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# Hypotriglyceridemic Potential of Fermented Mixed Tea Made with Third-Crop Green Tea leaves and Camellia (*Camellia japonica*) Leaves in Sprague–Dawley Rats

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**ABSTRACT:** Fermented mixed tea made with third-crop green tea leaves and camellia leaves by a tea-rolling process has been developed. The objective of this study was to investigate hypotriglyceridemic potential of the mixed tea in rats. The mixed tea contained theasinensins and theaflavins. Rats fed the mixed tea extract at the level of 1% exerted significantly lower body weight and adipose tissue weight compared to animals fed third-crop green tea or camellia tea extract alone for 4 weeks. Serum and hepatic triglyceride was significantly and dose-dependently decreased by the mixed tea. This decrease was associated with lowered lipogenic enzyme activities in the liver. Furthermore, an oral administration of 4 or 8% of the mixed tea extract followed by fat emulsion suppressed the increment of serum triglyceride level. These results suggest that the mixed tea has hypotriglyceridemic action, partially via delaying triglyceride absorption in the small intestine and repressing hepatic lipogenic enzymes.

**KEYWORDS:** *camellia leaves, theaflavins, theasinensins, green tea leaves, hypotriglyceridemic effect*

## INTRODUCTION

Tea has long been enjoyed worldwide, and green tea is the most favorable drink in Japan. Growing epidemiological data have implied that green tea consumption is negatively related to risks of coronary artery diseases, hypertension, prostate cancer, and breast cancer.[1−3] Green tea is rich in various polyphenols, among which are mainly catechins and tannins.[4] It is also known that during processing and extracting, heat epimerization produces catechin derivatives such as (−)-epigallocatechin-3-gallate (EGCG), (−)-epigallocatechin (EGC), and (−)-epicatechin-3-gallate (ECG).[5] These tea catechins and their derivatives have been reported to have antioxidant, anticancer, antiglycemic, and antilipidemic properties.[4] On the other hand, black tea is processed by polyphenol oxidase fermentation, decreasing 60−70% of catechins during processing.[6] Black tea manufacture has a process of fermentation which converts tea flavanols to their polymeric polyphenols, generating black tea polyphenols such as theaflavins, theasinensins, and thearubigins. Lin et al.[7] have reported using HepG2 cells that theaflavins alter lipid metabolism via suppressing fatty acid synthesis and stimulating fatty acid oxidation. The other study showed theaflavin-induced lipase inhibition.[8] Shii et al.[9] have reported that tea-rolling of green tea and loquat leaves oxidizes catechins in green tea, producing theaflavins and theasinensins. Tea leaves are harvested several times a year. After the first harvest, green tea gradually loses its commercial value because of decreasing an amount of theanine and adding bitterness but increasing catechin content. Camellia (*Camellia japonica*) is an evergreen tree grown nationwide in Japan, and camellia seeds have been squeezed as edible oil or hair dressing oil. Camellia leaves have been traditionally used as a tea and hemostatic. We have developed mixed tea made with third-crop green tea leaves and camellia leaves by tea-rolling. The mixed tea is a fermented tea which has a lesser amount of catechins and a relatively larger amount of black tea polyphenols such as theaflavins as compared to an ordinary green tea. However, no nutritional and functional characteristic of the mixed tea has been thus far known in comparison with ordinary tea, green tea, and camellia tea.

Thus, in this study, we tried to test how the mixed tea made with third-crop green tea leaves and camellia leaves affects lipid metabolism in rats in comparison with green tea or camellia tea alone. The study here shows that the mixed tea possesses hypotriglyceridemic potential and that this hypotriglyceridemic action may be in part due to the luminal events critical to fat absorption in the small intestine and decreased lipogenesis in the liver.

## MATERIALS AND METHODS

**Preparation of Tea.** Green tea leaves and camellia leaves were provided by the Nagasaki Prefectural Agricultural and Forestry Experiment Station, Higashisonogi Tea Branch (Nagasaki, Japan). Fresh green tea leaves (27 kg) were partially dried by blowing air (70

