Fecal Lipid Excretion after Consumption of a Black Tea Polyphenol Containing Beverage—Randomized, Placebo-Controlled, Double-Blind, Crossover Study—

Hiroshi Ashigai,*a Yoshimasa Taniguchi,b Mihoko Suzuki,c Emiko Ikeshima,a Tomoka Kanaya,a Kanako Zembutsu,d Shimpei Tomita,d Mika Miyake,a and Ikuo Fukuharaec


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Obesity is a serious medical condition worldwide. Inhibition of lipid absorption is very important in preventing obesity. In a previous study, we found that postprandial elevation of triacylglycerol was suppressed by the intake of black tea polyphenol (BTP). We also reported that BTP caused lipid excretion into feces in an animal study. The present study is a clinical trial that examined lipid excretion. In this randomized, placebo-controlled, double-blind, crossover study, in the first test period participants were asked to drink either a beverage containing 55 mg BTP or a control beverage without BTP 3 times a day for 10 d. After an 11-d interval, for the second test period, they then drank the alternate test beverage 3 times a day for 10 d. During the test periods, the participants were asked to eat meals standardized according to calorie and fat content. Stool samples were obtained during the last 3 d of each test period for fecal lipid measurements. Total lipid excretion increased from 5.51±1.73 to 6.87±1.91 g/3 d after BTP intake in comparison with intake of the control beverage. These results indicated that BTP increased lipid excretion.

Key words black tea polyphenol; fecal lipid; clinical study

Metabolic syndrome is a serious risk factor for cardiovascular disease such as arteriosclerosis2–3 and is caused by hyperlipidemia, hyperglycemia, and hypertension. Arteriosclerosis is related to cardiovascular disease and cerebral infarction, which are widely recognized as major public health problems. Reducing the risk of arteriosclerosis, especially dyslipidemia, which was reported to be the strongest risk factor for arteriosclerosis,3 is extremely important. In some patients, the major abnormality in dyslipidemia is a postprandial serum triglyceride elevation.

Black tea is one of the most famous beverages in the world. Traditionally, there are three types of tea: green tea (unfermented), oolong tea (partially fermented), and black tea (fully fermented).4 In previous reports, administration of black tea improved blood glucose levels in streptozocin-diabetic rats5 and blood lipid levels in animal experiments.6–8 Also, increasing black tea consumption was associated with lower levels of serum glucose.9 Moreover, we reported that black tea polyphenol was associated with lower levels of blood glucose.9

The major polyphenols in green tea are catechins: (−)epicatechin, (−)epicatechin-3-gallate, (−)epigallocatechin, and (−)epigallocatechin-3-gallate.4 These compounds are oxidized during the fermentation process (tea leaf fermentation). In this process, theaflavins and thearubigins are synthesized from catechin derivatives.4 They influence the taste and color of black tea. Theaflavins consist of the following four compounds: theaflavin (TF-1), theaflavin-3-gallate (TF-2A), theaflavin-3′-gallate (TF-2B), and theaflavin-3,3′-gallate (TF-3). Thearubigins are widely complex mixtures of highly polymerized polyphenols. We prepared an extract from black tea, BTP, which affected lipid excretion in mice.10,11

Some food ingredients, such as maltodextrin12 and mannanoligosaccharide,13,14 have been reported to promote lipid excretion during clinical studies. In addition, some polyphenols, such as catechin15 and oolong tea polyphenol16 which inhibits pancreatic lipase, have been reported to promote lipid excretion. So we assumed that BTP might promote lipid excretion in humans. For this report, we studied the effect of BTP on lipid excretion in a randomized, double-blind, placebo-controlled, crossover study. We also evaluated the safety of BTP ingestion.

MATERIALS AND METHODS

Ethics This clinical study adhered to the ethical standards of the Helsinki Declaration 1964 as modified by subsequent revisions and the ethical guidelines for epidemiological research of the Ministry of Education, Culture, Sports, Science and Technology of Japan and the Ministry of Health, Labour and Welfare of Japan. The experimental protocol (protocol no. 14113) was approved by the Board of Evaluation for Research Studies Involving Human Subjects at Miyawaki Clinic. This study was registered with the UMIN Clinical Trials Registry as UMIN000018499 and was conducted in compliance with the protocol as registered. This clinical trial was performed by a contract research organization, New Drug Research Center (Eniwa, Hokkaido) from October 2014 to December 2014 at

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the Fukuhara Clinic.

