Ingestion of a tea rich in catechins leads to a reduction in body fat and malondialdehyde-modified LDL in men[1–3]

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ABSTRACT

Background: Catechins, the major component of green tea extract, have various physiologic effects. There are few studies, however, on the effects of catechins on body fat reduction in humans. It has been reported that the body mass index (BMI) correlates with the amount of malondialdehyde and thiobarbituric acid–reactive substances in the blood.

Objective: We investigated the effect of catechins on body fat reduction and the relation between oxidized LDL and body fat variables.

Design: After a 2-wk diet run-in period, healthy Japanese men were divided into 2 groups with similar BMI and waist circumference distributions. A 12-wk double-blind study was performed in which the subjects ingested 1 bottle oolong tea/d containing 690 mg catechins (green tea extract group; $n = 17$) or 1 bottle oolong tea/d containing 22 mg catechins (control group; $n = 18$).

Results: Body weight, BMI, waist circumference, body fat mass, and subcutaneous fat area were significantly lower in the green tea extract group than in the control group. Changes in the concentrations of malondialdehyde-modified LDL were positively associated with changes in body fat mass and total fat area in the green tea extract group.

Conclusion: Daily consumption of tea containing 690 mg catechins for 12 wk reduced body fat, which suggests that the ingestion of catechins might be useful in the prevention and improvement of lifestyle-related diseases, mainly obesity. *Am J Clin Nutr* 2005; 81:122–9.

KEY WORDS Green tea extract, catechins, humans, body fat, malondialdehyde-modified LDL, double-blind controlled study

INTRODUCTION

High body fat increases the risk of diabetes, hyperlipidemia, and hypertension, which leads to arteriosclerotic disease. There is an increased risk of death associated with these diseases as well as with increased body fat (1–3). Body fat increases with an increase in dietary lipid intake (4, 5). Therefore, recommendations of lifestyle changes, and of changes in dietary content in particular, are often made for primary prevention and improvement of these diseases.

Polyphenols have recently attracted attention because of their physiologic activity. Green tea, long consumed in Asian countries (mainly Japan and China), contains low-molecular-weight polyphenols consisting mainly of flavanol (flavan-3-ol) monomers, which are referred to as catechins. There are several isomers of this compound: catechin, catechin gallate (Cg), gallocatechin, gallocatechin gallate (GCg), epicatechin, epicatechin gallate (ECg), epigallocatechin, and epigallocatechin gallate (EGCg). Normally, 10–20% of the catechins in green tea leaves are epigallocatechin and EGCg (6). A portion of ingested EGCg is absorbed and widely distributed throughout the body (7). The ingestion of tea extract or catechins induces antioxidant (8), antiviral (9), antiplaque-forming (10), and anticancer (11) activities, as well as decreases in blood pressure (12) and blood sugar (13). Lipid metabolism studies in animals, tissues, and cells have found that tea extract and catechins reduce triacylglycerol and total cholesterol concentrations (14, 15), inhibit hepatic and body fat accumulation (16, 17), and stimulate thermogenesis (18). In humans, there have been few studies on the effects of catechins on body fat, but the effects on energy expenditure (EE) and oxidative consumption have been examined (19, 20). One study of the effects of catechins on body weight found a tendency toward decreased body weight and waist circumference, but no comparative controls were included in the study (21).

Studies reported that body mass index (BMI; in kg/m^2) correlates with the amount of thiobarbituric acid-reactive substances (TBARS) and malondialdehyde in the blood (22, 23). These studies suggested that obesity might be related to an increase in lipid oxidizability. To examine the hypothesis that continuous ingestion of catechins reduces body fat in humans, we performed a double-blind controlled study in healthy men ($n = 38$) who were normal-weight to overweight and in whom the intake of catechins and caffeine from other foods was minimized. The effects of catechins on the blood variables and oxidized LDL and the association between body fat variables and oxidized LDL were investigated.

SUBJECTS, MATERIALS, AND METHODS

Subjects

The subjects were 38 male employees of Kao Corporation (Tokyo), aged 24–46 y, whose body weight was normal to overweight, according to the criteria of the National Institutes of Health for BMI (24).

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The study was performed under the supervision of an occupational health physician, in accordance with the regulations of the Kao Corporation Ethics Committee for Internal Clinical Studies and in conformity with the Helsinki Declaration. The conditions and procedures of the investigation were reviewed with all subjects before they gave written informed consent.

