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"Benifuuki" green tea, containing O-methylated EGCG, reduces serum low-density lipoprotein cholesterol and lectin-like oxidized low-density lipoprotein receptor-1 ligands containing apolipoprotein B: A double-blind, placebo-controlled randomized trial

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ABSTRACT

Dyslipidaemia is a significant cardiovascular risk factor. Green tea catechins are known to have cholesterol-lowering effects. We investigated the beneficial effects of "Benifuuki," containing O-methylated catechin, on cardiovascular risk factors, specifically low-density lipoprotein (LDL) cholesterol levels. One-hundred fifty-five participants who met the inclusion criteria were divided into 3 groups: "Benifuuki," "Yabukita," or barley infusion drinkers. We evaluated the changes in parameters after 12 weeks. Serum LDL cholesterol levels in the "Benifuuki"-consuming participants were significantly lower than those in barley infusion-consuming participants without a green tea habit. Furthermore, the lectin-like oxidized low-density lipoprotein receptor-1 containing apolipoprotein B (LAB) levels in "Benifuuki" drinkers were significantly lower than those in the barley infusion group and the "Benifuuki" baseline LAB level. In participants without a green tea habit, "Benifuuki" significantly reduced the serum LDL cholesterol level and the LAB levels compared to those observed after barley infusion consumption.

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Abbreviations: EGCG, (–)-Epigallocatechin-3-O-gallate; EGCG″3Me, (–)-epigallocatechin-3-O-(3-O-methyl)-gallate; HDL, high-density lipoprotein; LAB, lectin-like oxidized low-density lipoprotein receptor-1 containing apolipoprotein B; LDL, low-density lipoprotein; LOX-1, lectin-like oxidized low-density lipoprotein receptor-1

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1. Introduction

Recent lifestyles have caused a worldwide increase in obesity, hypertension, hyperlipidaemia, and cardiovascular disease (Braitman, Adlin, & Stanton, 1985; Ford, Giles, & Dietz, 2002; Kannel, Dawber, Kagan, Revotskie, & Stokes, 1961). Hyperlipidaemia is the most significant risk factor for cardiovascular disease, and it is well known that lowering serum low-density lipoprotein (LDL) cholesterol levels helps prevent cardiovascular disease (Pedersen et al, 1994).

Green tea is a popular beverage in Japan, and some studies have shown that increased consumption of green tea can reduce cardiovascular events (Kuriyama et al., 2006). Green tea catechin, one of the polyphenols present in green tea, plays an important role in the mechanism underlying this reduction in the risk of cardiovascular events. Catechin prevents lipase activity and lipid micellization in the process of digestion, and decreases serum cholesterol and triglyceride levels (Ikeda et al., 1992, 2005; Muramatsu, Fukuyo, & Hara, 1986; Sone et al., 2011; Wu et al., 2012). Green tea catechins consist mainly of (–)-epicatechin (EC), (–)-epigallocatechin (EGC), (–)-epigallocatechin-3-O-gallate (EGCG), and (–)-epicatechin-3-O-gallate (ECG). Of these four components, EGCG decreases the serum cholesterol level the most (Koo & Noh, 2007). Besides green tea, barley infusion (*Hordeum vulgare*) is widely consumed in Japan. However, unlike green tea, such as "Yabukita (*Camellia sinensis* var. Yabukita)" that contains high levels of EGCG, barley infusion does not contain any catechin. The composition of catechins in green tea varies depending on the source of the tea leaves, cultivation conditions, and processing. The green tea "Benifuuki (*Camellia sinensis* var. Benifuuki)" contains not only EGCG but also (–)-epigallocatechin-3-O-(3-O-methyl)-gallate (EGCG″3Me) in abundance. The latter is absent in "Yabukita," which is consumed the most in Japan.

EGCG″3Me is absorbed within the digestive tract more efficiently than EGCG and has a high retention in the blood (Maeda-Yamamoto, Ema, & Shibuichi, 2007). Hence, EGCG″3Me is expected to have higher physiological activity. In addition, its inhibitory effect on histamine release and its potent anti-allergic activity have been reported (Maeda-Yamamoto et al., 2009). Furthermore, methylated catechins have anti-obesity, anti-tumour, and anti-inflammatory effects (Inagaki et al., 2009), which also explain the interest in "Benifuuki" as a health-improving beverage. However, it is not yet clear whether "Benifuuki" has a beneficial effect on lipid metabolism, especially on the LDL cholesterol level. Therefore, in the present study, we investigated this potential effect of "Benifuuki" and compared the results to those obtained with "Yabukita" and barley infusion.

