation by suppressing fatty acid synthesis and stimulating fatty acid oxidation. A placebo controlled clinical trial by Maron et al.[13] showed a theaflavin-enriched green tea extract (20% as TFs by weight) was hypocholesterolemic and hypotriglyceridemic. Despite these promising findings, to our knowledge, the mechanism underlying the hypotriacylglycerolemic response induced by TFs has not been examined in vivo.

The antidiabetic properties of TFs and TSA are attributed to inhibition of α-glucosidase activity,[14] with a single dose of TF-

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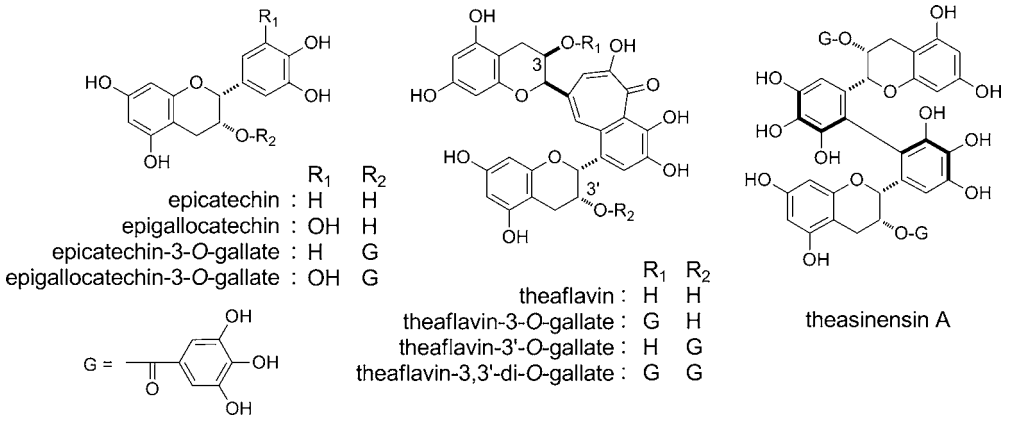

Figure 1. Structures of catechins, theaflavins, and theasinensin A.

3-*O*-Gal sufficient to suppress glucose production from maltose. Therefore, it is expected that supplementing the diet with TFs and TSA fractions could alter glucose availability and metabolism. However, the in vivo responses of serum glucose to feeding TFs and TSA fractions remain elusive despite the relevance to individuals with hyperglycemia. Therefore, this study investigated how TFs and TSA prepared from a fermented tea made with a third crop of green tea and loquat leaves affect serum glucose level in the KK-A^y mouse model for type 2 diabetes and serum and hepatic triacylglycerol concentrations in Sprague−Dawley (SD) rats fed a high fat diet.

MATERIALS AND METHODS

Chemicals. TFs TF-3-*O*-Gal, TF-3′-*O*-Gal, and TF-3, 3′-di-*O*-Gal and TSA were prepared by enzymatic oxidation of tea catechins.[19,20] Structures of catechins, TFs, and TSA are depicted in Figure 1. Other reagents were of analytical grade and used without further purification.

Materials. Fresh green tea leaves (*Camellia sinensis* var. *sinensis*, cv. Yabukita) were collected at Nagasaki Agriculture and Forestry Technical Development Center, Tea Research Station (Nagasaki, Japan). Fresh loquat leaves were collected at Nagasaki Agriculture and Forestry Technical Development Center, Fruit Tree Research Station (Nagasaki, Japan).