Materials

Green tea extract (GTE) was prepared by using 2 extraction methods. To prepare one version (GTE-A), green tea leaves underwent extraction with hot water, and the extract was reduced to a powder by using the spray-dry method. The powder was dissolved in methanol-water, discolored with the use of an octadecyl silica column, and freeze-dried (10). To prepare another version (GTE-B), which is decaffeinated, the GTE obtained with hot water was reduced to a powder by using the spray-dry method, and then the extract was dissolved in hot water and mixed with an equal volume of chloroform. The aqueous phase was recovered with 3 volumes of ethanol, and the extract was freeze-dried after removal of the solvent (10). The total catechin content was 33.0 g/100 g in the GTE-A powder and 85.6 g/100 g in the GTE-B powder. For preparation of the test beverage, 9 g oolong tea leaves underwent extraction with 100 mL distilled water at 80 °C for 5 min, and the extract was used as the base beverage. Two types of beverage were prepared: one beverage containing a high amount of catechins and a control beverage. The beverage with a high catechin content was prepared by adding both GTEs to the base beverage and adjusting the total catechin content to ≈700 mg/340 mL base beverage. For the control beverage, both GTEs were added to the base beverage, and the total catechin content was adjusted to ≈20 mg/340 mL base beverage; this is the same ratio of catechin components to the caffeine content as in the beverage with a high catechin content. To avoid oxidation and to maintain quality throughout the study period, 50 mg ascorbic acid/100 mL beverage was added to the beverages, and the beverages were sterilized by high-temperature heat sterilization at 138 °C for 30 min. Aliquots of 340 mL were distributed in 340-mL polyethylene terephthalate bottles.

The amounts of catechin, Cg, gallate catechin, GCg, epicatechin, ECg, epigallocatechin, and EGCg in the test beverage were measured by using HPLC carried out on an L-column ODS (4.6 mm diameter × 250 mm length; Chemicals Evaluation and Research Institute, Tokyo). The HPLC conditions were as follows: column temperature, 35 °C; sample size, 10 μL (0.1 mol acetic acid solution/L); mobile phase, 0.1 mol acetic acid/L (solution A), 0.1 mol acetic acid-acetonitrile/L (solution B); gradient conditions, 0–5 min ratio of solution A to solution B = 97:3, 5–37 min A:B = 80:20, 37–43 min A:B = 80:20, 43–43.5 min A:B = 0:100, and 43.5–48.5 min A:B = 0:100; flow rate, 1.0 mL/min; and measurement wavelength, 280 nm. Catechin, Cg, gallocatechin, GCg, epicatechin, ECg, epigallocatechin, and EGCg in proportions of 40, 100, and 200 μg/mL with a purity of ≥98% (Kurita Water Industries Ltd, Tokyo) in 0.1 mol acetic acid-methanol/L were used as standards. The calibration curves were prepared from the peak area of 10 μL of each standard solution.

The water content of the test beverage was ascertained by measuring the weight before and after drying at atmospheric pressure and at 105 °C by using a forced-circulation warm-air dryer. The protein content of the test beverage was calculated by using nitrogen × 6.25, and the amount of nitrogen was measured by using the Kjeldahl method. For protein quantification, the amount of nitrogen in caffeine (amount of caffeine × 56/194, with 56 and 194 being the MW of 4 nitrogens and caffeine, respectively) was subtracted from the total amount of nitrogen.

For lipid quantification, the test beverage was deproteinized by using a 7% copper sulfate solution, and the pH was adjusted to 6–7; the sample then underwent extraction with diethylether for 16 h with the use of a Soxhlet extractor. The lipid content of the test beverage was ascertained by measuring the weight before and after removal of the solvent. The ash content was ascertained by measuring the weight of the sample with the use of the direct ash method by heating at 550 °C.

Dietary fiber was quantified by using the enzyme-weight method. Dried powder of the test beverage (10 g) was combined with 40 mL phosphate buffer (0.08 mol/L; pH 6.0) and 0.1 mL of a thermostable α-amylase (Termamyl 120 L; Novo-Nordisk A/S, Bagsværd, Denmark), which was then incubated in a boiling water bath for 30 min. After cooling, the solution was adjusted to pH 7.5 ± 0.1 with the use of sodium hydroxide and combined with 0.1 mL of 50 mg protease/mL (product no. P-5380; Sigma-Aldrich Co, St Louis) in phosphate buffer (pH 6.0), and the mixture was incubated at 60 °C for 30 min. After cooling, the solution was adjusted to pH 4.3 ± 0.3 with the use of hydrochloric acid and combined with 0.1 mL amyloglucosidase solution (product no. A-9913, Sigma-Aldrich Co), and the mixture was incubated at 60 °C for 30 min. Next, the solution was combined with 4 volumes of 95% ethanol at 60 °C, kept at room temperature for 1 h, and filtered. The residue was washed 3 times with 78% ethanol, ≥2 times with 95% ethanol, and ≥2 times with acetone, and then it was dried at 105 °C overnight, and the weight was measured. This procedure was repeated twice—first for measurement of the ash content after incineration treatment and again for protein quantification by using the Kjeldahl method. The dietary fiber content was calculated by subtracting the protein content from the ash content.

Sodium was measured by atomic absorption spectrometry at a wavelength of 589.6 nm with the use of an acetylene-air flame. To quantify the sodium content, the dried powder of the test beverage was combined with 1% HCl and allowed to stand overnight.

For ascorbic acid measurement, a 10% solution was filtered, and 1 mL of the filtrate was combined with 1 mL of 5% metaphosphoric acid. This solution was oxidized by combining the sample with a few drops of 0.2% dichlorophenolindophenol and 2 mL of 2% thiourea–5% metaphosphoric acid, which was followed by the addition of 0.5 mL of 2% 2,4-dinitrophenylhydrazone in 4.5 mol sulfuric acid/L. This solution was then heated at 40 °C for 16 h to produce osazone and combined with 3 mL ethyl acetate; the sample was shaken for 1 h and quantified by using HPLC. The HPLC conditions were as follows: column, Silica-1100-N (4.6 mm diameter × 100 mm length; Senshu Scientific Co, Ltd, Tokyo), column temperature, 35 °C; mobile phase, ethyl acetate:hexane:acetic acid:water = 60:40:5:0.05; flow rate, 1.5 mL/min; and measurement wavelength, 495 nm.