2. Materials and methods

2.1. Participants

By local advertisement and cooperation with other hospitals, 10160 healthy volunteers were enrolled in the study. Before enrolment, the participants underwent thorough screening, including verification of their medical history, physical examination, and clinical laboratory tests (including blood chemistry) (Fig. 1). Only healthy participants who had no current illnesses

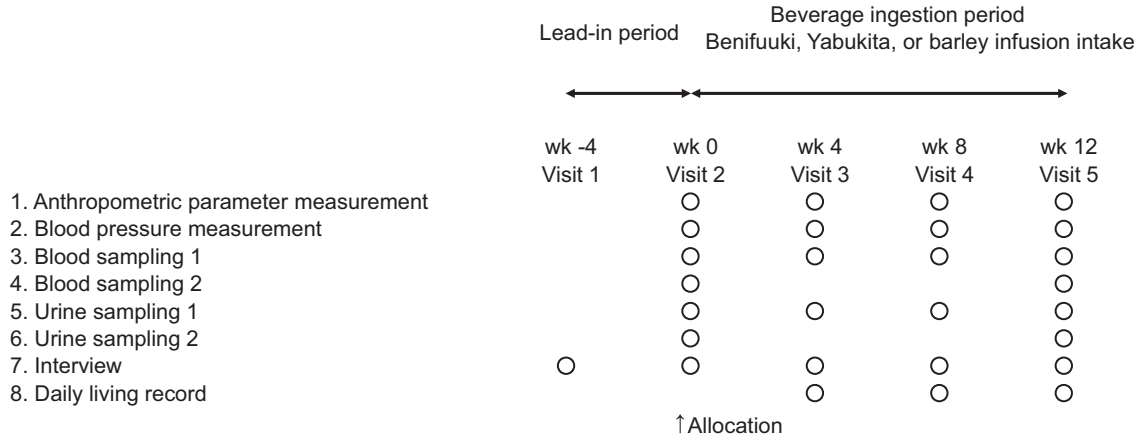

Fig. 1 – Schematic representation of the study protocol. (1) Anthropometric parameter measures included height, body weight, waist circumference, and body fat ratio. (2) The systolic blood pressure, diastolic blood pressure, and heart rate were measured. (3) The following parameters were measured in blood sample 1: triglycerides, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, haemoglobin A1c, glucose, immunoreactive insulin, γ-glutamyl transpeptidase, aspartate aminotransferase, alanine aminotransferase, iron, white blood cell count, red blood cell count, haemoglobin, haematocrit, and platelet count. (4) The following parameters were measured in blood sample 2: ferritin, high-sensitive C-reactive protein, malondialdehyde-modified low-density lipoprotein, soluble lectin-like oxidized LDL receptor-1, lectin-like oxidized LDL receptor-1 ligand containing apolipoprotein B, LOX-index, MCP-1, TNF-a, pentosidine, and adiponectin. (5) The following parameters were measured in urine sample 1: qualitative assessment of glucose, protein, urobilinogen, ketone bodies, and blood. (6) The following parameters were measured in urine sample 2: urinary 8-OH-dG. (7) In the subject interviews, a physician evaluated the physical condition and presence of symptoms of adverse effects due to catechin ingestion. (8) The subjects recorded the intake of the test beverage and the daily symptoms.

with high LDL cholesterol levels were enrolled. Individuals within an age range of 20 to 80 years and who met the following inclusion criteria were included: a serum LDL cholesterol level over 3.10 mmol/L and body mass index (BMI) over 25 kg/m^2. The exclusion criteria included current treatment for arrhythmia, hepatic damage, chronic kidney disease, cerebrovascular disease, rheumatism, diabetes mellitus, lipid disorder, and/or anaemia; a history of severe allergy to specific foods and drugs or a history of heart failure or cardiac infarction; pregnancy or intention to become pregnant; lactation; and other conditions judged by the doctor to be unsuitable for inclusion (e.g., use of supplements affecting lipid parameters [so-called dietary supplements]). One hundred and sixty-nine participants (64 men and 105 women), aged between 24 and 72 years (mean age: 52.6 ± 10.7 years, median: 51 years), were recruited from late October 2013 to March 2014. The complete date range for patient recruitment and follow-up was from 27 October 2013 to 31 May 2014.

The participants were residents of communities in Osaka, Kyoto, and Hyogo Prefecture in Japan. The baseline sampling was performed from November 2013 to March 2014. The follow-up period was 12 weeks. The study was started after the number of participants enrolled had reached the required number as planned beforehand.

