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Evaluation of the Hypolipemic Property of *Camellia sinensis* Var. *ptilophylla* on Postprandial Hypertriglyceridemia

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A naturally decaffeinated tea, *Camellia sinensis* var. *ptilophylla* (cocoa tea), has long been popular in southern China as a healthy beverage. Our experiments indicate that a single oral administration of 500 mg/kg of cocoa tea extract suppresses increases in plasma triacylgycerol (TG) levels when fed with 5 mL/kg of olive or lard oil in mice and that the inhibition rates are 22.9% and 31.5%, respectively, compared with controls. Under the same condition, cocoa tea extract did not affect the level of plasma free fatty acid. Likewise, the extract reduced the lymphatic absorption of lipids at 250 and 500 mg/kg. Also, cocoa tea extract and polyphenols isolated from cocoa tea inhibit pancreatic lipase. These findings suggest that cocoa tea has hypolipemic activity, which may be due to the suppression of digestive lipase activity by the polyphenols contained within the tea.

KEYWORDS: ***Camellia sinensis* var. *ptilophylla*; cocoa tea; triglyceride (TG); pancreatic lipase**

INTRODUCTION

Tea is the most popular beverage in the world (*1*). Green tea and oolong tea are mostly consumed in Japan and China, while black tea is preferred in America and Europe (*2*). All types of tea are manufactured from the same plant species, *Camellia sinensis* L., which was first discovered in China, where it has been used as a daily beverage for thousands of years because of its beneficial health effects (*3*). Tea contains a large quantity of polyphenols and caffeine (*4*). Tea polyphenols have been reported to have various biological and pharmacological functions, such as anti-HIV (*5*), antioxidative (*6*), antimutagenic (*7*), anticarcinogenic (*8*), antitopoisomerase (*9*), antiobesity (*10*), and hypocholesterolemic activities (*11*). In addition, caffeine is the most widely consumed behaviorally active substance and a large percentage of the human population chronically consumes caffeine in the diet (*12*). It is well-known that caffeine provides positive effects on human psychomotor and cognitive performance including increased alertness, energy, and ability under habitual caffeine intake at low to moderate doses (*13*). In contrast, high doses of caffeine can cause negative effects such as nervousness, anxiety, restlessness, insomnia, and tachycardia (*14*), which is an important reason marketable demand for decaffeinated coffee or tea is increasing year after year, despite the loss of key flavor compounds during the industrial decaffeinating process (*15*).

Camellia sinensis var. *ptilophylla* (cocoa tea), the naturally decaffeinated tea plant, which contains theobromine instead of caffeine (*16*), is a wild species of Sect, *Thea sinensis Linnaeus dyer* of the genus *Camellia*, an endemic tree that grows in cloudy and foggy highlands in the Longmen area of southern China (*17*).

In the Longmen area, the local people have habitually drank cocoa tea for a long time. Where the leave of cocoa tea is not only drunk as a healthy beverage but also used as a traditional remedy for ailments, enhanced mental efficiency and recovery from mental fatigue have been noted (*18*). Recently, epidemiological research revealed that these areas have few patients with hyperglycemia and diabetes mellitus. However, experimental studies on the hypolipemic effect of cocoa tea have not been reported. Iis study, we examined the effects of cocoa tea on postprandial hypertriglyceridemia.

MATERIALS AND METHODS

Materials and Preparation of Cocoa Tea Extract. Caffeine, theobromine, gallic acid, (+)-catechin (C), (+)-gallocatechin (GC), (−)-catechin gallate (CG), (−)-epicatechin gallate (ECG), (−)-epicatechin (EC), (−)-gallocatechin gallate (GCG), (−)-epigallocatechin (EGC), and (−)-epigallocatechin gallate (EGCG) were purchased from Wako Pure Chemical Ind. (Osaka, Japan). Other components including 1,3,4,6-tetra-*O*-galloyl-*β*-D-glucopyranose (1,3,4,6-GA-glc) and (−)-gallogatechin-3,5-di-*O*-gallate (GC-3,5-diGA) used in this study had previously

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been isolated from cocoa tea. Olive oil and lard oil were purchased from Nacalai Tesque Inc. (Kyoto, Japan). Pancreatic lipase (type VI-S, from porcine pancreas) and 4-methylumbelliferyl oleate (4-MU) were purchased from the Sigma Chemical Co. (St. Louis, MO), respectively.