Preparation of TFs and TSA Fractions. TFs and TSA fractions were prepared based on the modified method of Tanaka et al.[21] from fermented tea obtained by tea-rolling processing of a third crop of green tea leaves and loquat leaves as described in detail elsewhere.[22] Briefly, fresh tea leaves (27 kg) were partially dried by blowing air (70 °C) for 20 min in a primary tea-rolling dryer (60k type, Kawasaki Co., Ltd., Shimada, Japan). The temperature of the leaves did not exceed 40 °C during this process. Then the leaves were mixed with fresh loquat leaves (3 kg) and kneaded with a tea roller (60k type, Kawasaki Co., Ltd.) at room temperature for 20 min. Finally, the leaves were heated at 100 °C in a tea dryer (120k type, Kawasaki Co., Ltd.) for 30 min to terminate enzymatic oxidation. The water content of the final tea leaves was less than 5%. We extracted fermented tea leaves (1.93 kg) with H_2O (30 L) at room temperature overnight and then filtered. The filtrate was directly applied to Sephadex LH-20 column. The plant debris remained on the filter paper was further extracted twice with 60% acetone, and the extract was concentrated using a rotary evaporator. Resulting insoluble precipitates, mainly chlorophylls, was removed by filtration. To the filtrate methanol was added to clear reddish-brown turbidity, which mainly composed of thearubigins, theaflavins, and caffeine. The final concentration of methanol was about 20%. The filtrate was applied to the aforementioned Sephadex LH-20 column. After washing of the column with 20% methanol, caffeine and flavonoid glycosides were eluted with 40% methanol. The fractions containing EC, EGC, EGCG, ECG, and theasinensin B were obtained by elution with 60−100% methanol (20% stepwise). TSA, TFs, and thearubigins were eluted with 60% acetone. The last fraction was subjected to size-exclusion chromatography (SEC) using Sephadex LH-20 with acetone-7 M urea (3:2, v/v, adjusted to pH 2 with conc. HCl). Thearubigins with larger molecular weights were eluted faster than TSs and TFs, and the fractions containing thearubigins (detected at origin on thin layer chromatography) were combined. After evaporation of acetone, the aqueous solution was applied to a Diaion HP20SS column, and the column was then washed with H_2O to remove urea and HCl. Polyphenols adsorbed on the gel were eluted using 80% methanol to give thearubigins. Fractions of SEC containing TSA and TFs were combined and concentrated. The aqueous solution was applied to a Diaion HP20SS column with H_2O containing increasing proportions of methanol (10% stepwise) to yield TSA and the fraction mainly containing a mixture of TFs.

Purity of TFs and TSA Fractions. The purity of TFs and TSA fractions was checked using a high-performance liquid chromatography (HPLC) by the method of Tanaka et al.[21] Figure 2 shows chromatograms of TFs and TSA fractions. The purity of TFs and TSA in the fractions was 12% and 59%, respectively. Ingredients other than TFs and TFA were catechins with polymerization of more than 3. The remaining of TFs fraction is mainly thearubigins with relatively smaller molecular-weights compared to those of thearubigin fractions.

Animals. Male KK-A^y mice, type 2 diabetic animals, were purchased from Clea Japan, Tokyo, Japan. Male SD rats were obtained from Japan SLC, Inc., Hamamatsu, Japan. Mice and rats were individually housed in plastic and stainless-steel cages, respectively, under a controlled atmosphere (temperature, 22 ± 1 °C ; humidity, 55 ± 5%; light cycle, 08:00−20:00). Animal studies were carried out under the Guidelines for Animal Experiments at the University of Nagasaki (Nagasaki, Japan) and under Law No. 105 and Notification No. 6 of the Government of Japan.

Effect of Feeding TFs and TSA Fractions on Serum Glucose Level in Diabetic Mice. After five-week-old male KK-A^y mice were fed a commercial chow (type MF, Oriental Yeast Co., Ltd., Tokyo, Japan) for 2 weeks, they were divided into three groups of equal body weight and serum glucose level. A control diet was prepared according to AIN-76 compositions,[23] and the composition was as follows (in weight %): casein, 20; corn starch, 15; corn oil, 10; cellulose, 5; mineral mixture (AIN-76), 3.5; vitamin mixture (AIN-76), 1; DL-methionine, 0.3; choline bitartrate, 0.2; and sucrose to 100. TFs or TSA fraction was added at the level of 0.1% to the control diet at the expense of sucrose. Mice had free access to the diets and deionized water for 6 weeks. Food intake and body weight of each animal were recorded every 2 days. Glucose in serum obtained from the tail vein was enzymatically measured with a commercial kit (Glucose C−II Test Wako, Wako Pure Chemical Industries, Osaka, Japan) every 2 weeks after 6 h of fasting. At 6 wk, mice were sacrificed by withdrawing blood from the aorta under sodium pentobarbital anesthesia after 6 h of fasting, and the liver was excised. After separation of the serum, triacylglycerol concentration was enzymatically measured with a