2.2. *Setting*

The study was conducted according to the principles of the "Declaration of Helsinki." All participants in the study gave their informed consent. The study was a randomized, double-blind, placebo-controlled trial and was approved by the Ethics Committees of Osaka Medical College (No. 1285) (date of approval: from 3 September 2013 to 31 March 2016) and Osaka Medical College Health Science Clinic (No. 2011-CR-10) (date of approval: 29 June 2013). The trial was registered at the University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR, No. UMIN000011901). The authors confirm that all on-going and related trials for this drug/intervention are registered.

2.3. *Interventions, randomization, and blinding*

"Benifuuki" green tea was used as the active test tea, and "Yabukita" green tea or barley infusion as the placebo tea. All three types of teas were manufactured by Asahi Soft Drinks, Co., Ltd. (Tokyo, Japan). Barley infusion is not obtained from *Camellia*, but it is made from the extract of the plant, like green tea. We used barley infusion, which does not contain catechins, as one of the control beverages in the study. The tea was served as an extract powder. The extracted tea powder (100 mg) was added to 25 mL of distilled water, centrifuged at 1200 *g* for 5 min at 4 °C, and the supernatant was diluted five-fold with distilled water. Twenty microlitres of the sample, filtered through a membrane filter (DISMIC-13HP, PTFE; pore size = 0.45 μm; Advantec, Osaka, Japan), was injected into a high-performance liquid chromatography (HPLC) apparatus. HPLC was performed with a Shimadzu LC-10A pump coupled with a UV–vis detector (SPD-M10Avp; Shimadzu Corp., Kyoto, Japan) using a reverse-phase Wakopak Navi C18-5 column (4.6 mm i.d. × 150 mm; granule diameter, 5 μm; Wako Pure Chemical Industries, Ltd., Osaka, Japan) with Wakopak Navi C18-5 (4.6 mm i.d. × 10 mm; granule diameter, 5 μm; Wako Pure Chemical Industries, Ltd., Osaka, Japan) as a guard column and eluted with an eluent (as described below) at a flow rate of 1 mL/min at 40 °C. Catechin levels were measured at 272 nm. HPLC analysis was performed using a linear gradient system with mobile phase A (DW/MeCN/H3PO4, 400:10:1) and mobile phase B (MeOH/mobile phase A, 1:2). Linear gradient elution was performed as follows: 100% mobile phase A for 2 min; 20% mobile phase A for 27 min; maintain 20% mobile phase A for 10 min; and return to 100% mobile phase A for 7 min. Quantification was carried out using the external standard method. Quantification of catechins and caffeine was performed after data acquisition. Each 9 g of tea extract powder contained catechins and *O*-methylated EGCG in the following amounts, respectively: barley infusion extract powder, 0.0 mg and 0.0 mg; "Yabukita" green tea extract powder, 603 mg and 0.0 mg; and "Benifuuki" green tea extract powder, 607.5 mg and 49.5 mg. Each aluminium pack contained 3 g powder, which was for one-time use. The 9 g (3 g × 3) sample of the test tea was the daily dose. The participants were instructed to consume 3 g of test tea extract powder three times a day at mealtimes for 12 weeks by dissolving the powder in 200 mL of water (a daily total of 9 g tea extract powder and 600 mL of water). The catechin and caffeine contents of the test beverages are presented in Table 1. The participants were prohibited from consuming any tea other than the test or placebo tea during the study period; however, they were allowed to consume any non-tea beverages.

A computer-generated randomization sequence allocated participants in a 1:1:1 ratio into the barley infusion, "Yabukita" green tea, or "Benifuuki" green tea groups by the permuted block

Table 1 – The catechins and caffeine content of the test beverages per day.

Component	"Benifuuki" (mg)	"Yabukita" (mg)	Barley infusion (mg)
O-methylated EGCG (EGCG″3Me + GCG″3Me)	49.5	0	0
(–)-Epigallocatechin-3-O-(3-O-methyl)-gallate (EGCG″3Me)	42.3	0	0
Gallocatechin-3-O-(3-O-methyl)-gallate (GCG″3Me)	7.2	0	0
Eight types of catechins	558	603	0
Gallocatechin (GC)	39.6	52.2	0
(–)-Epigallocatechin (EGC)	147.6	132.3	0
Catechin (C)	11.7	27	0
(–)-Epicatechin (EC)	54	40.5	0
(–)-Epigallocatechin-3-O-gallate (EGCG)	210.6	243	0
Gallocatechin-3-O-gallate (GCG)	34.2	57.6	0
(–)-Epicatechin-3-O-gallate (ECG)	55.8	44.1	0
Catechin-3-O-gallate (CG)	4.5	6.3	0
Total catechins	607.5	603	0
Caffeine	125.1	129.6	0

method (block size was kept constant). Each tea was wrapped individually in an aluminium package labelled with an allocation number, which was created by a third party not directly involved in the study to ensure that the study investigators and participants were blinded to the type of tea provided.