Cocoa tea and green tea leaves were obtained from plants growing in a natural field in the highlands of the Longmen area of Guangdong province, southern China, in April. Dry leaves were treated with 20 parts of hot water for 10 min at 90 °C. After filtration and evaporation of the water, the residue was powdered under frozen−decompression conditions. The recovery rate of cocoa tea was 21.2%, and that for green tea was 23.7%. The extract residue was immediately dissolved in water before the experiments.

Reverse-Phase HPLC Analysis of Polyphenols and Alkaloids in Cocoa Tea. The concentrations of theobromine, gallic acid, flavanols, and other polyphenols in the cocoa tea were analyzed by HPLC (LC-10Ai System (Shimadzu Co., Ltd.), under the following conditions: column, Cosmosil 5PE-MS (Nakalai Tesuque, Kyoto, Japan, 4.6 mm × 150 mm, 5 mm); mobile phase, eluent A 0.05% trifluoroacetic acid (TFA) in water; eluent B 0.05% TFA in acetonitrile; flow rate, 2 mL/min; column temperature, 40 °C; detection wavelength of 280 nm (*19*). The analysis was performed with a gradient program of eluent B content of 10% for 5 min, 21% for 8 min, 90% for 1 min, and 90% for 6 min. Quantification of caffeine, theobromine, catechins, and gallic acid was performed using standard calibration curves for marketed compounds. The data were obtained as average values from three experiments.

Animals. The 7-week-old male ICR mice and Sprague−Dawley rats (SD rats) were purchased from Charles River Japan Inc. (Tokyo, Japan). The animals were housed in groups in plastic cages, kept in a specific-pathogen-free animal room at 23 ± 1 °C with a 12-h light-dark cycle of lights on from 6:00 to 18:00 and were provided with standard laboratory chow (CE-2; Clea Japan, Inc.) and tap water. The animals were kept for 1 week before the experiment. The care and treatment of the animals were conformed to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH publication No. 85-23, revised 1985) and Japanese Experimental Animal Research Association standards, as defined in the Guidelines for Animal Experiments (1987).

Plasma Lipid Tolerance Test and Measurement of Plasma Lipid Levels. For the lipid absorption test, mice were deprived of food for 20 h before the experiment. An olive and lard oil solution was orally administered at 5 mL/kg of body weight, 30 min after 0.1 mL/10 g of cocoa tea extract was treated. An equal volume of distilled water was administered to the control mice. The mice were analyzed for chronological changes in plasma TG and free fatty acid (FFA) levels, with seven animals at each time point. Blood samples were taken from the heart under anesthesia with diethyl ether and were put in a tube containing 2% sodium heparin. Then the tubes were centrifuged at 5000 rpm for 5 min to obtain supernatant as a sample. All samples were stored at −20 °C until the assay. Plasma TG was measured using a Hitachi 7070 Automatic Serum Analyzer (Hitachi Co., Ltd., Japan) by the glycerol kinase/glycerol-3-phosphate oxidase (GK-GPO) method (*20*), and plasma FFA was determined as previously described (*21*). The assay kit was purchased from International Reagents Corp., Kobe, Japan.