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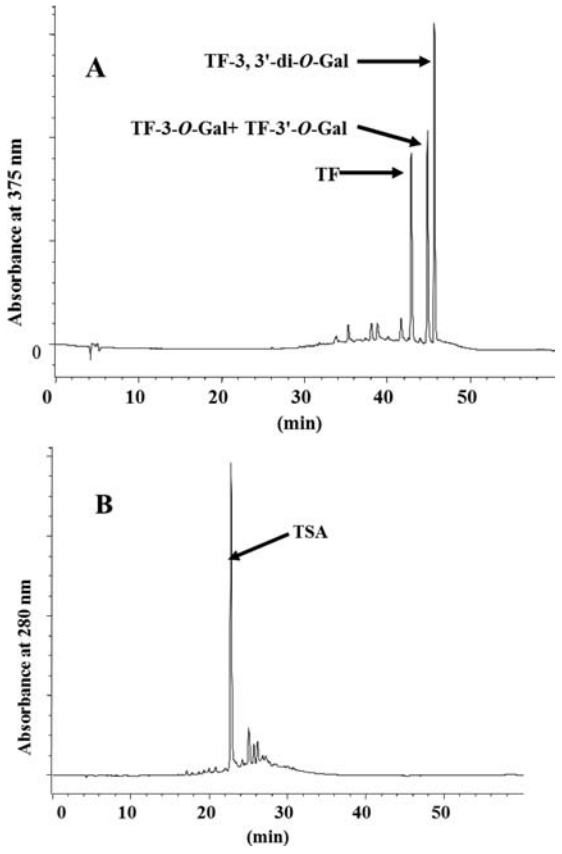

Figure 2. HPLC chromatograms of the fraction theaflavins and theasinensin A. (A) theaflavins fraction, (B) theasinensin A fraction.

commercial kit (Triglyceride E-Test Wako, Wako Pure Chemical Industries, Osaka, Japan). Approximately 0.5 g of liver was extracted with chloroform:methanol (2:1, v/v) according to the method of Folch et al.[24] Hepatic triacylglycerol concentration was measured enzymatically as described above.

Effect of Feeding TFs and TSA Fractions on Lipid Metabolism in SD Rats. Five-week-old male SD rats were fed a commercial pellet diet (CE-2, Clea Japan) for 2 weeks and were subsequently fasted overnight. After a blood sample was withdrawn from the tail vein of rats weighing about 200 g (as 0 time), the solution of TFs or TSA fraction (200 mg/5 mL 20% ethanol/kg body weight) was orally administered. As a control, 5 mL of 20% ethanol was orally administered to rats. At 5 min after TFs or TSA containing solution or ethanol administration, a 10% soybean oil emulsion (Intralipid 10%, Terumo, Co., Tokyo) was orally given at a dose of 15 mL/kg body weight. Blood was withdrawn from the tail vein at 1, 2, 3, 4, and 6 h after an emulsion administration. Serum triacylglycerol was enzymatically measured using a commercial kit as described above.

Four-week-old male SD rats weighing about 140 g were divided into three groups of equal body weight. The control diet was prepared according to AIN-76 composition[23] and contained (in weight %): casein, 20; corn starch, 15; corn oil, 1; lard, 24; cellulose, 5; mineral mixture (AIN-76), 3.5; vitamin mixture (AIN-76), 1; DL-methionine, 0.3; choline bitartrate, 0.2; and sucrose to 100. TFs or TSA fraction was added at the level of 0.2% to the control diet at the expense of sucrose. Rats had free access to the diets and deionized water for 4 weeks. Food consumption and body weight were recorded every other day. Feces were collected for 2 days before sacrifice. After rats were fasted for 6 h, blood was collected with decapitation, and the liver was immediately excised and weighed. The concentration of serum and hepatic triacylglycerol was enzymatically assayed using a commercial kit. A small aliquot of the excised liver was homogenized in 6 volumes of 0.25 M sucrose solution containing 1 mM EDTA in a 10 mM Tris-HCl buffer (pH 7.4). After sedimentation of the nuclei fraction, the supernatant was centrifuged at 100,000*g* for 60 min to precipitate microsomes, and the remaining supernatant was used as cytosol fraction. The mitochondrial and microsomal pellets were resuspended in the same 0.25 M sucrose solution. Activities of cytosolic fatty acid synthase (FAS),[25] glucose 6-phosphate dehydrogenase (G6PDH),[26] malic enzyme,[27] microsomal phosphatidic acid phosphohydrolase (PAP),[28] and mitochondrial carnitine palmitoyltransferase (CPT)[29] were determined. Protein was assayed by the method of Lowry et al.[30] using bovine serum albumin as a standard. The amount of fatty acid excreted into the feces was measured by the method of Jeejeebhoy et al.[31]