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Table 1. Chemical Composition of Green Tea, Black Tea and the Mix Tea (mg/g Dried Leaf)[a]

| | green tea | black tea | mix tea |
|---|---|---|---|
| gallic acid | 0.14 ± 0.01 | 2.73 ± 0.02 | 1.81 ± 0.01 |
| theogallin | 1.06 ± 0.02 | 3.59 ± 0.02 | 1.01 ± 0.02 |
| theobromine | 0.91 ± 0.02 | 1.58 ± 0.06 | 0.82 ± 0.01 |
| gallocatechin | 4.48 ± 0.21 | 2.38 ± 0.05 | 3.95 ± 0.04 |
| theasinensin B | ND[b] | 4.94 ± 0.04 | 6.98 ± 0.68 |
| epigallocatechin | 49.8 ± 0.5 | 11.6 ± 1.0 | 4.55 ± 0.05 |
| catechin | 1.91 ± 0.11 | 1.42 ± 0.23 | ND |
| caffeine | 27.7 ± 0.3 | 36.6 ± 0.2 | 25.4 ± 0.4 |
| theasinensin A | ND | 9.24 ± 0.49 | 5.01 ± 0.06 |
| epicatechin | 11.9 ± 0.8 | 4.74 ± 0.57 | 3.71 ± 1.06 |
| epigallocatechin-3-*O*-gallate | 76.9 ± 0.9 | 43.4 ± 0.9 | 16.9 ± 0.2 |
| gallocatechin-3-*O*-gallate | 4.35 ± 0.09 | 3.32 ± 0.67 | 1.13 ± 0.01 |
| epicatechin-3-*O*-gallate | 16.7 ± 0.2 | 18.8 ± 0.7 | 7.59 ± 0.12 |
| polymeric polyphenol | 8.97 ± 0.08 | 45.8 ± 3.9 | 57.2 ± 6.2 |
| total theaflavin | ND | 3.52 ± 0.12 | 4.77 ± 0.07 |

[a]Values are mean ± SEM ($n$ = 4). [b]ND: not detected.

°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. The leaves were then mixed with fresh camellia tea leaves (3 kg) and kneaded with a tea roller (60k type, Kawasaki Co., Ltd., Shimada, Japan) at room temperature for 20 min. Finally, the leaves were heated at 110 °C in a tea dryer (120k type, Kawasaki Co., Ltd., Shimada, Japan) for 30 min to terminate enzymatic oxidation. The water content of the final tea leaves was less than 5%. To prepare green tea and camellia tea, we steamed third-crop green tea leaves and camellia leaves and kneaded by blowing hot air at room temperature. The water content of the final green tea leaves or camellia leaves was less than 5%. Green tea leaves and camellia tea leaves were treated at 100 °C to inhibit oxidation, thus not being called a fermented tea. The mixed tea leaves, green tea leaves, and camellia tea leaves were extracted with 50 volumes of hot water. The extract was lyophilized, powdered, and incorporated into experimental diets as described below.

**Extraction and Analysis of Catechins and Polyphenols.** Separation of catechins and polyphenols was carried out as described elsewhere.[9] Briefly, a portion of tea products (0.5 g) was extracted with 60% ethanol containing 0.1% of trifluoroacetic acid (15 mL) at room temperature in a shaking incubator for 2 days. After filtration, an aliquot (50 μL) was analyzed by HPLC. The HPLC was performed on a Cosmosil 5C18-ARII (Nacalai Tesque, Kyoto, Japan) column (250 mm × 4.6 mm i.d., 5 μm) with gradient elution from 4 to 30% (39 min) and from 30 to 75% (15 min) $CH_3CN$ in 50 mM $H_3PO_4$; flow rate, 0.8 mL/min; temperature, 35 °C; detection, JASCO photodiode array detector MD-2010 (JASCO, Tokyo, Japan). For a comparison of contents of catechins and black tea polyphenols, black tea, a fermented tea, was similarly extracted.

**Animals.** Four-week-old Sprague−Dawley (SD) rats were purchased from Japan SLC (Shizuoka, Japan), kept individually in stainless steel cages under a controlled atmosphere (temperature, 22 ± 1 °C; humidity, 55 ± 5%; light cycle, 08:00−20:00) and fed a commercial pellet diet (CE-2, Clea Japan, Tokyo) for a week. Animal studies were carried out under the Guidelines for Animal Experiments of University of Nagasaki (Nagasaki, Japan), and under Law No. 105 and Notification No. 6 of the Government of Japan.

**Effect of Different Tea on Lipid Metabolism (Experiment I).** To see if the mixed tea has potential in reducing blood lipids in comparison with third-crop green tea or camellia tea alone, we assigned rats for the following four groups of 6−7 animals: (1) a control group (C) that was given a modified AIN-76 diet with sucrose as a major source of carbohydrate, (2) a green tea group (G) that was given the C diet but containing 1% third-crop green tea extract, (3) a camellia tea group (CA) that was given the C diet but containing 1% camellia tea extract, and (4) a mixed tea group (M) that was given the C diet but containing 1% mixed tea extract. Rats had free access to the diets and deionized water for 4 weeks. Food intake and body weight of each animal were recorded every 2 days. Animals were sacrificed by decapitation after 6 h fasting at 4 weeks. Blood was withdrawn and was centrifuged at 3000 rpm for 20 min after clotting. The liver was excised, frozen immediately in liquid nitrogen, and stored at −80 °C before analysis. Serum and hepatic lipid concentrations were measured. Hepatic enzymes involved in lipid metabolism were also determined.