2.4. *Primary and secondary endpoints*

The original primary endpoints were serum total cholesterol and serum LDL cholesterol levels. The secondary endpoints were (1) body weight, body fat ratio [measured using the body composition analyser (bioelectrical impedance analysis [BIA]; InnerScan® 50V, Tanita Co., Tokyo, Japan)], waist, and blood pressure [measured twice after a 3-minute rest using a sphygmomanometer (ES-P2000BR®, Terumo Co., Tokyo, Japan)]; (2) triglyceride levels, lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) index (obtained by multiplying the LAB [LOX-1 ligand containing ApoB] by the sLOX-1 [soluble LOX-1] level); (3) fasting plasma glucose, HbA1c (described by both the NGSP HbA1c [as %] and SI, IFCC-recommended units [as mmol/mol)), and insulin levels; (4) high sensitivity C-reactive protein (CRP), catechin-sensing molecule level; (5) each tea's *in vivo* antioxidant action, and pentosidine and urinary 8-OHdG level; (6) levels of the cytokines monocyte chemoattractant protein-1 (MCP-1), tumour necrosis factor (TNF) α, high sensitivity CRP, and adiponectin; (7) aspartate aminotransferase (AST), alanine aminotransferase (ALT), and γ glutamyl transpeptidase (γGTP) level; (8) serum iron and ferritin level; (9) adiponectin level; (10) urinalysis; and (11) diet survey. The participants were requested to fill in questionnaires on their health, lifestyle, and food intake. They were also asked to fill in a questionnaire on the strength of the green tea that they usually drank and the frequency of green tea consumption. Biochemical tests were performed in the laboratory of the LSI Medience Co., Tokyo, Japan. We employed the Food Frequency Questionnaire as used in the Osaki National Health Insurance cohort (Kuriyama et al., 2006) to evaluate the intake of tea catechins aside from those present in the test beverages. We evaluated each endpoint after analysis of all the data. We evaluated the participants' daily catechin intake via a diet survey before the start of the study, and we performed a subgroup analysis according to the presence or absence of a habit of daily tea drinking.

2.5. *Participant instructions*

Overeating and over-drinking were avoided as much as possible during the study period from one week before the start of the test beverage ingestion. Participants were instructed not to change their everyday lifestyle, including eating habits, smoking, and exercise, as much as possible. All participants were instructed not to consume unregulated drugs and supplements, and other types of tea, which could have a potential influence on the body fat or serum lipid level measured during the examination period from one week before the start of test beverage ingestion. The participants were told that they were not permitted to have a meal but could drink water after 22:00 on the night preceding examination. They were further instructed to keep a record of their adherence to the test beverage and their physical condition (daily living record).

2.6. *Sample size determination*

The target number of cases was determined by referring to previous human intervention trials. LDL-cholesterol and oxidized LDL cholesterol levels were significantly reduced by daily catechin ingestion as shown in human intervention trials of 22 to 240 participants (group size was 22 to 120 participants) (Hirano-Ohmori et al., 2005; Inami et al., 2007; Nagao, Hase, & Tokimitsu, 2007). It is also known that green tea catechins reduce the serum cholesterol levels of hyperlipidaemic patients by a mean value of about 0.129 mmol/L (5 mg/dL). Previous studies on the effect of "Benifuuki" on lipid metabolism did not exist. "Benifuuki," which is similar to "Yabukita" but with a greater level of O-methylated catechins, is expected to be as or more potent and could reduce mean serum cholesterol levels by approximately the same degree as other green teas. This observed reduction is regarded as a clinically relevant improvement. Moreover, clinical knowledge suggests that the standard deviation of the reduction in serum cholesterol levels with both types of tea is 0.103 mmol/L (4 mg/dL) (Kim et al., 2011). Sample size calculations were based on the primary outcomes of serum total cholesterol and LDL cholesterol levels. The sample size calculations assumed that the statistical analysis would be based on a comparison between the barley infusion, "Yabukita," and "Benifuuki" groups (assuming 13% failure rate in each group). The significance level was set at $\alpha = 0.05$. If there is truly no difference in the change in serum total cholesterol and LDL cholesterol levels between the barley infusion, "Yabukita," and "Benifuuki" groups, then 35 participants are required for 80% confidence. The calculated number of 150 participants in the present human intervention trial was based on these previous studies.