Infusion of Lipid Emulsion and Measurement of the Lymphatic Absorption of TG in Rats. The thoracic lymph duct was cannulated as previously described (*22*). Briefly, male SD rats deprived of food for 18 h were anesthetized with sodium pentobarbital. While under anesthesia, the rats were kept under a heating light to maintain their body temperature at 35 °C. After an abdominal incision was made along the midline, the thoracic lymph duct was cannulated with polyethylene tubing (SV.31 tubing, i.d., 0.50 mm; o.d., 0.80 mm; Dural Plastics, Auburn, Australia). A second indwelling infusion catheter (Silastic medical grade tubing, i.d., 1.0 mm; o.d., 2.1 mm; Dow Corning, Midland, MI) was placed in the stomach for the administration of a test emulsion and secured by a purse-string suture (4-0 silk, Ethicon, Somerville, NJ). After the abdominal incision was closed, the rats were placed in restraining cages in a heated chamber at 30 °C for postoperative recovery for 24 h. During this period, the rats were intragastrically administered a continuous infusion of a maintenance solution containing 139 mM glucose and 85 mM NaCl by means of a precision infusion pump (model 22, Harvard Apparatus, South Natick, MA) at 3.4 mL/h until the end of the experiment. The same solution was given as drinking water. After 24 h, rats with a constant lymph flow rate were administered with emulsion containing 200 mg of triolein (95%, Sigma Chemical, St. Louis, MO) and the cocoa tea extract. Thoracic lymph was continuously collected into 50 mL polypropylene tubes containing 75 mg of disodium EDTA for the 24 h postdosing period. All lymph samples collected/hour were pooled, and the total volume of lymph that had been collected was gravimetrically determined. Aliquots of the pooled lymph samples from each hourly collection period were dispensed into Eppendorf tubes and stored at −80 °C before analysis. The TG was measured using a Hitachi 7070 automatic serum analyzer (Hitachi Co., Ltd., Tokyo, Japan) by the glycerol kinase/glycerol-3-phosphate oxidase (GK-GPO) method (*20*).

Table 1. Amounts of Theobromine and Polyphenols in Cocoa Tea and Green Tea[a]

components	cocoa tea (mg/g of powdered extract)	green tea (mg/g of powdered extract)
theobromine	139	2.3
caffeine	0	67
GCG	199	3.7
EGC	43	87
EGCG	37	93
ECG	0	19
CG	0	4.1
1,2,4,6GA-g lc	55	0
GC-3,5diGA	33	0

[a] The data are average values for three experiments.

Measurement of Pancreatic Lipase Activity. Pancreatic lipase activity was measured using 4-MU as a substrate. A 25 μL volume of sample solution dissolved in water and 50 μL of 0.1 mM 4-MU solution dissolved in a buffer consisting of 13 mM Tris-HCl, 150 mM NaCl, and 1.3 mM $CaCl_2$ (pH 8.0) were mixed in the well of a microtiter plate, and 25 μL of the lipase solution at 50 U/mL in the above buffer was then added to start the enzyme reaction. After incubation at 25 °C for 30 min, 0.1 mL of 0.1 M sodium citrate (pH 4.2) was added to stop the reaction. The amount of 4-methylumbelliferone released by the lipase was measured with a TECAN GENios microplate fluorescence reader (Salzburg, Austria) at an excitation wavelength of 355 nm and an emission wavelength of 460 nm (*23*). The IC_{50} of the test sample was obtained from the least-squares regression line of the plots of the logarithm of the sample concentration (0.01−10 μg/mL) versus the inhibition percent of pancreatic lipase activity. The inhibition experiments were repeated at least 3 times with deviations of 10% from the mean.

Statistical Evaluation. Data were expressed as means ± SD and evaluated by analysis of variance (ANOVA) using SPSS software (SPSS Inc., Japan, Tokyo). Differences between the group means were considered to be significant at $p < 0.05$ using the Tukey or Dunnet procedure generated by this program.

RESULTS

HPLC Analysis of Polyphenols and Alkaloids in Green Tea and Cocoa Tea. The chemical compositions of tea polyphenols and alkaloids in green and cocoa tea extracts were analyzed by HPLC. As shown in **Table 1**, the compositions of tea polyphenols in cocoa tea were almost similar to that of green tea extract. Cocoa tea contained much more GCG and less EGC and EGCG than green tea. Also, cocoa tea contained a high level of theobromine, while the content of theobromine in green tea was markedly low. Caffeine was plentiful in green tea but not in cocoa tea. Finally, 1,2,4,6-GA-glc and GC-3,5 diGA were present in cocoa tea but not in green tea.