**Effect of Different Dose of the Mixed Tea on Lipid Metabolism (Experiment II).** To see if the mixed tea alters lipid metabolism in a dose-dependent manner, animals were assigned for the following three groups of 6−7 rats: (1) a control group (C) that was given a modified AIN-76 diet with sucrose as a major source of carbohydrate, (2) a mixed tea group that was given the C diet but containing 0.5% mix tea extract, and (3) a mixed tea group that was given the C diet but containing 1% mixed tea extract. Animals were fed each diet for 4 weeks and sacrificed by decapitation after 6 h fasting. Analyses were carried out as outlined in experiment I.

**Oral Fat Tolerance Test (Experiment III).** To determine whether the mixed tea affects triglyceride absorption from the small intestine, we carried out an oral fat tolerance test as described below. For the preparation of the mixed tea extract for an oral fat tolerance test, the dried mixed tea (1.5 kg) were crushed in a Waring blender (Torrington, CT) and extracted with acetone−$H_2O$ (6:4, v/v, 18 L) at room temperature three times. The extract was concentrated under reduced pressure and then used. The mixed tea extract was lyophilized and dissolved in 0.2% CMC solution. As a control, 0.2% of CMC solution without the mixed tea extract was prepared. Then 4 or 8% of the mixed tea extract (200 or 400 mg/kg body weight) were orally administered to rats fasted overnight. Intralipid containing 10% soybean oil (Terumo, Tokyo, Japan) was used as a fat emulsion, and 15 mL/kg of body weight was administered 5 min after a single administration of the mixed tea extract or a vehicle. Blood was taken before and 1, 2, 3, 4, and 6 h after an administration of fat emulsion. Triglyceride was measured enzymatically as described below.

**Serum and Tissue Biochemical Analyses.** Serum lipids were assayed enzymatically using a commercial kit (Cholesterol E-Test, Triglyceride E-Test, Phospholipid C-Test, and NEFA C-Test, Wako Pure Chemical Industries, Osaka, Japan). Serum glucose was also analyzed enzymatically (Glucose CII test, Wako Pure Chemical Industries, Osaka, Japan). Serum insulin was measured by ELIZA assay (Hi range Speedy, Morinaga Institute of Biological Science, Yokohama, Japan). Liver lipids were extracted by the method of Folch et al.,[10] and concentrations of cholesterol, phospholipid, and triglyceride were then measured enzymatically as described above.

Table 2. Effects of the Diets on Growth and Tissue Weights (Experiment 1)[a]

| group | control | green tea | camellia tea | mix tea |
|---|---|---|---|---|
| Body Weight | | | | |
| final (g) | 407 ± 15b | 404 ± 7b | 412 ± 14b | 359 ± 7a |
| food efficiency (g gain/g intake) | 0.36 ± 0.01b | 0.34 ± 0.01b | 0.35 ± 0.01b | 0.30 ± 0.01a |
| relative liver weight (g/100 g/body weight) | 4.70 ± 0.20b | 4.48 ± 0.07b | 4.41 ± 0.16b | 3.35 ± 0.11a |
| Relative White Adipose Tissue Weight (g/100 g/Body Weight) | | | | |
| perirenal | 1.96 ± 0.11b | 1.38 ± 0.17a | 2.03 ± 0.19b | 0.95 ± 0.10a |
| epididymal | 1.37 ± 0.08ab | 1.23 ± 0.08ab | 1.49 ± 0.08b | 1.11 ± 0.06a |
| mesenteric | 1.23 ± 0.05b | 1.06 ± 0.03ab | 1.30 ± 0.14b | 0.81 ± 0.11a |
| total | 4.56 ± 0.12bc | 3.67 ± 0.23ab | 4.82 ± 0.35c | 2.87 ± 0.25a |
| relative brown adipose tissue weight (g/100 g/body weight) | 0.15 ± 0.01b | 0.14 ± 0.01ab | 0.17 ± 0.01b | 0.10 ± 0.02a |

[a]Values are means ± SEM for 6−7 rats per group. Letters a−c show significant difference at $p < 0.05$.