2.7. *Statistical methods*

The significance of the differences in the mean values and in the proportions of the parameters between the barley infusion, "Yabukita" green tea, and "Benifuuki" green tea groups was assessed using the Kruskal–Wallis test, Dunn multiple comparison test, and the chi-square test as appropriate (JMP® v11.2.1; SAS Institute Inc., Cary, NC, USA). The significance of the differences in the mean values and in the proportions of the parameters between pre- and post-intervention was assessed by paired *t*-test as appropriate. Parameters that showed a significant difference in the Kruskal–Wallis test were analysed by the Dunn's multiple comparison test. The significance level was set at $p < 0.05$. The intention-to-treat analysis was also performed. Subgroup analysis was performed between the two participant groups with and without a habit of daily tea drinking. All participants were prohibited from consuming any tea other than the test tea, irrespective of their habit of daily tea drinking, during the study period, and such prohibition might have affected the outcomes.

3. Results

3.1. *Participant flow and follow-up*

A total of 169 participants were allocated to the "Benifuuki" group (n = 56), "Yabukita" group (n = 60), and barley infusion

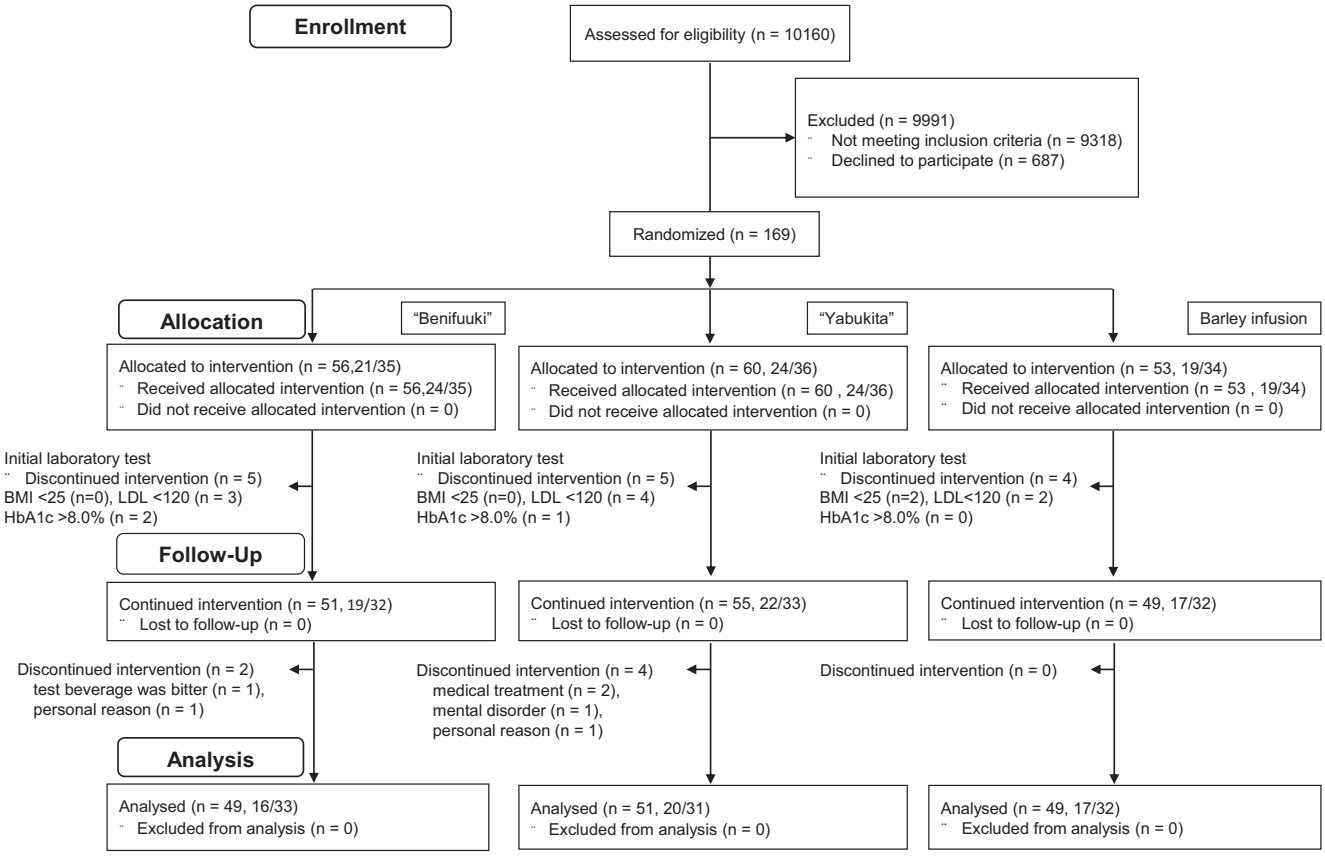

Fig. 2 – Flowchart of the study (n = all number, men/women).