Effect of Cocoa Tea Extract on Postprandial Hypertriglyceridemia. As shown in **Figure 1A**, the plasma TG reached a maximum level 3 h after the oral administration of 5 mL/kg

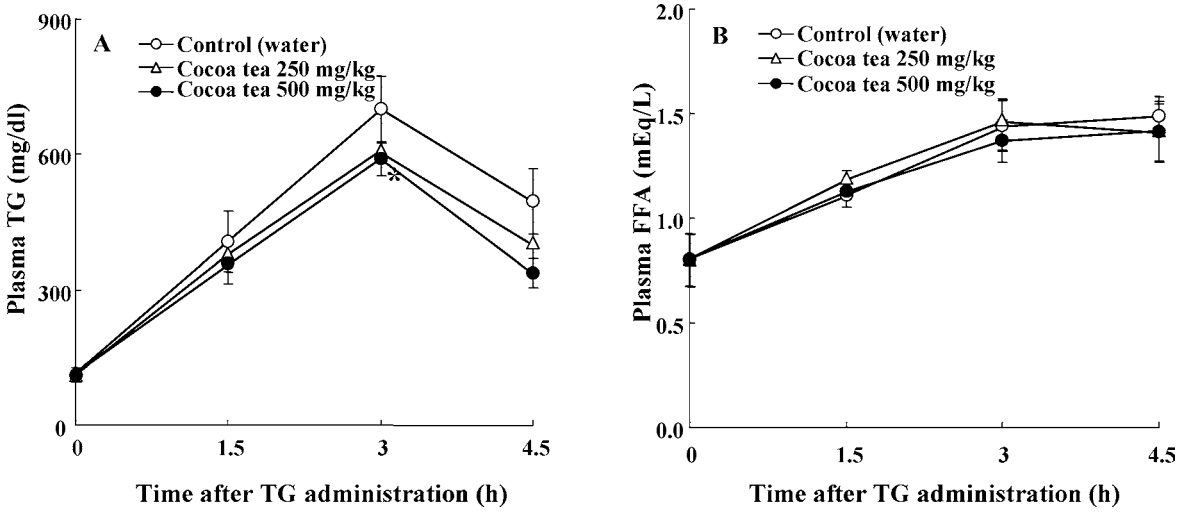

Figure 1. Inhibitory effect of a single oral administration of cocoa tea extract on plasma TG levels in olive oil-loaded ICR mice. The 7-week-old male ICR mice were used in the lipid absorption test. Samples were dissolved in water before use, and the solution was orally administered to animals at 0.1 mL/10 g of body weight. Olive oil was orally administered at 5 mL/kg of body weight, 30 min after the oral administration of 500 and 250 mg/kg, respectively, of cocoa tea extractto mice that had been deprived of food for 20 h. Blood TG and FFA levels were chronologically analyzed, and the results represent the mean ± SD of the values obtained from seven mice at each time point. The asterisk shows significant differences from the control determined by the Dunnet procedure ($p < 0.05$).