Table 3. Effctcts of the Diets on Serum and Hepatic Biochemical Parameters[a]

| group | control | green tea | camellia tea | mix tea |
|---|---|---|---|---|
| Serum | | | | |
| triglyceride (mg/dL) | 215 ± 19b | 202 ± 35b | 249 ± 33b | 53.3 ± 6.5a |
| phospholipid (mg/dL) | 228 ± 10b | 209 ± 9ab | 191 ± 15ab | 168 ± 17a |
| cholesterol (mg/dL) | 102 ± 7 | 92.3 ± 5.3 | 78.2 ± 8.1 | 85.6 ± 5.8 |
| nonesterifed fatty acid (mEq/L) | 0.67 ± 0.06 | 0.60 ± 0.05 | 0.57 ± 0.02 | 0.66 ± 0.11 |
| glucose (mg/dL) | 121 ± 7 | 136 ± 4 | 124 ± 3 | 115 ± 4 |
| insulin (mg/mL) | 2.12 ± 0.39a | 4.03 ± 0.33b | 5.20 ± 0.74b | 1.60 ± 0.10a |
| Hepatic | | | | |
| triglyceride (mg/g) | 46.6 ± 6.2 | 41.0 ± 5.6 | 37.7 ± 1.6 | 27.2 ± 6.2 |
| phospholipid (mg/g) | 17.0 ± 0.8b | 16.2 ± 0.8b | 17.3 ± 0.7b | 25.3 ± 3.0a |
| cholesterol (mg/g) | 6.57 ± 0.64ab | 6.54 ± 0.53ab | 4.96 ± 0.57a | 7.66 ± 0.86b |

[a]Values are mean ± SEM for 6−7 rats per group. Letters a,b show significant difference at $p < 0.05$.

Table 4. Effects of the Diets on Hepatic Enzymes[a]

| | | (nmol/min/mg protein) | | |
|---|---|---|---|---|
| group | control | green tea | camellia tea | mix tea |
| fatty acid synthase | 50.1 ± 9.1b | 35.7 ± 2.1ab | 29.8 ± 2.1a | 19.9 ± 1.9a |
| malic enzyme | 54.2 ± 5.0b | 48.3 ± 3.8b | 41.4 ± 3.5ab | 31.0 ± 3.4a |
| glucose 6-phosphate dehydrogenase | 21.9 ± 2.3 | 21.7 ± 1.0 | 21.8 ± 1.8 | 18.2 ± 0.7 |
| Phosphatidate Phosphohydrolase | | | | |
| microsome $Mg^{2+}$ dependent | 6.61 ± 0.46b | 4.84 ± 0.69b | 1.52 ± 1.37a | 1.11 ± 0.38a |
| Carnitine Palmitoyltraseferase | | | | |
| liver | 2.31 ± 0.39 | 2.66 ± 0.33 | 3.01 ± 0.50 | 2.25 ± 0.24 |
| brown adipose tissue | 2.08 ± 0.63 | 1.23 ± 0.48 | 2.74 ± 0.50 | 1.46 ± 0.56 |

[a]Values are mean ± SEM for 6−7 rats per group. Letters a,b show significant difference at $p < 0.05$.

**Hepatic Enzyme Activity.** The liver was excised, frozen immediately by liquid nitrogen, and stored at −80 °C before analysis. The liver was homogenized in 6 volumes of a 0.25 M of sucrose solution containing 1 mM of EDTA in a 10 mL of Tris-HCl buffer (pH 7.4) to prepare hepatic subcellular fractions. Activities of cytosol fatty acid synthase (FAS)[11] and glucose 6-phosphate dehydrogenase (G6PDH)[12] and malic enzyme (ME)[13] were measured. Microsomal phosphatidic acid phosphohydrolase (PAP)[14] and mitochondrial carnitine palmitoyltransferase (CPT)[15] were also determined. Brown adipose tissue was homogenized, and CPT activity was measured. Protein was assayed by the method of Lowry et al.[16]

**Fecal Lipid Analyses.** In experiments I and II, feces were collected for 2 days before sacrifice, lyophilized, and ground. A small aliquot of feces was extracted by the method of Ikeda et al.[17] and measured for lipid excretion with a gas chromatography. Neutral and acidic sterols were measured by 5α-cholestane and 23-nordeoxycholic acid as an internal standard, respectively. The excretion of fatty acid into feces was determined by a titration method.[18]

**Statistics.** All values are expressed as mean ± SEM. Statistical analyses were performed by one-way ANOVA, followed by the Tukey−Kramer test. Values were considered to be significantly different when the $p$-value was less than 0.05.