Statistical Analysis. Data were expressed as means ± SEM and were analyzed by ANOVA followed by the Tukey-Kramer test. Values were considered to be significantly different when the *p*-value was less than 0.05.

RESULTS

Antihyperglycemic Effects of Theaflavins and Theasinensin A Fractions in KK-A^y Mice. As shown in Table 1, there were no differences in body weight gain, food intake, and relative liver weight among the groups. Serum glucose level in KK-A^y mice fed the control diet consistently increased after the start of feeding the experimental diet to 6 weeks (Figure 3). On the other hand, feeding TFs and TSA fractions suppressed the increment in serum glucose on and after 4 weeks, and the level

Table 1. Effect of Feeding Theaflavins and Theasinensin A Fractions on Growth Parameters and Triacylglycerol Concentration in Serum and Liver of KK-A^y Mice[a]

	control	theaflavins	theasinensin A
Growth Parameters			
initial body weight (g)	33.5 ± 0.6	32.5 ± 0.6	33.4 ± 0.6
body weight gain (g)	10.6 ± 0.8	8.86 ± 0.93	10.0 ± 0.9
food intake (g/day)	5.50 ± 0.24	5.76 ± 0.29	5.41 ± 0.18
relative liver weight (g/100 g body weight)	5.25 ± 0.18	5.18 ± 0.21	4.99 ± 0.20
Triacylglycerol Concentration			
serum (mg/dL)	183 ± 20	281 ± 21	285 ± 22
liver (mg/g)	59.9 ± 5.3	52.3 ± 8.0	57.6 ± 6.8

[a]Data are means ± SEM for/mice. Mice were given the diet containing 0.1% theaflavins or theasinensin A fraction for 6 weeks.

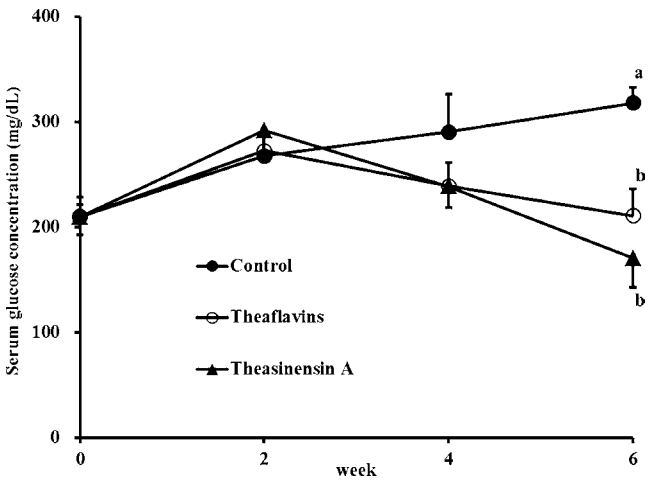

Figure 3. Effects of feeding theaflavins and theasinensin A fractions on serum glucose concentration in KK-A^y mice. Data are means ± SEM for seven mice. Letters a,b show a significant differences at $p < 0.05$.

in the TFs and TSA groups at 6 weeks was significantly lower than that in the control group. Serum triacylglycerol concentration tended to be higher in the TFs and TSA groups than in the control group at 6 weeks but did not show a significant difference (Table 1). Hepatic triacylglycerol level was comparable among the groups.