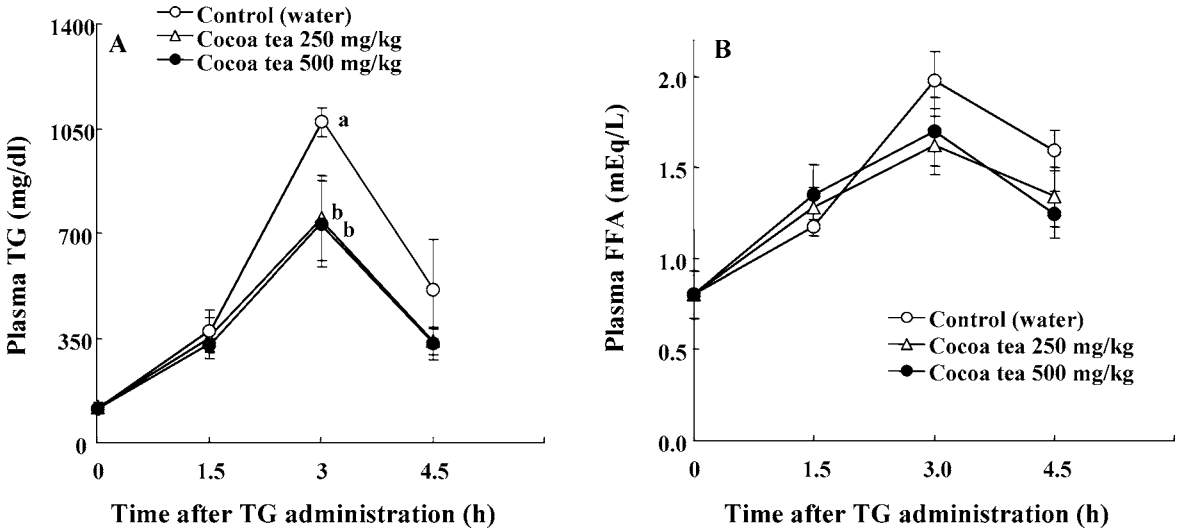

Figure 2. Inhibitory effect of a single oral administration of cocoa tea extract on plasma TG levels in lard oil-loaded ICR mice. The 7-week-old male ICR mice were used in the lipid absorption test. Samples were dissolved in water before use, and the solution was orally administered to animals at 0.1 mL/10 g of body weight. Lard oil was orally administered at 5 mL/kg of body weight, 30 min after the oral administration of 500 and 250 mg/kg, respectively, of cocoa tea extract to mice that had been deprived of food for 20 h. Blood TG and FFA levels were chronologically analyzed, and the results represent the mean ± SD of the values obtained from seven mice at each time point. The different letters indicate significant differences among the groups determined by the Tukey procedure ($p < 0.05$).

of olive oil and then gradually decreased. The change in the plasma FFA level almost showed the same pattern (**Figure 1B**). Compared with the control group, 500 mg/kg of cocoa tea extract significantly reduced plasma TG levels 3 h after the administration of olive oil. The mean value of the cocoa tea group was 589.7 ± 36.5, 22.9% lower than that of the control group (764.7 ± 72.9). On the other hand, cocoa tea extract had no effect on the plasma FFA levels. In this study, the hypolipemic effect was also observed by using 5 mL/kg of lard oil loaded mice as shown in **Figure 2A**. Cocoa tea extract at 500 and 250 mg/kg lowered the plasma TG level to 31.5% at 3 h, the peak time of the plasma TG level compared with the control group after the oral administration of lard oil ($p < 0.05$). Similarly, cocoa tea extract did not cause a significant decrease in the plasma FFA level.

Effects of Cocoa Tea Extract on Lymphatic TG Absorption. Our experiments confirmed that plasma TG concentrations linearly increased after triolein was infused by gastric intubation. The time course of the TG concentration in the thoracic lymph is presented in **Figure 3A**, and **Figure 3B** shows the cumulative recovery of TG in the thoracic lymph. The maximum concentration of lymphatic TG was observed 2−4 h after infusion of the triolein emulsion. Cocoa tea extract drastically lowered TG absorption. Two hours after lipid emulsion infusion, the average amount of TG in the lymph of the triolein emulsion was 52.2 ± 7.6 mg/h in the control group, while it was 21.9 ± 7.1 mg/h and 28.9 ± 6.9 mg/h for the 500 and 250 mg/kg of cocoa tea group, respectively. Furthermore, the cumulative absorption of TG after 4 h was 145.7 ± 13.7 mg in the control group, 94.9 ± 19.6 mg in the 250 mg/kg group, and 74.6 ± 25.4 mg in the