## RESULTS

**Analysis of Polyphenols.** Table 1 shows a comparison of catechin and polymeric polyphenol contents. Green tea consisted of mainly EGC and EGCG. The level of EGCG was highest in green tea and lowest in the mixed tea, and that in black tea was in between. On the contrary, during the tea-rolling process, EGCG and EGC dramatically decreased while polymeric polyphenol content considerably increased in the mixed tea. The level of total theaflavins in the mixed tea was greater than that in black tea (4.77 ± 0.07 vs 3.52 ± 0.12 mg/g dried leaf). No difference was found in caffeine content among tea preparations. Because no established method of analyzing individual polyphenol in camellia tea was available, only total polyphenol content was measured. The level of it was 42.9 ± 4.07 (mg/g leaf).


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**Effect of Different Tea on Lipid Metabolism (Experiment I).** As shown in Table 2, the M group was significantly lower in final body weight and food efficiency compared to the C, G, and CA groups. Perirenal and mesenteric adipose tissues and total white adipose tissue (g/100 g BW) were significantly smaller in the M group than those in the C group. Similarly, the mixed tea gave smaller brown adipose tissue than the control group did.

Table 3 shows that compared to the control group, the M group significantly reduced serum triglyceride while the G and CA groups did not. The similar trend was also observed on phospholipid; the M group had lower phospholipid than the C group did. However, no difference for serum cholesterol was found among the groups. The M group tended to lower hepatic triglyceride but did not reach a significant difference against the C group (Table 3). The mixed tea significantly increased phospholipid in the liver compared to the other three groups. Hepatic cholesterol in the M group tended to be higher, and was significantly higher than the CA group.

Activities of enzymes involved in lipid metabolism in the liver are depicted in Table 4. FAS activity tended to be lower when tea was added to diets, and the M group exhibited the lowest FAS activity among the groups. The similar trend was observed on malic enzyme; the CA and M groups decreased the activity, but only the M group showed a significant difference against the C group. PAP was also significantly lowered in the CA and M groups when compared to the C and G group. However, the diets did not show a significant change in a lipogenic enzyme, G6PDH. The activity of CPT, the rate-limiting enzyme of fatty acid oxidation, was not altered by the diets.

As shown in Table 5, adding tea to the diets did not influence fecal weight in any groups. No alteration to fecal lipid excretion was found among the groups.

**Table 5. Fecal Weight and Fecal Lipids[a]**

| group | control | green tea | camellia tea | mix tea |
|---|---|---|---|---|
| fecal weight (g/day) | 2.30 ± 0.15 | 2.53 ± 0.19 | 2.47 ± 0.22 | 3.53 ± 0.45 |
| fatty acid excretion (mg/day) | 59.6 ± 7.4 | 59.1 ± 5.1 | 45.7 ± 8.3 | 40.1 ± 2.5 |
| neutral steroids (mg/day) | 9.31 ± 1.11 | 13.0 ± 1.48 | 10.0 ± 1.12 | 9.37 ± 1.02 |
| acidic steroids (mg/day) | 2.26 ± 0.13 | 3.13 ± 0.21 | 3.19 ± 0.46 | 3.53 ± 0.45 |
| total steroids (mg/day) | 11.6 ± 1.1 | 16.1 ± 1.6 | 13.2 ± 1.0 | 12.9 ± 1.2 |

[a]Values are mean ± SEM for 6−7 rats per group.

**Effect of Different Dose of the Mixed Tea on Lipid Metabolism (Experiment II).** There was no effect of tea on final body weight and food efficiency (Table 6). Adding tea to the diets tended to decrease liver weight (g/100 g BW). There was a dose-dependent decrease in adipose tissue weight; the 1.0% mixed tea diets significantly lowered perirenal and total adipose tissue weight compared to the control group. No difference was seen for brown adipose tissue weight among the groups.

Table 7 shows that serum triglyceride was dose-dependently decreased when the diets were added the mixed tea, and the 1.0% mixed tea diets exhibited a significant difference as compared to the control diet. On the contrary, irrespective of the level of the mixed tea, no difference in serum total cholesterol, free fatty acid and phospholipid was detected among the groups. The mixed tea dose-dependently reduced hepatic triglyceride and total cholesterol, and the difference between the control and 1.0% mixed tea-fed rats was statistically significant. The mixed tea did not change the level of phospholipid.