Hypotriacylglycerolemic Effects of Theaflavins and Theasinensin A Fractions in SD Rats. Figure 4 shows the

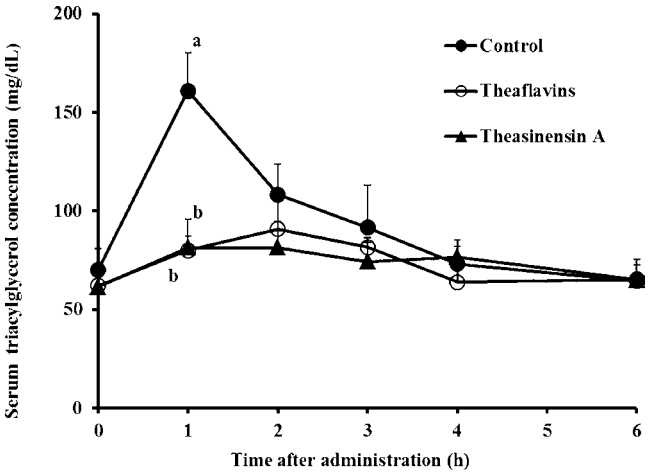

Figure 4. Effects of feeding theaflavins and theasinensin A fractions on serum triacylglycerol concentration after an oral administration of fat emulsion to SD rats. Data are shown as means ± SEM for 5−6 rats. Letters a,b show a significant difference at $p < 0.05$.

increment in serum triacylglycerol after an oral administration of vehicle, TFs, or TSA and fat emulsion. Serum triacylglycerol level in the control group increased, reached to the maximum level at 1 h, and gradually decreased afterward. The TFs and TSA groups slightly increased serum triacylglycerol. Serum triacylglycerol in the TFs and TSA groups was significantly lower than that in the control group at 1 h, but there was no significant difference between the TFs and TSA groups.

When TFs or TSA fraction was given to rats at the level of 0.2% for 4 weeks, no effect of the diets was found in body weight gain, food intake, and relative liver weight in rats (Table 2). The concentration of serum triacylglycerol did not reveal a significant difference among the groups. On the contrary, hepatic triacylglycerol level was significantly lower in rats fed the TSA diet than in rats fed the control diet, and that in the TFs group was intermediate between the control and TSA groups (Table 2). Weight of feces and fatty acid excretion into feces collected for 2 days before sacrifice are presented in Table 2. Fecal weight was similar among the groups. Fatty acid excretion was accelerated more in rats fed TFs and TSA diets than in those fed the control diet, and the difference between the control and TFs groups was statistically significant.

Table 3 shows activities of hepatic enzymes involved in lipid metabolism. The activities of FAS, G6PDH, and malic enzyme in the hepatic cytosol fraction exhibited no significant difference among the groups. The activity of PAP, an enzyme of triacylglycerol synthesis, in the hepatic microsome fraction of rats fed the TFs and TSA diets was significantly lowered than that in rats fed the control diet, and that in the TFs and TSA groups was approximately half the control group. No significant difference for the activity of CPT in hepatic mitochondria was seen among the groups.

Table 2. Effect of Feeding Theaflavins and Theasinensin A Fractions on Growth Pararneters,Triacylglycerol Concentration in Serum, Liver, and Dry Fecal Weight and Fatty Acid Excretion in SD Rats[a]

	control	theaflavins	theasinensin A
Growth Parameters			
initial body weight (g)	141 ± 4	142 ± 3	143 ± 3
body weight gain (g)	333 ± 11	352 ± 10	336 ± 7
food intake (g/day)	16.0 ± 0.7	16.7 ± 0.6	16.2 ± 0.7
relative liver weight (g/100 g body weight)	4.06 ± 0.08	4.06 ± 0.12	4.06 ± 0.06
Triacylglycerol Concentration			
serum (mg/dL)	124 ± 16	136 ± 30	128 ± 15
liver (mg/g)	121 ± 7a	101 ± 6ab	82.2 ± 5.7b
Fecal Excretion			
dry fecal weight (g/day)	1.69 ± 0.10	2.46 ± 0.46	1.79 ± 0.14
fatty acid excretion (mg/day)	74.2 ± 6.2a	123 ± 10b	107 ± 16ab

[a]Data are shown as means ± SEM for 6−7 rats. Rats were given the diet containing 0.2% theaflavins or theasinensin A fraction for 4 weeks. Letters show a significant difference at $p < 0.05$.