Participants Healthy adults were recruited through the New Drug Research Center, Inc., and written informed consent was obtained from each individual. Healthy males and females, aged 20 to 64 years old, were enrolled. The exclusion criteria were as follows: possible onset of allergy symptoms; fecal evacuation less than 5 times per week; food intake less than twice per day; under treatment for or with a history of drug addiction or alcoholism; extremely irregular dietary habits; any history of serious disease (e.g., heart disease, respiratory disorder, digestive disturbance, endocrine disorder, metabolic disturbance, food allergy); kidney disease, liver disease, infectious disease, viral infection; constant use of pharmaceuticals for a chronic malady; surgical history related to a digestive organ; donation of more than 200 mL of blood or blood components within the month prior to this study or over 400 mL blood or blood component within the three months prior to this study; graveyard shift worker or irregular shift worker; irregular life style during the test period; constant use of pharmaceuticals, dietary supplements or functional foods affecting lipid metabolism or intestinal function; excessive alcohol consumption; and possible pregnancy, pregnancy, or lactation. Also excluded there those unable to stop drinking the day before the examination day; participation or possible participation in another clinical study; determination of anemia on the examination day prior to the beginning of the study; those judged ineligible on the examination day prior to the beginning of the study by the site investigators for any reason; and those working for a functional food company.

Test Beverage Preparation of the BTP extract and HPLC analysis were performed according to previously published methods.\textsuperscript{10,11} As a result, the BTP extract contained 5% BTP.

We prepared black tea beverages (350 mL) with or without BTP (Table 1). Food coloring was used so that the two beverages would not differ in appearance and other means were taken to ensure that the two beverages were similar in taste. The BTP drink contained 55 mg (TF-1 eq)/bottle of BTP.

Study Design The present study was designed as a randomized, double-blind, placebo-controlled, crossover study. The site investigator enrolled the participants. Participants were divided into two groups by stratified randomization by the assigning controller, and each group was assigned either the BTP beverage or placebo beverage before the first trial period. The assigning controller kept the assignment list in a sealed container until the trial was complete. Participants, investigators, and any persons concerned with the study, excluding the assigning controller, remained blinded. Participants had to drink the BTP beverage or placebo beverage with a standardized meal 3 times per day for 10 d. During the last 3 d, all feces were collected (Fig. 1). We prepared the standardized meals to create a high-fat diet, and nutrient components of the standardized meals are listed in Table 2 according to the Japanese law of nutrient representation. Participants were required to eat the same standardized meals in both test periods. During the test period, they were also forbidden to excessively drink or eat a fatty diet on the day before examination day; to vigorously exercise on the day before the examination day; to smoke from 1 h prior to the beginning of the check-up on examination day; and to drink and eat from 21:00 on the day before the examination day to the end of the examination day. Also prohibited during the test period was the use of pharmaceuticals, dietary supplements or functional foods that could affect lipid metabolism or intestinal function; drinking black tea with the exception of the test beverage; donating blood or blood components; drinking excessive amounts of alcohol; eating anything except the standardized meals during the study period; eating or drinking immoderately during the interval period; and participating in other clinical studies. Participants were asked to maintain the intake of the test beverage as instructed; eat standardized meals only during the test periods; maintain daily life as usual except for intake of the test drink and standardized meals; keep a diary every day and submit it on the examination day; contact the doctor immediately if they felt ill during and after the test period;

Table 1. Nutrient Components of Test Beverages

<table>
<thead>
<tr>
<th>Scale</th>
<th>Energy kcal</th>
<th>Protein g</th>
<th>Fat g</th>
<th>Sugar g</th>
<th>Cathechin mg</th>
<th>BTP mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>BTP</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>55</td>
</tr>
<tr>
<td>Placebo</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2. Energy and Fat Intake during Treatment Periods

<table>
<thead>
<tr>
<th>Scale</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy intake kcal/d</td>
<td>2350±50</td>
<td>2050±50</td>
</tr>
<tr>
<td>Fat intake g/d</td>
<td>84.5±1.0</td>
<td>68.0±51.0</td>
</tr>
</tbody>
</table>

Fig. 1. Schematic Representation of Test Schedule
contact the clinic immediately if unable to visit the hospital on the examination day; and keep the test information secret. On the examination day, the site investigator interviewed the participants and collected blood and urine samples.