Caffeine was quantified by using HPLC after the dried powder was dissolved in methanol and adjusted to the specified volume. The HPLC conditions were as follows: column, Mightysil RP-18 Aqua (5 μm particle size; 4.5 mm × 150 mm diameter; Kanto Kagaku, Tokyo); column temperature, 50 °C; mobile phase, the ratio of 0.03 mol acetate buffer/L (pH 4) to acetonitrile = 850:30;

flow rate, 2.0 mL/min; and measurement wavelength, 270 nm. Tannin was measured as tannic acid by using the Folin-Denis method.

Carbohydrates were calculated as the residue of these substances. Energy was calculated by using the energy conversion coefficients (ie, protein, 16.7 kJ/g; lipid, 37.7 kJ/g; and carbohydrate, 16.7 kJ/g).

Protocol

Before the study, all subjects were taught to calculate their energy and lipid intakes by using 2 guidebooks, the *Standard Tables of Food Consumption in Japan, 4th edition* (25), and the *New Calorie Guide Book for Daily Diet* (26). The energy intake (EI) required for living activity strength 1 (a Japanese term, equivalent to a physical activity level of 1.3), based on the subject's advance activity report, was calculated by using the equation below from the standard table (25):

$$A\ (\text{kJ/d}) = 1.35B \times 10.9 \qquad (1)$$

where A is measured in kJ/d, $B = a \times \text{weight (kg)}^{0.444} \times \text{height (cm)}^{0.663} \times 88.83/10000 \times 24 \times 4.184$, and a ($\text{kcal/m}^2 \cdot \text{h}^{-1}$) is the standard value of basal metabolic rate at living activity strength 1 (37.5 for age 20–29 y: 36.5 for age 30–39 y: and 35.6 for age 40–49 y).

During the study period, the subjects were instructed to ingest 90% of the individual EI calculated above and to ingest 60 g lipids/d, which is the average intake in Japan, as calculated from the 1998 National Nutrient State report (27). They were instructed to stay within ± 10% of those amounts.

On weekdays (Monday–Friday), the subjects ate the same set menu for breakfast between 0730 and 0830 and the same set menu for supper between 1700 and 1800 at the company cafeteria. For lunch, the subjects could select one of several dishes on the cafeteria menu that were controlled for EI and lipid intake within the standard ranges given above. Lunch was eaten between 1130 and 1330. On weekends, holidays, and other days when they were unable to eat at the company cafeteria, the subjects controlled their own daily EI and lipid intake within the standard ranges by using the guidebooks.

The consumption of foods and beverages containing large amounts of catechins, polyphenols, or caffeine, such as green tea, oolong tea, wine, and coffee, was prohibited under the protocol. The subjects were allowed to drink ≤27.5 mL alcohol/d, which equals ≈500 mL beer/d. The subjects were advised to maintain their current level of exercise.

Anthropometry, computed tomography (CT), and inquiries by the occupational health physician were performed every 4 wk. Blood samples were collected during week 0 and week 12.

In the run-in period, dietary restriction was begun 2 wk before the start of consumption of the test beverage. The subjects were divided into 2 groups with similar BMI and waist circumference distributions, and the study was performed by using a double-blind protocol. The control group consumed the control beverage, and the GTE group consumed the beverage with high catechin content. The subjects consumed the contents of a 340-mL bottle of the test beverage during supper (within 30 min) daily for 12 wk after the run-in period.

Lifestyle check

The subjects recorded their daily dietary intakes in a dietary diary by using the calorie and lipid list of the cafeteria menu and the guidebooks. They also calculated their EI and lipid intakes. The dietary diary was retrieved every week, and the results were reported back to the subjects the next week. Daily activity and physical condition were recorded by using the checklist every 4 wk, and, depending on the report, the physician checked the subject's condition and gave advice.

Anthropometric measurements

Body weight, waist circumference, and hip circumference were measured. The body fat ratio was measured by using the bioimpedance analysis method (Tanita Body Fat Analyzer, model no. TBF-401; Tanita Co, Tokyo), and skinfold-thickness measurements were performed by using the caliper method. Body fat mass and lean body mass were calculated from the ratio of body weight to body fat. Waist circumference at the umbilical level was measured while the subjects were standing, according to the criteria of the Japan Society for the Study of Obesity. For skinfold-thickness measurements, the subcutaneous fat thickness was measured at the lower end of the scapula on the back and in the intermediate region on the extensor side of the arm, and the sum of the 2 values was calculated.