DISCUSSION

The increase in obesity, hypertriglyceridemia, hypercholesterolemia, fatty liver disease, and type 2 diabetes in advanced industrial countries has created interest in identifying natural products that are effective at reducing serum lipids and glucose. To our knowledge, this is the first report demonstrating in vivo the antihyperglycemic and hypotriacylglycerolemic actions of major black tea polyphenols enriched in TFs and TSA.

The antihyperglycemic benefits of natural products have been attributed to inhibition of α-glucosidase,[32] α-amylase activities,[33] and glucose transport.[34] Among these, α-glucosidase inhibition is a reasonable target. Daily intake of therapeutic α-glucosidase inhibitors, such as an acarbose, is effective in reducing hyperglycemia, consequently reducing the risk of developing diabetes.[35] The reduced serum glucose levels measured in the diabetic mice fed the diet supplemented with the partially purified TFs and TSA are consistent with the reported inhibition of amylase,[14] which would reduce systemic availability. On the basis of the in vitro findings of Matsui et al.,[14] the TF-3-*O*-Gal present in the TFs fraction was expected to provide greater inhibition of α-glucosidase activity than the TSA fraction. The lack of differences in antihyperglycemic responses between the two black tea polyphenol fractions used indicate TFs and TSA may provide similar in vivo inhibition of α-glucosidase activity. Importantly, this is the first long-term study showing a prolonged antihyperglycemic influence of tea polyphenols.

Higher levels of dietary fat are considered to contribute to the increased incidence of obesity.[36] Reducing the amount of fat absorbed by the small intestine is a target of interventions, as evidenced by the proliferation of "fat blockers" such as Orlistat. Our study indicated that TFs and TSA fractions from black tea provide a natural means to suppress postprandial hypertriacylglycerolemia. The inhibition of pancreatic lipase by TFs and TSA[14] is consistent with the higher fecal excretion of fatty acid of rats fed the TFs and TSA fractions. Hence, reduced lipid digestion and absorption are the most likely explanation for the hypotriacylglycerolemic influences of the black tea polyphenols used in the present study.

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Table 3. Effect of Feeding Theaflavins and Theasinensin A Fractions on Hepatic Enzyme Related to Lipid Metabolism in SD Rats[a]

	nmol/min/mg protein		
	control	theaflavins	theasinensin A
Lipogenic Enzyme			
cytosol			
fatty acid synthase	4.35 ± 0.46	4.04 ± 0.87	4.17 ± 0.44
glucose 6-phosphate dehydrogenase	19.6 ± 2.2	17.4 ± 2.7	17.0 ± 0.8
malic enzyme	29.0 ± 4.3	26.1 ± 3.1	25.0 ± 2.1
Microsome			
phosphatidic acid phosphohydrolase	9.12 ± 1.98a	4.23 ± 0.55b	4.01 ± 1.45b
Fatty Acid-Oxidizing Enzyme			
mitochondria			
carnitine palmitoyltransferase	3.78 ± 0.49	5.20 ± 1.35	3.88 ± 0.61

[a]Data are shown as means ± SEM for 6−7 rats. Rats were given the diet containing 0.2% theaflavins or theasinensin A fraction for 4 weeks. Letters show a significant difference at $p < 0.05$.

Feeding the KK-A^y mice diets containing 0.1% of the TFs or TSA fraction for 6 weeks did not reduce triacylglycerol concentration in serum and liver. This paradoxical response may reflect how this strain of mouse has impaired triglyceride metabolism[37] or the responses of mice fed the TFs and TSA fractions may differ from those of rats. Alternatively, the lower levels of polyphenols used in the diets fed to the mice may explain the different results. Specifically, Ohta et al.[38] reported that visceral fat and serum and hepatic triacylglycerol levels were reduced when obese rats were fed a diet containing 0.2% apple polyphenol. This is consistent with the present results for rats fed 0.2% TFs or TSA. Additional studies report a hypotriglycerolemic response when rats are fed diets with more than 0.2% of polyphenol.[39,40] It is possible an effect on lipid metabolism requires more than 0.1% of polyphenols be included in the diet.