**Measurement of Fat in Feces** Total fecal lipid content was measured according to Van De Kamer’s method.\(^{17}\) Five grams of feces was extracted in 10 mL of 6.7% KOH: 80% (v/v) ethanol. After striation, the solution was neutralized by 5 mL of 25% HCl and petroleum ether 10 mL was added to the solution, and the solution was shaken. We aliquoted 2 mL of the petroleum ether layer and mixed it with 95% ethanol, then we titrated the solution with thymol blue and 0.1 M KOH. The reagents were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

**Measurement of Physical Parameters and Circulatory Parameters** Body height, body weight, body fat percentage, systolic blood pressure, diastolic blood pressure, and pulse rate were measured at each check. Body mass index (BMI) was calculated from body height and body weight. Body fat percentage was measured using a body composition analyzer (TBF-310, Tanita Corp., Tokyo, Japan) and systolic blood pressure, diastolic blood pressure, and heart rate were measured using a HBP-9020 (OMRON COLIN Corp., Tokyo, Japan).

**Blood Count and Biochemical Tests** The following were measured in fasting blood samples: white blood cell count (WBC), red blood cell count (RBC), hemoglobin (Hb), hematocrit (Ht), platelet count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) for hematology, total protein, albumin, total bilirubin, aspartate aminotransferase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), \(\gamma\)-glutamyltransferase (\(\gamma\)-GT), total cholesterol, high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol, triglycerides, blood glucose, uric acid, urea nitrogen, creatinine, sodium (Na), chloride (Cl), potassium (K) and hemoglobin A1c (HbA1c). All except HbA1c were analyzed at each examination. HbA1c was analyzed at the pre-examination only. Data were analyzed by SRL Inc. (Hokkaido, Japan). Quantitative urinalysis of protein, glucose, urobilinogen, bilirubin, specific gravity, pH, ketones, and urine occult blood reaction were analyzed using fasting urine samples. Urinalysis was performed by SRL Inc.

![Flow Diagram of the Progress through the Study](image-url)
**Statistical Analysis** Results were described as mean±standard deviation of the mean using statistical software, SAS 9.3 (SAS Institute Inc., Cary, NC, U.S.A.). Significant differences between fecal fat measurements, physical parameters, circulatory parameters, and results of blood studies during intake of the placebo and BTP were estimated using paired t-tests. Also significant differences in those values between the time previous to intake of the test beverage (initial) and after intake of the test beverage (final) were estimated using paired t-tests. In urinalysis, quantitative parameters were estimated using paired t-tests, and semi quantitative parameters were estimated using the Wilcoxon signed-rank test.

**RESULTS**

**Participants** Participants were recruited on October 14 and 15, 2014. Sixty-seven individuals were evaluated for eligibility to participate and 25 healthy adults (13 males and 12 females) were enrolled. Figure 2 is a flow diagram of the study from the assessment until the final analysis. Table 3 provides information on the participants’ background. No participant dropped out of the study, and all underwent a full analysis as a safety evaluation. One participant was excluded from the efficacy evaluations by the site investigator before the study became double-blind in accordance with the exclusion criteria.
in vitro study. Generally, lipid absorption consists of two processes: lipid hydrolysis followed by micelle formation. Lipids are hydrolyzed by pancreatic lipase to fatty acids and monoacylglycerol. Fatty acid and monoacylglycerol then form micelles with bile acids and phospholipids before the micelle is transported through the digestive tract. We suggest that BTP inhibits pancreatic lipase and lipid absorption. As a result, BTP intake promotes lipid excretion and inhibits serum triglyceride elevation in healthy men and women.

The safety evaluation showed some significant differences between the placebo and BTP groups in quantitative parameters. The physical examination revealed that the final body weight and BMI decreases in both groups. But their changes were within the standard value. Some adverse events were also found, but the site investigator deemed that they were not related to test beverages, which indicated that the BTP-containing beverage was safe.

As a limitation of this study, lipid excretion through the use of BTP might have been increased as the participants ate standardized meals that had high lipid concentrations.

In conclusion, we confirmed that the intake of BTP promotes lipid excretion. The increase in lipid excretion may be due to inhibition of pancreatic lipase and lipid absorption. Therefore, BTP is a beneficial substance for patients with metabolic disorders.

Conflict of Interest HA, YT, EI, TK and MM are employees of Kirin Co., Ltd., the study sponsor. MS is an employee of Kirin Beverage Co., Ltd. KZ and ST are employee of New Drug Research Center, Inc. as clinical research officer, IF is the site investigator.

Supplementary Materials The online version of this article contains supplementary materials.

REFERENCES
(2009).