Measurement of fat by computed tomography

Within 3 d of the anthropometric measurements, the subjects underwent CT imaging (TCT-300; Toshiba Medical Co, Tokyo) of the abdominal transverse section at the L4-L5 level at the Yabuki Clinic (Tochigi, Japan). CT imaging was performed under the conditions for visceral fat measurement by using FAT SCAN software (version 2; N2 System Co, Osaka, Japan), which was developed on the basis of the method reported by Tokunaga et al (28). The X-ray conditions were tube voltage of 120 kVp and mAs of 360; the film was processed at a window level of 0 and a window width of 1000. By using the abovementioned software, the visceral fat area (VFA) and subcutaneous fat area (SFA) were obtained from the abdominal CT image, and these areas were summed to obtain the total fat area (TFA).

Blood sampling and clinical analysis

Alcohol consumption was prohibited starting 3 d before blood sampling, and eating or drinking anything other than water was prohibited after 2100 the day before sampling. Fasting blood was collected from a vein on the flexor side of the arm between 0900 and 1000. The blood samples were analyzed by SRL Inc (Tokyo).

Concentrations of the following variables were measured in week 0 and week 12: triacylglycerol (Pureauto S TG-N; Daiichi Pure Chemicals Co, Ltd, Tokyo), total cholesterol (L type Wako CHO · H; Wako Pure Chemicals Co, Ltd, Osaka, Japan), HDL cholesterol (Cholestest HDL; Daiichi Pure Chemicals Co, Ltd), LDL cholesterol (Cholestest LDL; Daiichi Pure Chemicals Co, Ltd), remnant-like lipoprotein cholesterol (RLP-cholesterol JIMRO II; Japan Immunoresearch Laboratories Co, Ltd, Gunma, Japan), free fatty acid [(FFA) NEFA-SS EIKEN; Eiken Chemical Co, Ltd, Tokyo], total ketone body (Total ketone body KAINOS; KAINOS Laboratories Inc, Tokyo), blood sugar (Quick auto neo GLU-HK; Shino-Test Co, Tokyo), insulin (EIKEN Insulin; Eiken Chemical Co, Ltd), leptin (Human Leptin RIA Kit; Linco Research Inc, St Charles, MO), and total plasminogen activator inhibitor-1 [(PAI-1) LPIA · tPAI test; Dia-Iatron Co, Ltd, Tokyo]. We measured vitamin A by using a modification of the method reported by Henry et al (29): the serum sample was

combined with methanol, mixed, and centrifuged. The supernatant was separated by ODS reversed-phase HPLC, and vitamin A was detected at excitation wavelengths of 325 nm and emission wavelengths of 480 nm. We measured vitamin E by using the method of Abe et al (30). We also measured malondialdehyde-modified LDL [(MDA-LDL) by using an enzyme-linked immunosorbent assay using anti–MDA-LDL antibody (ML25; 31)], aspartate transaminase (Transaminase-HR II; Wako Pure Chemical Co, Ltd), glutamic-pyruvic transaminase (Transaminase-HR II; Wako Pure Chemical Co, Ltd), blood urea nitrogen (Pureauto S UN; Daiichi Pure Chemicals Co, Ltd), calcium (Orthocresolphthalein complexone method; 32), iron (Neo Fe Shino-Test; Shino-Test Co, Tokyo), inorganic phosphate by using the method of Drewes et al (33), and sodium, potassium, and chlorine by using an ion-selective electrode method (34).

For blood sugar measurements, blood was collected by using a sodium fluorine–containing blood sampling tube and the plasma was separated. For PAI-1, blood was sampled by using a sodium citrate-containing blood sampling tube, and the plasma was separated by centrifugation (1900 × *g* for 15 min at 4 °C). For the other variables, blood was collected by using separating medium–containing blood sampling tubes, and the blood was centrifuged (1900 × *g* for 15 min at 4 °C) to separate the serum.

Statistical analysis

All evaluation variables were presented as means ± SEMs. For statistical analysis, STATVIEW for WINDOWS software (version 4.58; Abacus Concepts, Berkeley, CA) was used. Baseline measurements were assessed by using the unpaired *t* test. The effect of the GTE was assessed by using two-factor repeated-measures analysis of variance (ANOVA), and variables were measured each week. To compare the magnitude of the decreases in the anthropometric values and in body composition at the endpoint, the differences between the values at the initial measurement and those at week 12 were analyzed by using an unpaired *t* test. Linear regression analysis was used to evaluate the association between MDA-LDL and body fat variables at week 12. A P value < 0.05 was considered to be significant.

RESULTS

Analysis of the test beverages

The contents and analyzed ingredient values of catechin, Cg, gallocatechin, GCg, epicatechin, ECg, epigallocatechin, and EGCg in the test beverages are shown in **Table 1**. The total catechin content was 689.9 mg/340 mL beverage in the high-catechin beverage and 21.8 mg/340 mL in the control beverage.

Dietary condition of the subjects

Three subjects were excluded from the analysis because of a marked change in their living environment during the study (control group, $n = 18$; GTE group, $n = 17$). The daily EI was 8.7 ± 0.1 MJ at week 0 and 8.8 ± 0.1 MJ at week 12 in the control group and 8.6 ± 0.1 MJ at week 0 and 8.6 ± 0.1 MJ at week 12 in the GTE group. Those daily energy values were very close to the Japanese standard, which is 9.0–9.4 MJ in men 20–40 y old at living activity strength 1 (35), and 90% of the values were 8.3–8.5 MJ. The mean (± SE) daily lipid intake was 59.2 ± 0.8 g at week 0 and 61.4 ± 0.9 g at week 12 in the control group and 61.5 ± 1.4 g at week 0 and 59.6 ± 0.8 g at week 12 in the GTE group. There were no significant differences between the 2 groups. The mean values of each variable were calculated every 4 wk and analyzed by using a two-factor repeated-measures ANOVA, and there was no significant difference between groups.