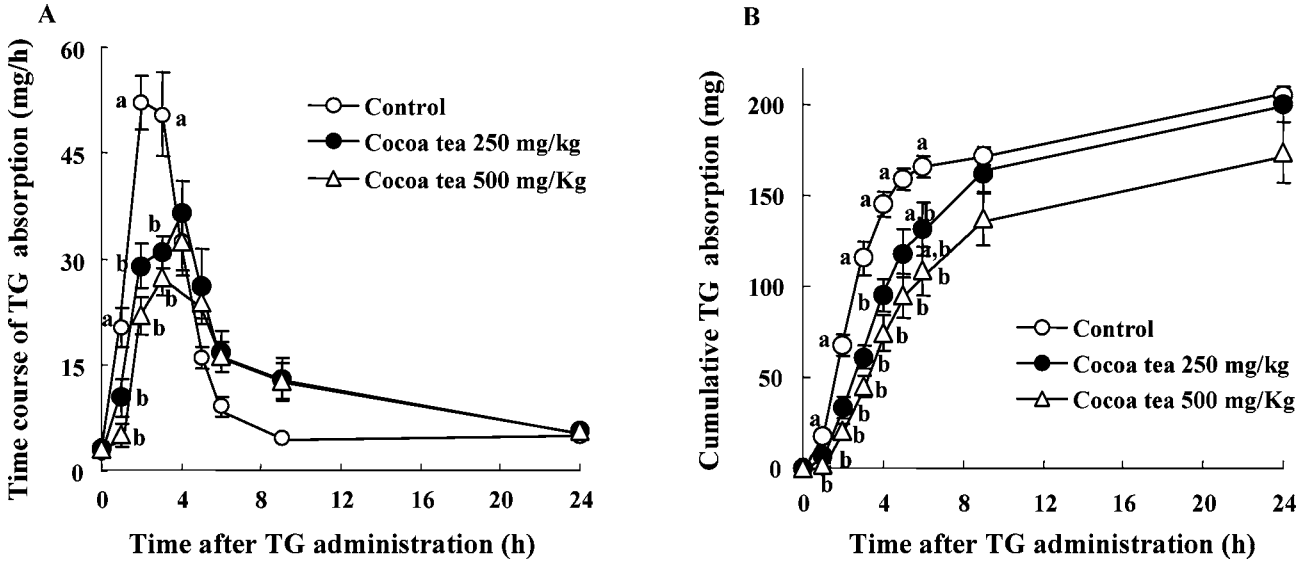

Figure 3. Inhibitory effect of cocoa tea extract on the lymphaticabsorption of TG in triolein-loaded SD rats. The 7-week-old male SD rats were used in the lipid absorption test. Samples were dissolved in lipid emulsion before use, and the solution was infused into the stomach. Thoracic lymph was then continuously collected for 24 h after infusion. Lymphatic TG levels were chronologically analyzed, and the results represent the mean ± SD of the values obtained from five rats at each time point. (A) shows the time course of the lymphatic absorption of TG for 24 h. (B) shows the cumulative lymphatic absorption of TG for 24 h. The different letters indicate significant differences among the groups determined by the Tukey procedure ($p < 0.05$).

Table 2. Pancreatic Lipase Inhibition of Cocoa Tea Extract and Its Major Components

compds	IC_{50} (μg/mL)	compds	IC_{50} (μg/mL)
cocoa tea extract	0.63	CG	0.38
green tea extract	0.72	GCG	0.24
theobromine	>10	EGCG	0.16
catechine	>10	ECG	0.14
EGC	>10	1,2,4,6GA-glc	0.16
GC	>10	CG-3,5diGA	0.13
EC	>10		

[a] Pancreatic lipase activity was measured using 4-methylumbelliferyl oleate (4-MU) as a substrate.

500 mg/kg group. The total absorption of TG 4 h after triolein infusion in the two cocoa tea groups was 34.9 and 48.8% of the control, respectively. The rates of absorption did not rise further in all groups.

Inhibition of Pancreatic Lipase by Cocoa Tea Extract and Catechins in vitro. The inhibitory effect of cocoa tea extract and its major compounds against pancreatic lipase was investigated. The 50% inhibitory concentration (IC_{50}) is shown in **Table 2**. ECG and EGCG exhibited more potent activity than did GCG and CG. Also, caffeine and theobromine did not show any obvious effects until 10 mg/mL. Moreover, cocoa tea extract showed higher inhibitory activity with an IC_{50} of 0.63 μg/mL, this being at the same level of the green tea extract (IC_{50} = 0.72 μg/mL).