**Table 6. Effects of the Diets on Growth and Tissue Weights (Experiment II)[a]**

| group | control | 0.5% mix tea | 1.0% mix tea |
|---|---|---|---|
| Body Weight | | | |
| final (g) | 436 ± 4 | 443 ± 17 | 409 ± 13 |
| food efficiency (g gain/g intake) | 0.37 ± 0.010 | 0.36 ± 0.01 | 0.35 ± 0.01 |
| relative liver weight (g/100 g/body weight) | 3.90 ± 0.06 | 3.62 ± 0.19 | 3.50 ± 0.16 |
| Relative White Adipose Tissue Weight (g/100 g/body weight) | | | |
| perirenal | 2.80 ± 0.29b | 2.16 ± 0.26ab | 1.62 ± 0.17a |
| epididymal | 1.85 ± 0.09 | 1.67 ± 0.15 | 1.50 ± 0.12 |
| mesenteric | 1.46 ± 0.12 | 1.24 ± 0.14 | 1.04 ± 0.09 |
| total | 6.11 ± 0.46b | 5.08 ± 0.49ab | 4.16 ± 0.35a |
| relative brown adipose tissue weight (g/100 g/body weight) | 0.18 ± 0.01 | 0.17 ± 0.02 | 0.17 ± 0.02 |

[a]Values are mean ± SEM for 6−7 rats per group. Letters a,b show significant difference at $p < 0.05$.

**Table 7. Effects of the Diets on Serum and Hepatic Biochemical Parameters[a]**

| group | control | 0.5% mix tea | 1.0% mix tea |
|---|---|---|---|
| Serum | | | |
| triglyceride (mg/dL) | 148 ± 21b | 113 ± 39ab | 86.6 ± 8.1a |
| phospholipid (mg/dL) | 150 ± 18 | 143 ± 11 | 134 ± 5 |
| cholesterol (mg/dL) | 105 ± 7 | 106 ± 11 | 96.6 ± 5.4 |
| nonesterifed fatty acid (mEq/L) | 1.06 ± 0.15 | 1.08 ± 0.06 | 1.14 ± 0.11 |
| glucose (mg/dL) | 171 ± 14 | 167 ± 15 | 166 ± 12 |
| insulin (mg/mL) | 0.867 ± 0.094 | 0.678 ± 0.106 | 0.569 ± 0.074 |
| Hepatic | | | |
| triglyceride (mg/g) | 39.1 ± 4.9b | 27.1 ± 3.2ab | 19.4 ± 2.0a |
| phospholipid (mg/g) | 18.1 ± 0.6 | 18.5 ± 0.6 | 18.5 ± 0.4 |
| cholesterol (mg/g) | 36.6 ± 2.8b | 32.4 ± 1.6ab | 29.3 ± 1.2a |

[a]Values are mean ± SEM for 6−7 rats per group. Letters a,b show significant difference at $p < 0.05$.

There was a trend toward decreasing lipogenic enzymes such as FAS, but the difference was not significant (Table 8). No effect of diets on fecal weight and fecal lipids was seen (data not shown).

**Oral Fat Tolerance Test (Experiment III).** The mixed tea extract was orally administered to rat followed by fat emulsion. The concentration of serum triglyceride was measured periodically as illustrated in Figure 1a. When rats were given 0.2% CMC as a control, serum triglyceride rapidly increased, reached its peak at 2 h, and then gradually decreased. Serum triglyceride in 4% mixed tea-administered rats gradually increased for the first 2 h, but the increment was suppressed. The 8% mixed tea suppressed the increase in triglyceride


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**Table 8. Effects of the Diets on Hepatic Enzymes**[a]

| | (nmol/min/mg protein) | | |
|---|---|---|---|
| group | control | 0.5% mix tea | 1.0% mix tea |
| fatty acid synthase | 13.2 ± 1.9 | 7.8 ± 1.9 | 8.0 ± 2.1 |
| malic enzyme | 34.2 ± 2.9 | 30.7 ± 3.0 | 30.0 ± 3.9 |
| glucose 6-phosphate dehydrogenase | 47.1 ± 6.2 | 39.9 ± 3.1 | 42.1 ± 10.1 |
| Phosphatidate Phosphohydrolase | | | |
| microsome $Mg^{2+}$ dependent | 6.57 ± 1.96 | 3.76 ± 0.80 | 1.92 ± 0.39 |
| Carnitine Palmitoyltraseferase | | | |
| liver | 2.31 ± 0.39 | 2.66 ± 0.33 | 3.01 ± 0.50 |
| brown adipose tissue | 2.60 ± 0.80 | 3.60 ± 0.80 | 3.60 ± 1.00 |

[a]Values are mean ± SEM for 6−7 rats per group.

further, and the difference between the 8% group and the control group at 1, 2, and 6 h was statistically significant. As a result, as shown in Figure 1b, area under the curve (AUC) of triglyceride decreased in a dose-dependent manner and the difference reached significant between the control and the 8% mixed tea groups.