group (n = 53). The study lasted 12 weeks. Of these participants, 20 were excluded from the study for the following reasons: (1) At baseline, 14 participants (5 in the "Benifuuki," 5 in the "Yabukita," and 4 in the barley infusion group) did not meet the inclusion criteria. (2) During the study period, 1 participant in the "Benifuuki" group did not tolerate the bitterness of the test tea, and 1 other consumed tea other than the test tea. (3) Lastly, 4 participants in the "Yabukita" group commenced drug treatment for hypertension or dyslipidaemia, had psychological reasons to leave the study, or experienced a substantial change in their living environment. Consequently, 149 participants (53 men and 96 women; 49 [16 men and 33 women] in the "Benifuuki" group, 51 [20 men and 31 women] in the "Yabukita" group, and 49 [17 men and 32 women] in the barley infusion group) completed the study (Fig. 2). These participants all met the inclusion and exclusion criteria, and none of them was taking any drugs for dyslipidaemia.

3.2. *Baseline data and outcome*

At baseline, among several of the parameters evaluated, only the γGTP and LAB levels were significantly different between the three groups (Table 2) (Supplementary Table S1). None of the subjects took methylated catechin before the start of the trial, and the amount of total catechin intake showed no significant difference among the three groups in pre-intervention by the Kruskal–Wallis test (Benifuuki 280.0 ± 423.1 mg/day, Yabukita 451.9 ± 769.9 mg/day, barley infusion 294.1 ± 578.8 mg/day; p = 0.7260). The intake rate of the test tea was 96.0 ± 5.4%, 94.9 ± 6.5%, and 96.1 ± 5.8% in the "Benifuuki," "Yabukita," and barley infusion groups, respectively, and there was no significant difference between the three groups.

Among the three groups, only the "Benifuuki" group showed a significant decrease in the serum cholesterol and LDL cholesterol levels at the end of the study compared with those at baseline (Table 2). At the end of the 12-week study, the primary endpoints, serum total cholesterol levels, and LDL cholesterol levels did not differ significantly between the three groups (Figs 3 and 4). Among the secondary endpoints, the LAB level significantly decreased in the "Benifuuki" group at the end of the study compared with that at baseline (Fig. 5). In addition, the LAB level significantly decreased at the end of the study in the "Benifuuki" group compared with that in the barley infusion group ("Benifuuki" vs. barley infusion = –1.0 ± 2.6 mg cs/L vs. 0.2 ± 3.3 mg cs/L, p = 0.0405) (Table 3) (Fig. 5). No significant difference in the urinalysis, participant interview, and daily living record was observed between the three groups (data not shown).

No significant difference in the daily calorie intake at the start and the end of the study was observed in the "Benifuuki" group, as evaluated using the Food Frequency Questionnaire. In contrast, the "Yabukita" and barley infusion groups showed a significant decrease in the daily calorie intake at the end of the study compared with that at baseline.

Table 2 – Baseline characteristics and changes in the anthropometric values and biochemical parameters after drinking "Benifuuki," "Yabukita," or barley infusion for 12 weeks. *ALL*, all participants; *YES*, participants with a habit of daily tea drinking; *NO*, participants without a habit of daily tea drinking.