DISCUSSION

Some clinical studies have indicated a correlation between hyperlipidemia and life style-related diseases (*24*). Hence, keeping blood TG level within the normal range is important for the prevention of diseases such as arteriosclerosis, cerebral apoplexy, and myocardial infarction. Furthermore, it has been indicated that various teas affect physiological function (*25*). Green tea polyphenol (EGCG) intake decreases the absorption of TG and cholesterol (*26*). However, the influence on blood lipid levels of cocoa tea has not been reported yet. Thus, we investigated whether cocoa tea extract reduces plasma lipid levels in ICR mice loaded with olive oil or lard oil. The cocoa tea extract significantly suppressed blood lipid levels 3 h after the oral administration of each respective oil as shown in **Figures 1A** and **2A**. At that time, the blood lipid level reached a maximum. It is suggested that cocoa tea suppresses the postprandial hyperlipemia. Since administration of the extract did not affect plasma FFA levels (**Figures 1B** and **2B**), which may be derived from cytoplasmic TG, de-novo lipogenesis, and TG derived from lipoproteins, it is possible that the hypolipemic activity is due to the suppression of lipid absorption.

Moreover, the effects of cocoa tea on the intestinal absorption of lipids were examined in conscious rats with lymph cannula. The plasma TG concentration of the control linearly increased after triolein infusion with gastric intubation. TG was rapidly absorbed in the control and reached 52.2 ± 7.6 mg/h after 2 h (**Figure 3A**). In contrast, the cocoa tea groups moderately absorbed TG. The total absorption of TG in the 500 and 250 mg/kg groups was 34.9 and 48.8% of that of the control for 4 h after triolein infusion, respectively, as shown in **Figure 3B**. The results also revealed that the cocoa tea extract suppresses intestinal TG absorption.

It is well-known that it is useful to reduce the intestinal absorption of dietary lipids by inhibiting metabolic processes implicated in lipid digestion and absorption to decrease blood TG (*27*). Since ingested lipid is usually insoluble oil, the basic process of lipids absorption is the conversion of lipid into water-soluble compounds which can be efficiently absorbed. Lipid is usually absorbed as fatty acid and 2-monoglyceride generated by pancreatic lipase, which is the principal lipolytic enzyme localized in the digestive tract (*28*, *29*). Thus, we examined whether cocoa tea reduces the plasma TG level by inhibiting digestive lipase. As shown in **Table 2**, the cocoa tea and green tea extracts showed submicro order values of IC_{50} against pancreatic lipase. Polyphenols such as EGCG, GCG, and ECG contained in cocoa and green tea also potently inhibited the lipase, while the other polyphenols, EGC, C, and EC, did not. 1,3,4,6-GA-glc and GC-3,5-diGA contained in cocoa tea and

not green tea exhibited potent lipase inhibiting activity. These results indicate that cocoa tea reduces plasma lipid levels by inhibiting pancreatic lipase and limiting the intestinal absorption of lipid. Much evidence from animal studies indicates that tea polyphenols lower blood levels of lipids (*30*), suggesting that tea polyphenols may inhibit pancreatic lipolytic enzymes and thereby interfere with lipid digestion and absorption.

Lipids are an important energy source, but excess intake may induce obesity and hyperlipidemia (*31*). Obesity has become a serious public health problem since it is directly associated with various chronic diseases such as coronary heart disease and diabetes in developed societies (*32*). Inhibiting fat absorption from the intestine is an effective way to prevent these diseases. Evaluation of the hypolipemic properties of cocoa tea using rats with thoracic lymph cannulae provided direct evidence that cocoa tea can reduce the lymphatic absorption of TG. Furthermore, the hypolipemic activity exhibited by cocoa tea extract probably involves the control of absorption of lipid by suppressing the activity of pancreatic lipase and slowing the hydrolysis of lipid emulsions. The above findings indicate that chronically drinking cocoa tea should be beneficial for the prevention of life-style related diseases caused by hyperglycemia.

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