## DISCUSSION

Emerging evidence suggests that foods rich in polyphenols may have beneficial effects on lipid and glucose metabolism in humans.[2] We show here that the mixed tea made with third-crop green tea leaves and camellia leaves may have potential to control lipid metabolism and adiposity. Green tea has been used in Asian countries for centuries, and data has been accumulating that green tea can work as antioxidant, anticarcinogenic, and antiinflammatory properties, thus reducing risks of cardiovascular diseases, diabetes, and various types of cancer.[1,3,19,20] The mixed tea we have developed contains black tea polyphenols such as theaflavins and theasinensins in addition to catechins (Table 1). Catechins are a major component in green tea, with EGCG being the most pharmacologically active, and have been reported to inhibit lipase activity,[8,21,22] decrease micelle formation,[23] and enhance low-density lipoprotein (LDL) receptor activity,[24,25] thus resulting in hypolipidemic action. Therefore, we tested to see how the mixed tea extract affects triglyceride absorption. A fat tolerance test showed that a single oral administration of the mixed tea extract resulted in a significant decreased triglyceride in blood as compared to the vehicle (Figure 1a), indicating that hypotriglyceridemic effects of the mixed tea is, in part, attributed to the inhibited triglyceride absorption in the intestine. Studies have shown that micelle formation may be impaired in the presence of green tea extract, especially EGCG, theaflavins, and theasinensins,[8,26,27] thus resulting in enhanced fecal excretion of lipids.[28] However, this was not the case in our study; the mixed tea did not change fecal excretion of lipids (Table 5). The reason may be that the dose of green tea extract and diets we used (low-fat, cholesterol-free) were different from those by Chan et al.,[28] showing that jasmine green tea extract (5.7 g/kg diet) did increase fecal excretion of lipid in high-fat-fed hamsters. As shown by Ikeda et al.,[29] catechins might simply have delayed absorption of lipid but might not have inhibited lipid absorption or enhanced lipid excretion into feces even though in vitro studies have shown antilipase activity by catechins.[8,26,27] It is known that the luminal content of catechins and black tea polyphenols was high and that the bioavailability of those compounds was low when green tea extracts were orally administered to rats.[30] Thus, an interaction between catechins and dietary fat in the lumen may slow down an incorporation of fat into the circulation. This may be supported by the report showing that EGCG and its polymeric form of catechins such as theaflavins are responsible for the changes in the physiological characteristics of fat emulsification,[26] hence expecting a delayed transfer of fat into circulation and lowered accumulation of fat in adipose tissues. In fact, the mixed tea significantly reduced relative white adipose tissue weight in experiment I (perirenal and mesenteric and total white adipose tissue) as compared to the control diet (Table 2). In experiment II, visceral fat deposition (mesenteric and perirenal adipose tissues) was dose-dependently lower in the mixed tea group compared to the control group (Table 6). These results imply that the mixed tea may be potential as hypotriglyceridemic and antiobese therapy.

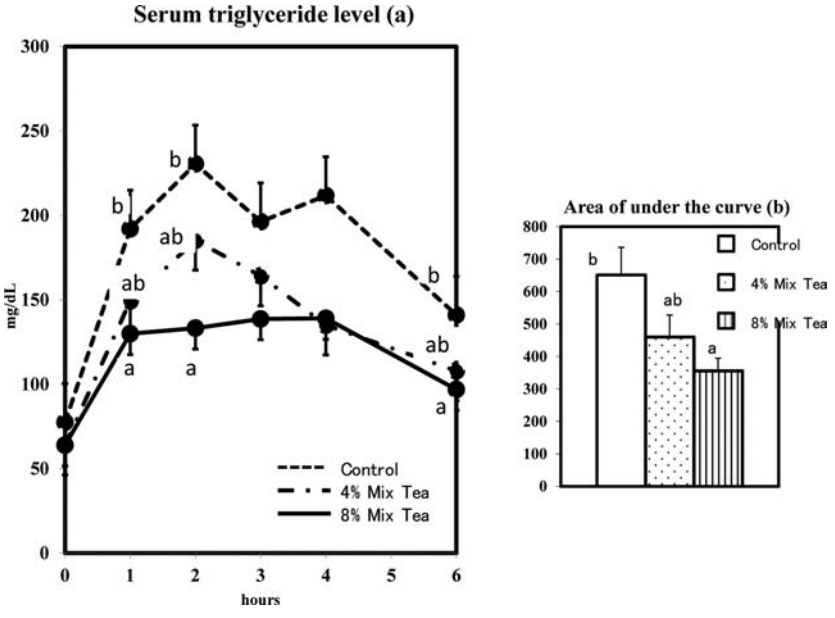


**Figure 1.** Effects of mix tea on serum triglyceride levels. Values are mean ± SEM ($n$ = 8−11) . Letters a,b show significant difference at $p < 0.05$.