TABLE 1
Composition of test beverages[1]

	Control beverage[2]	High-catechin beverage[2]
Total catechin (mg/100 mL)	6.4 (100.0)	202.9 (100.0)
Catechin (mg/100 mL)	0.5 (7.8)	13.5 (6.7)
Epicatechin (mg/100 mL)	0.3 (4.7)	13.1 (6.5)
Catechin gallate (mg/100 mL)	0.2 (3.1)	9.6 (4.7)
Epicatechin gallate (mg/100 mL)	0.0 (0.0)	15.1 (7.4)
Gallocatechin (mg/100 mL)	1.7 (26.6)	40.4 (19.9)
Epigallocatechin (mg/100 mL)	1.1 (17.2)	30.2 (14.9)
Gallocatechin gallate (mg/100 mL)	1.7 (26.6)	41.0 (20.2)
Epigallocatechin gallate (mg/100 mL)	0.9 (14.0)	40.0 (19.7)
Water (g/100 mL)	99.5	99.1
Protein (g/100 mL)	0.1	0.1
Fat (g/100 mL)	0.0	0.0
Carbohydrate (g/100 mL)	0.3	0.4
Dietary fiber (g/100 mL)	ND	ND
Ash (g/100 mL)	ND	ND
Sodium (mg/100 mL)	10	10
Ascorbic acid (mg/100 mL)	50	50
Tannin (mg/100 mL)	10	260
Caffeine (mg/100 mL)	23	22
Calories (kJ/100 mL)	6.7	8.4

[1] ND, not detectable.
[2] Percentage of total catechin in parentheses.

Effects on the anthropometric values and body composition

Because one subject in the control group did not consent to CT imaging at weeks 4 and 8, analyses of the control group for weeks 4 and 8 were performed in 17 subjects. There was no significant difference in the initial value of any variable between the 2 groups (**Table 2**). There was a significant time-by-group interaction for body weight, BMI, waist circumference, body fat mass, and SFA (Table 2). The decrease in waist circumference between the initial measurement and that at week 12 in the GTE group was significantly greater than that in the control group (−3.4 ± 0.5 cm and −1.6 ± 0.4 cm, respectively; Table 2). The decrease in skinfold thickness between the initial measurement and that at week 12 in the GTE group was significantly greater than that in the control group (−3.3 ± 0.7 mm and −1.3 ± 0.7 mm, respectively; Table 2). The decrease in TFA between the initial measurement and that at week 12 in the GTE group was significantly greater than that in the control group (−26.7 ± 6.0 cm^2 and −6.7 ± 5.8 cm^2, respectively; Table 2). The decrease in SFA between the initial measurement and that at week 12 in the GTE group was significantly greater than that in the control group (−16.7 ± 3.0 cm^2 and −4.4 ± 4.1 cm^2, respectively; Table 2).

Effects on blood variables and side effects

There was no significant difference between the groups in the initial value of any variable (**Table 3**). There was a significant

TABLE 2
Changes in anthropometric variables and body composition after consumption of either control or high-catechin beverages for 12 wk[1]