	Interventions	Week 0	Week 12	Δ value at week 12[a]	Paired *t* test	p-value of multiple comparison test[c]		
						Drinking tea habit		
					p-value[b]	*ALL*	*YES*	*NO*
Baseline characteristics								
Sex (men/women)	"Benifuuki"	16/33						
	"Yabukita"	20/31				0.7811	0.6569	0.7649
	Barley infusion	17/32						
Age	"Benifuuki"	51.3 ± 10.4						
	"Yabukita"	52.4 ± 10.4				0.5375	0.4297	0.9558
	Barley infusion	53 ± 10.6						
Diet survey								
Total daily intake	"Benifuuki"	1979 ± 173.2	1962.6 ± 200.3	−16.4 ± 112.6	0.3127			
(kcal/day)	"Yabukita"	2081.5 ± 294.0	2027.0 ± 283.9	−54.5 ± 127.1	0.0035**	0.4200	0.4626	0.3466
	Barley infusion	2060.6 ± 271.3	2005.3 ± 258.3	−55.4 ± 169.5	0.0267*			
Protein (g/day)	"Benifuuki"	80.3 ± 7.3	79.5 ± 7.8	−0.8 ± 4.5	0.2249			
	"Yabukita"	81.8 ± 9.1	80.6 ± 8.5	−1.2 ± 5.8	0.1407	0.5153	0.8263	0.3445
	Barley infusion	82.7 ± 11.0	79.4 ± 8.6	−3.4 ± 8.4	0.0072**			
Fat (g/day)	"Benifuuki"	61.1 ± 5.4	60.3 ± 4.9	−0.8 ± 3.2	0.0970			
	"Yabukita"	61.8 ± 5.3	61.5 ± 5.5	−0.3 ± 3.8	0.5711	0.9208	0.9756	0.6951
	Barley infusion	62.4 ± 6.3	60.8 ± 5.2	−1.7 ± 5.5	0.0415*			
Carbohydrate (g/day)	"Benifuuki"	260.4 ± 22.9	257.3 ± 32	−3.1 ± 28.5	0.4467			
	"Yabukita"	285.8 ± 56.4	269.9 ± 47.4	−15.9 ± 29.1	0.0003***	0.1576	0.3263	0.1715
	Barley infusion	277.3 ± 43.0	266.6 ± 36.5	−10.7 ± 31.2	0.0209*			
Anthropometric values								
Body weight (kg)	"Benifuuki"	71.8 ± 10.0	71.5 ± 9.7	−0.3 ± 1.6	0.1610			
	"Yabukita"	72.9 ± 12.4	72.7 ± 12.2	−0.2 ± 1.4	0.3152	0.0646	0.4374	0.0807
	Barley infusion	72.5 ± 11.4	72.9 ± 11.6	0.4 ± 1.5	0.0415*			
Body mass index	"Benifuuki"	27.6 ± 2.3	27.5 ± 2.3	−0.1 ± 0.6	0.2078			
(kg/m^2)	"Yabukita"	28.3 ± 3.2	28.2 ± 3.2	−0.1 ± 0.5	0.4237	0.0824	0.5207	0.0866
	Barley infusion	28.0 ± 2.7	28.1 ± 2.8	0.2 ± 0.6	0.0511			
Waist circumference	"Benifuuki"	94.0 ± 6.7	94.3 ± 6.9	0.3 ± 3.2	0.5263			
(cm)	"Yabukita"	95.0 ± 8.3	95.2 ± 8.3	0.3 ± 2.7	0.4942	0.7754	0.9331	0.3396
	Barley infusion	95.9 ± 6.4	96.6 ± 5.7	0.7 ± 4	0.1878			
SBP (mm Hg)	"Benifuuki"	127.7 ± 14.2	127 ± 14.7	−1.6 ± 9.7	0.5696			
	"Yabukita"	129.1 ± 17.1	125.5 ± 17.5	−2.5 ± 13.0	0.0244*	0.1625	0.5093	0.0799
	Barley infusion	131.0 ± 20.5	125.2 ± 22.7	−6.5 ± 16.3	0.0087**			
DBP (mm Hg)	"Benifuuki"	83.8 ± 11.7	82.8 ± 11.6	−1.6 ± 8.0	0.5696			
	"Yabukita"	84.0 ± 11.3	80.6 ± 11.9	−3 ± 9.6	0.0244*	0.2616	0.8214	0.1199
	Barley infusion	86.5 ± 10.7	81.7 ± 11.3	−4.5 ± 8.8	0.0087***			
Pulse (bpm)	"Benifuuki"	72.5 ± 9.2	73.8 ± 9.7	1.7 ± 9.0	0.2466			
	"Yabukita"	72.9 ± 8.9	74 ± 9.6	0.9 ± 9.8	0.3680	0.2078	0.5584	0.2442
	Barley infusion	73.6 ± 9.2	72.4 ± 9.2	−1.8 ± 7.3	0.2345			
Body fat ratio (%)	"Benifuuki"	33.8 ± 7.3	29.2 ± 8.4	−4.6 ± 6.8	<.0001****			
	"Yabukita"	33.9 ± 7.6	31.0 ± 10.5	−2.9 ± 6.3	0.0020**	0.2457	0.1594	0.5475
	Barley infusion	34.6 ± 7.8	31.0 ± 9.4	−3.9 ± 5.6	<.0001****			
Lipid parameters								
Total cholesterol	"Benifuuki"	6.39 ± 0.73	6.24 ± 0.68	−0.16 ± 0.55	0.0496*			
(mmol/L)	"Yabukita"	6.27 ± 0.74	6.16 ± 0.79	−0.12 ± 0.69	0.2397	0.4583	0.7885	0.021*
	Barley infusion	6.20 ± 0.70	6.26 ± 0.78	0.06 ± 0.69	0.5610			
Triglyceride (mmol/L)	"Benifuuki"	1.73 ± 0.94	1.69 ± 0.96	−0.04 ± 0.94	0.7693			
	"Yabukita"	1.45 ± 0.79	1.45 ± 0.96	0.00 ± 0.82	0.9801	0.6559	0.9690	0.4051
	Barley infusion	1.48 ± 0.59	1.58 ± 0.72	0.10 ± 0.54	0.2086			
HDL cholesterol	"Benifuuki"	1.57 ± 0.40	1.55 ± 0.37	−0.02 ± 0.17	0.4459			
(mmol/L)	"Yabukita"	1.55 ± 0.39	1.55 ± 0.38	0.00 ± 0.18	0.8906	0.8433	0.6447	0.9756
	Barley infusion	1.57 ± 0.35	1.56 ± 0.37	−0.02 ± 0.18	0.5581			
LDL cholesterol	"Benifuuki"	4.05 ± 0.55	3.92 ± 0.62	−0.13 ± 0.45	0.0450*			
(Friedewald) (mmol/L)	"Yabukita"	4.06 ± 0.74	3.96 ± 0.75	−0.10 ± 0.62	0.2443	0.5044	0.6581	0.0162*
	Barley infusion	3.95 ± 0.55	3.97 ± 0.63	0.03 ± 0.58	0.7421			