The activities of FAS, G6PDH, and malic enzyme in the hepatic cytosolic fraction and CPT activity in hepatic mitochondrial fraction were similar among the groups. The activity of PAP, the rate limiting enzyme in triacylglycerol synthesis, was significantly lower in hepatic microsomes isolated from rats fed the diets with TFs and TSA compared with those from rats fed the control diet. Therefore, the reduction of serum and hepatic triacylglycerol concentration by TFs and TSA fractions may be at least in part due to lower hepatic lipid synthesis. This speculation is partially supported by in vitro finding[9] showing that TFs represses lipid accumulation in HepG2 cells. However, the inhibition of FAS protein level in the HepG2 cells[9,41] contrasts with the lack of hepatic cytosolic FAS inhibition of rats fed the diet with the TFs. Whether this reflects the use of different models (in vivo versus cultured cells) or species (rats versus human) is unknown. There is no clear explanation why PAP, but not FAS, was lower in rats fed the diet with TFs. More detailed studies are warranted to determine how black tea polyphenol fractions alter triacylglycerol metabolism via hepatic enzymes or substrate availability related to lipid metabolism.

The present study was not designed to elucidate the mechanisms of action for the antidiabetic and hypotriglcyidemic responses to TFs and TSA. However, the site of action for TFs and TSA is likely to be in the lumen of the intestine. Because TFs are characteristically produced as dimers of EGC and EC or their galloyl esters and TSA is produced as a dimer of ECG, these polymeric molecules have higher molecular weights than monomeric catechins, such as EGCG, which is characterized by a low absorption rate.[42] Hence, as previously reported by Mulder et al.,[43] TFs and TSA are not likely to be well absorbed in the small intestine. As a consequence, systemic levels of TFs and TSA may not be high enough to influence hepatic enzymes. Although not directly measured in the present study, it is likely the antihyperglycemic and hypotriaclyglycerolemic actions of TFs and TSA are mediated instead by inhibition of digestive enzymes such as lipase, α-glucosidase, and α-amylase[14,16] and the availability of substrates for metabolism. This may involve the binding of the polyphenols to the lipid bilayer, as proposed by Sirk et al.[44] Taken together, the beneficial actions of the TFs and TSA fractions are more likely to occur at the level of the small intestine than a direct influence on the liver.

The 12 and 59% purity of the TFs and TSA fractions, respectively, is a potential limitation of the present study. Yet, the TFs and TSA fractions increased fecal excretion of fatty acids and reduced the expected increase in serum lipids after oral administration of a fat emulsion. This corroborates the findings of Matsumoto et al.[11] that a black tea polyphenol fraction containing theaflavin (17% by weight) significantly lowers plasma triglyceride in rats fed a high fat/cholesterol diet. Importantly, our findings are in concordance with clinical data for the hypolipidemic response to a theaflavin-purified green tea extract (20% as TFs by weight).[13] Thus, the hypolipidemic and antihyperglycemic actions seen in rats may not require more costly higher levels of purities of TFs and TSA. This will need to be confirmed in clinical trials using human subjects.

In conclusion, our results provide novel data that TFs and TSA fractions possess an antihyperglycemic effect in diabetic mice, probably by suppressing glucose availability via inhibition of carbohydrate digestion and absorption. In addition, TFs and TSA fractions reduce fat digestion, thereby reducing serum and hepatic triglycerides. More detailed studies on the mechanisms underlying the health benefits of individual black tea polyphenols using purified TFs and TSA are warranted.

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS USED

ANOVA, analysis of variance; CPT, carnitine palmitoyltransferase; EC, (−)-epicatechin; ECG, (−)-epicatechin-3-gallate; EGC, (−)-epigallocatechin; EGCG, (−)-epigallocatechin-3-gallate; FAS, fatty acid synthase; G6PDH, glucose 6-phosphate dehydrogenase; HPLC, high performance liquid chromatography; iAGH, immobilized α-glucosidase; PAP, phosphatidic acid phosphohydrolase; SD, Sprague−Dawley; SEC, size-exclusion chromatography; SEM, standard error of the mean; TF, theaflavin; TF-3-*O*-Gal, theaflavin-3-*O*-gallate; TF-3′-*O*-Gal, theaflavin-3′-*O*-gallate; TF-3, 3′-di-*O*-Gal, theaflavin-3, 3′-di-*O*-gallate; TFs, theaflavins; TSs, theasinensins; TSA, theasinensin A

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