	Initial	4 wk	8 wk	12 wk	Change at 12 wk
Weight (kg)[2,3]					
Control group	73.8 ± 1.3	72.9 ± 1.3	72.7 ± 1.4	72.5 ± 1.4	−1.3 ± 0.3
GTE group	73.9 ± 1.8	72.6 ± 1.7	72.2 ± 1.7	71.5 ± 1.7	−2.4 ± 0.5
BMI (kg/m^2)[2,3]					
Control group	25.0 ± 0.4	24.7 ± 0.4	24.6 ± 0.4	24.6 ± 0.4	−0.4 ± 0.1
GTE group	24.9 ± 0.4	24.4 ± 0.4	24.3 ± 0.4	24.1 ± 0.4	−0.8 ± 0.2
Waist (cm)[2–4]					
Control group	87.8 ± 1.1	86.7 ± 1.1	86.6 ± 1.1	86.2 ± 1.2	−1.6 ± 0.4
GTE group	87.9 ± 1.4	86.6 ± 1.4	85.5 ± 1.3	84.5 ± 1.3	−3.4 ± 0.5
Hip (cm)[2]					
Control group	97.0 ± 0.8	95.9 ± 0.7	95.8 ± 0.8	95.8 ± 0.8	−1.1 ± 0.3
GTE group	97.4 ± 0.9	97.0 ± 0.9	96.0 ± 1.1	96.1 ± 1.1	−1.3 ± 0.3
Body fat mass (kg)[2,3]					
Control group	19.5 ± 1.0	18.8 ± 0.9	18.8 ± 1.0	18.8 ± 1.1	−0.7 ± 0.3
GTE group	19.7 ± 0.8	19.2 ± 0.9	18.0 ± 0.9	18.3 ± 0.9	−1.4 ± 0.3
Lean body mass (kg)[2]					
Control group	54.3 ± 0.7	54.1 ± 0.7	53.9 ± 0.7	53.7 ± 0.7	−0.6 ± 0.3
GTE group	54.2 ± 1.1	53.4 ± 1.0	54.1 ± 1.1	53.2 ± 1.0	−1.0 ± 0.4
Skinfold thickness (mm)[2,5]					
Control group	27.0 ± 1.5	25.3 ± 1.3	26.2 ± 1.5	25.7 ± 1.4	−1.3 ± 0.7
GTE group	27.9 ± 1.8	26.3 ± 1.6	25.9 ± 1.8	24.6 ± 1.5	−3.3 ± 0.7
TFA (cm^2)[2,5,6]					
Control group	261.0 ± 12.7	254.2 ± 13.1	246.4 ± 12.7	254.3 ± 13.6	−6.7 ± 5.8
GTE group	258.4 ± 11.0	246.3 ± 11.2	232.1 ± 9.9	231.7 ± 11.1	−26.7 ± 6.0
VFA (cm^2)[2,6]					
Control group	89.3 ± 5.8	88.9 ± 6.5	84.5 ± 5.0	87.0 ± 5.2	−2.4 ± 2.7
GTE group	83.1 ± 5.7	79.2 ± 5.4	73.2 ± 5.3	73.0 ± 5.3	−10.1 ± 4.0
SFA (cm^2)[2,3,5,6]					
Control group	171.7 ± 10.7	165.3 ± 10.7	161.9 ± 11.0	167.3 ± 11.0	−4.4 ± 4.1
GTE group	175.3 ± 8.2	167.1 ± 8.7	158.8 ± 8.3	158.7 ± 7.9	−16.7 ± 3.0

[1] All values are $\bar{x}$ ± SEM. GTE, green tea extract; TFA, total fat area; VFA, visceral fat area; SFA, subcutaneous fat area. Control group, $n = 18$; GTE group, $n = 17$. The initial values did not differ significantly between groups. Data from weeks 0, 4, 8, and 12 were compared by using two-factor repeated-measures ANOVA with time and group.
[2] Significant effect of time from week 0 to week 12, $P < 0.01$.
[3] Significant time-by-group interaction, $P < 0.05$.
[4,5] Significant difference between groups for change at 12 wk (unpaired t test): [4]$P < 0.01$, [5]$P < 0.05$.
[6] Control group, $n = 18$ at initial measurement and 12 wk; $n = 17$ at 4 and 8 wk.

time-by-group interaction for vitamin E and MDA-LDL concentrations (Table 3). Among the variables for which standard values for Japanese have been established (36), the mean values did not deviate from the standard values throughout the study period.

The occupational health physician assessed the subjects every 4 wk. The physician reported no side effects related to the study.

Effects on blood MDA-LDL and the association between MDA-LDL and body fat variables

Because the initial MDA-LDL values tended to differ between groups, the variable was compared on the basis of the change by week 12, and we took 100% as the initial value. The decrease in MDA-LDL between the initial measurement and that at week 12 in the GTE group was significantly greater than that in the control group (67.5 ± 6.1% and 89.5 ± 6.0%, respectively; $P < 0.05$). To investigate the association between MDA-LDL and body fat variables, linear regression analysis was performed within each group. The percentage value of MDA-LDL in week 12 was used as an independent value, and the percentage values of each anthropometric variable and abdominal fat area in week 12 were used as dependent values. In the GTE group, the percentage value of MDA-LDL at week 12 was significantly associated with the percentage values of body fat mass ($R^2 = 0.646$, $P = 0.0001$) and TFA ($R^2 = 0.273$, $P = 0.0313$) (**Figure 1**). In the control group, however, those associations were not observed ($P > 0.05$).

DISCUSSION

In the current study, the samples were prepared in beverage form so that the subjects could easily consume them every day for 12 wk. The base beverage was oolong tea, which is the most widely sold tea in Japan (37). The average consumption of green tea and of catechins in Japan is calculated to be ≈2 g tea leaves/d and 200–400 mg catechin/d (6). Before conducting this study, for clarification of the effect of catechins, the caffeine content in the test beverages was adjusted to a minimal amount, and the consumption of beverages and foods containing large amounts of catechins or caffeine was prohibited so as to minimize the effect of other food-derived catechins and caffeine. Dietary control, aimed at weight loss for both groups, was included as a benefit for subjects in the control group: the standard daily EI was set to 90% of the value calculated from the body surface area and daily living

TABLE 3
Changes in blood variables after consumption of either control or high-catechin beverages for 12 wk[1]