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Because the effects of catechins and black tea polyphenols on hepatic enzymes have been little known,[31,32] we measured the effects of the mixed tea on the activity of hepatic enzymes associated with lipid metabolism. As a result, lower FAS activity was seen in the mixed tea group than in the control group. Other lipogenic enzymes such as malic enzyme and PAP were also decreased by the mixed tea. In contrast, CPT, the rate-limiting enzyme of fatty acid oxidation, was not altered by the treatment with tea. EGCG and theaflavins inhibited activities of $\alpha$-amylase[33] and $\alpha$-glucosidase,[34] thus altering glucose absorption. Although the difference of insulin in this study was not significant, insulin tended to be decreased dose-dependently in the mixed tea group. Because insulin is a modulator of lipid synthesis via sterol regulatory element binding protein-1c (SREBP-1c),[35] decreased levels of insulin might have affected hepatic triglyceride synthesis in the mixed tea group. However, we did not measure activities of SREBP-1c and its target genes in this study. The underlying mechanism by which the mixed tea affects hepatic lipogenic enzymes remains elusive.

In regard to cholesterol metabolism, meta-analysis has shown that green tea consumption significantly reduces total and LDL-cholesterol, irrespective of the type of intervention, doses of green tea catechins, and study duration.[36] Studies have suggested that green tea extract affects a lymphatic absorption of cholesterol.[37,38] In studies using black tea polyphenols, hypocholesterolemic activity was observed in high fat-cholesterol-fed rats.[39] On the other hand, our study did not clearly show effects of the mixed tea on cholesterol metabolism. This is probably because the diet was cholesterol-free. Given that green tea modifies micelle formation,[23] cholesterol synthesis,[40] and LDL receptor activity,[24,25] further studies of the effects of the mixed tea on cholesterol metabolism are needed.

The question has arisen as to what component(s) in the mix tea is responsible for hypotriglyceridemic action. In contrast to green tea, the mixed tea contained black tea polyphenols such as theaflavins and theasinensins as shown in Table 1. These black tea polyphenols are produced by oxidizing tea leaves during the tea-rolling process.[9] Compared to catechins such as EGCG, few studies have explored functions of black tea polyphenols in vivo.[7,39] Theaflavins were effective in inhibiting lipase activity in vitro.[8] Lin et al.[7] have shown that theaflavins diminish lipid accumulation in HepG2 cells and in high-fat-fed rats by repressing lipid synthesis and stimulating fatty acid oxidation. Black tea polyphenols containing theaflavins reduced hepatic triglyceride in cholesterol-fed rats.[39] Furthermore, another study has reported that the dimeric compounds of the flavan-3-ol such as theaflavins and theasinensin account for lipase inhibition more than EGCG and (−)-gallocatechin-3-*O*-gallate (GCG).[8] In addition, theaflavin-enriched green tea extract has been reported to be effective in lowering cholesterol and triglyceride in subjects with mild to moderate hypercholesterolemia although the underlying mechanism was not shown.[41] Collectively, these findings suggest that polymeric forms of catechins such as theaflavins and theasinensins may be responsible for the mixed tea-induced hypotriglyceridemic action. Again, detailed studies using purified theaflavins and other black tea polyphenols are warranted.

In conclusion, the mixed tea made with third-crop green tea leaves and camellia leaves possesses hypotriglyceridemic potential in part via delayed lipid absorption in the small intestine and via repressed hepatic lipogenesis. This study implies that in addition to traditional usage of camellia seeds as edible oil or hair dressing oil, camellia provides health benefits as tea. Additional studies are needed to elucidate the precise mechanism underlying the effects of the mixed tea and to identify compounds responsible for hypotriglyceridemic and antiobesity actions.

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### Notes

The authors declare no competing financial interest.


## ABBREVIATIONS USED

ANOVA, analysis of variance; BW, body weight; CPT, carnitine palmitoyltransferase; ECG, (−)-epicatechin-3-gallate; EGC, (−)-epigallocatechin; EGCG, (−)-epigallocatechin-3-gallate; GCG, (−)-gallocatechin-3-*O*-gallate; FAS, fatty acid synthase; G6PDH, glucose 6-phosphate dehydrogenase; LDL, low-density lipoprotein; ME, malic enzyme; PAP, phosphatidic acid phosphohydrolase; SD, Sprague−Dawley; SEM, standard error of the mean; SREBP-1c, sterol regulatory element binding protein-1c

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