	Control group ($n = 18$)		GTE group ($n = 17$)	
	Initial	12 wk	Initial	12 wk
Triacylgylcerol (mmol/L)	1.38 ± 0.11	1.41 ± 0.12	1.29 ± 0.21	1.28 ± 0.16
Cholesterol (mmol/L)[2]				
Total	4.77 ± 0.16	5.07 ± 0.18	4.76 ± 0.15	4.92 ± 0.21
HDL	1.23 ± 0.07	1.30 ± 0.08	1.23 ± 0.06	1.29 ± 0.06
LDL	3.27 ± 0.13	3.10 ± 0.14	3.38 ± 0.18	2.99 ± 0.16
RLP	0.11 ± 0.01	0.12 ± 0.01	0.10 ± 0.01	0.10 ± 0.01
Free fatty acid (mmol/L)	0.44 ± 0.03	0.40 ± 0.04	0.40 ± 0.03	0.44 ± 0.03
Total ketone body (μmol/L)	47.3 ± 7.1	57.7 ± 10.7	51.3 ± 8.6	69.4 ± 11.6
Blood sugar (mmol/L)[2]	5.19 ± 0.12	5.47 ± 0.18	5.03 ± 0.07	5.15 ± 0.09
Insulin (pmol/L)[2]	60.7 ± 5.2	78.4 ± 8.5	67.5 ± 15.4	72.6 ± 10.5
Leptin (mg/L)[2]	0.04 ± 0.00	0.05 ± 0.01	0.03 ± 0.00	0.04 ± 0.00
PAI-1 (mg/L)	0.19 ± 0.03	0.19 ± 0.02	0.23 ± 0.04	0.18 ± 0.03
Vitamin A (IU/L)	1952 ± 89	2017 ± 98	1971 ± 127	1987 ± 143
Vitamin E (μmol/L)[2,3]	32.2 ± 1.3	30.3 ± 1.6	31.5 ± 1.4	27.1 ± 1.0
Malondialdehyde-modified LDL (U/L)[2,3]	158 ± 11	134 ± 8	193 ± 14	123 ± 9
Aspartate transaminase (μkat/L)[2]	0.42 ± 0.04	0.34 ± 0.03	0.38 ± 0.02	0.33 ± 0.02
Glutamic-pyruvic transaminase (μkat/L)[2]	5.95 ± 0.81	4.98 ± 0.65	5.09 ± 0.59	4.42 ± 0.40
Blood urea nitrogen (mmol/L)	4.95 ± 0.20	4.95 ± 0.22	4.74 ± 0.20	4.73 ± 0.20
Calcium (mmol/L)	5.05 ± 0.05	5.08 ± 0.03	5.04 ± 0.06	5.02 ± 0.04
Iron (mmol/L)	22.2 ± 1.4	22.4 ± 1.7	26.0 ± 2.2	27.2 ± 2.9
Inorganic phosphate (mmol/L)[2]	1.09 ± 0.04	1.21 ± 0.04	1.06 ± 0.04	1.22 ± 0.03
Sodium (mmol/L)	144 ± 1	143 ± 0	143 ± 1	143 ± 0
Potassium (mmol/L)	4.24 ± 0.06	4.37 ± 0.07	4.30 ± 0.11	4.28 ± 0.06
Chlorine (mmol/L)	106 ± 1	105 ± 0	105 ± 0	105 ± 0

[1] All values are $\bar{x}$ ± SEM. GTE, green tea extract; RLP cholesterol, remnant-like particles of cholesterol; PAI-1, plasminogen activator inhibitor 1. The initial values did not differ significantly between groups. Data from weeks 0 and 12 were compared by using two-factor repeated-measures ANOVA with time and group.
[2] Significant effect of time from week 0 to week 12, $P < 0.05$.
[3] Significant time-by-group interaction, $P < 0.05$.

activities. Under these conditions, body weight, BMI, waist circumference, body fat mass, and SFA were significantly lower in the GTE group than in the control group. At week 12, the decrease in waist circumference, skinfold thickness, TFA, and SFA from the initial measurements was significantly greater in the GTE group than in the control group. The percentage difference in the changes in weight, BMI, waist circumference, body fat mass, skinfold thickness, TFA, and SFA between the GTE and control groups was 1.5%, 1.5%, 2.0%, 3.7%, 6.9%, 7.9%, and 7.5%, respectively. These results indicate that catechins not only promoted EE (18, 19) but also mildly reduced body fat in humans, as suggested by Chantre et al (21). Substances related to lipid metabolism and metal ions in the blood were also investigated, but there was no significant difference between the groups in any variable except vitamin E and MDA-LDL; nor were there any side effects.

Dulloo et al (18) observed that the addition of 200 nmol EGCg/mL increased thermogenesis in brown fat tissue of rat and that there was a synergistic effect between caffeine and EGCg. They also found that consumption of GTE (270 mg EGCg/d) increased EE and the oxidation of lipids in another human study (19). In those reports, they suggested that those effects were due to an inhibitory effect of catechins on the activity of catechol-*O*-methyltransferase (COMT), which is a catecholamine-degrading enzyme, and to an inhibitory effect of caffeine on phosphodiesterase that results in an increase in noradrenalin-induced thermogenesis and the maintenance of that increase (20). Other studies, however, reported that plasma EGCg concentrations were only ≈4.5 nmol/mL after the intake of 525 mg EGCg/d in humans (38), and that the maximum in vitro inhibitory effect on the activity of COMT by 100 μmol flavonoids/L was 64% (39). These results indicate that the increase in thermogenesis and the reduction in body fat in humans cannot be completely explained by an inhibitory effect on COMT, and therefore another mechanism must be involved in the decrease in body fat induced by catechins.

Chemically modified, degenerated lipoproteins such as oxidative stress–induced MDA-LDL and 4-hydroxynonenal lysine–LDL are observed in atheroma in humans and rabbits (40). Holvoet et al (41) measured plasma MDA-LDL in humans and suggested both that an increase in plasma MDA-LDL can be used as a marker of unstable atherosclerotic cardiovascular disease and that blood MDA-LDL is an independent factor not correlated with LDL cholesterol. Previously, MDA-LDL was measured indirectly by using TBARS, and there are many reports that catechins prevent an increase in serum TBARS. In 2 reports, increases in BMI were significantly correlated with concentrations of TBARS (22) and malondialdehyde (23) in blood.

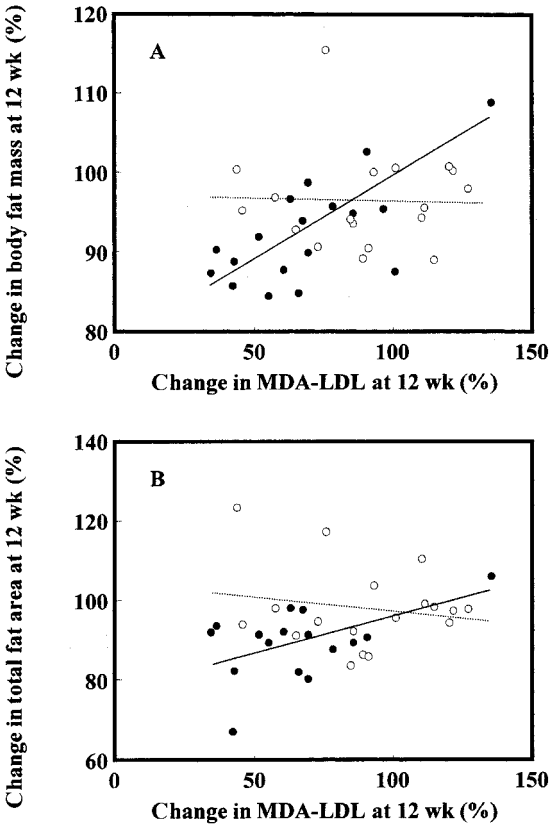

FIGURE 1. Associations between the percentage of malondialdehyde-modified LDL (MDA-LDL) concentration at week 12 and the percentage of body fat mass (A) and total fat area (B) at week 12 in the green tea extract (GTE) group (● and —; $n = 17$) and the control group (○ and - - -; $n = 18$).A: GTE group, $y = 78.62 + 0.21\times$ ($R^2 = 0.646$, P of slope $= 0.0001$); control group, $y = 97.07 - 0.07\times$ ($R^2 = 0.001$, P of slope $= 0.9080$). B: GTE group, $y = 77.36 + 0.18\times$ ($R^2 = 0.273$, P of slope $= 0.0313$); control group, $y = 104.41 - 0.08\times$ ($R^2 = 0.034$, P of slope $= 0.4664$).

The current studies suggest that obesity might be related to an increase in lipid oxidizability. In addition, there is an interaction between nuclear factor-κ B and peroxisome proliferator–activated receptors (PPARs). PPARs are important transcription factors for lipid metabolism; for example, mRNA of β-oxidation enzymes is up-regulated by PPAR-α (42–45). Because nuclear factor-κ B is regulated by a redox regulatory system, it is possible that such a system also regulates body fat metabolism. This hypothesis led us to measure MDA-LDL by using an enxyme-linked immunosorbent assay and an anti–MDA-LDL antibody (ML25) to investigate the antioxidizing activity of ingested catechins and also to compare body fat variables. In the GTE group, the changes in the concentrations of MDA-LDL were positively associated with the changes in body fat mass and TFA. These results suggest that the accumulation of body fat might be associated with an increase in lipid oxidizability and that a redox regulatory system might be involved individually in the body fat–and MDA-LDL–reducing effects of catechins. Further investigation is needed to clarify the relation between a redox regulatory system and the body fat–reducing mechanism.

Catechins reduce serum lipids by inhibiting small-intestine micelle formation in animals (46), and they limit the absorption of sugars by inhibiting α-glucosidase activity (47). There was no significant difference in serum lipids or blood sugar between the groups; however, the initial values might have been too low for any effects to be detected. Thus, the amount of catechins ingested in this study might not have been sufficient to inhibit micelle formation or α-glucosidase activity.

In conclusion, long-term consumption of beverages containing catechins inhibits the formation of oxidized lipids such as MDA-LDL, and this is a risk factor for developing arteriosclerosis. Moreover, catechin intake decreases body fat. These results suggest that catechins contribute to the prevention of and improvement in various lifestyle-related diseases, particularly obesity. These findings also suggest that regulation of a redox regulatory system might influence the accumulation of body fat.

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TN, YK, SS, and SM conducted the study. YK and SS contributed to data collection and database management. SM and TH assisted in the statistical analyses and contributed to data analyses. YH provided the samples, and TN designed and contributed to the writing of the manuscript. IT served as laboratory director, oversaw the writing of the manuscript, and was responsible for the diagnosis and recruitment of participants. None of the authors of this manuscript had any conflicts of interest.

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