(*continued on next page*)

Table 2 – (*continued*)

	Interventions	Week 0	Week 12	Δ value at week 12[a]	Paired *t* test	p-value of multiple comparison test[c]		
						Drinking tea habit		
					p-value[b]	*ALL*	*YES*	*NO*
Glycometabolism-associated parameters								
HbA1c (mmol/mol)	"Benifuuki"	37.9 ± 4.0	38.5 ± 5.1	0.6 ± 2.3	0.0642			
	"Yabukita"	38.1 ± 6.3	38.4 ± 5.7	0.3 ± 2.4	0.3104	0.9877	0.7727	0.6124
	Barley infusion	37.7 ± 3.7	38.5 ± 5.4	0.8 ± 3.2	0.1040			
HbA1c (%)	"Benifuuki"	5.61 ± 0.37	5.67 ± 0.47	0.06 ± 0.21	0.0642			
	"Yabukita"	5.64 ± 0.58	5.67 ± 0.52	0.03 ± 0.22	0.3104	0.9877	0.7727	0.6124
	Barley infusion	5.60 ± 0.34	5.67 ± 0.49	0.07 ± 0.29	0.1040			
Blood glucose	"Benifuuki"	4.90 ± 0.57	5.12 ± 0.75	0.22 ± 0.47	0.0026**			
(mmol/L)	"Yabukita"	4.89 ± 0.74	4.92 ± 0.76	0.03 ± 0.35	0.5792	0.2013	0.1489	0.0759
	Barley infusion	4.70 ± 0.56	4.97 ± 1.10	0.27 ± 0.88	0.0392*			
IRI (μU/mL)	"Benifuuki"	6.8 ± 2.9	7.9 ± 3.4	1.0 ± 2.5	0.0058**			
	"Yabukita"	7.2 ± 3.9	7.6 ± 3.0	0.4 ± 3.1	0.3173	0.8422	0.9350	0.8411
	Barley infusion	8.3 ± 4.0	10.5 ± 8.8	2.2 ± 8.3	0.0746			
HOMA-IR	"Benifuuki"	1.5 ± 0.7	1.8 ± 1.0	0.3 ± 0.7	0.0014**			
	"Yabukita"	1.6 ± 0.9	1.7 ± 0.9	0.1 ± 0.7	0.2401	0.6741	0.8229	0.6704
	Barley infusion	1.8 ± 0.9	2.6 ± 3.3	0.8 ± 3.1	0.0660			
LOX index-associated parameters								
sLOX-1 (ng/L)	"Benifuuki"	464.5 ± 118.7	593.7 ± 199.1	129.2 ± 223.2	0.0002***			
	"Yabukita"	485.5 ± 122.5	637.3 ± 207.1	152 ± 241.8	<.0001****	0.8767	0.6655	0.9726
	Barley infusion	498.7 ± 158.6	614.5 ± 181.5	115.7 ± 220.8	0.0006***			
LAB (mg cs/L)	"Benifuuki"	5.05 ± 1.81	4.01 ± 1.68	−1.04 ± 2.60	0.0070**			
	"Yabukita"	4.61 ± 1.76	4.18 ± 1.97	−0.44 ± 2.53	0.2253	0.0453*	0.0204*	0.6611
	Barley infusion	4.23 ± 2.09	4.66 ± 2.19	0.42 ± 3.25	0.3653			
LOX index	"Benifuuki"	2364.9 ± 1070.2	2426.5 ± 1308.6	61.5 ± 1764.7	0.8080			
	"Yabukita"	2241.8 ± 976.4	2724.1 ± 1560.3	482.4 ± 1818.3	0.0639	0.1767	0.1337	0.8494
	Barley infusion	2154.7 ± 1516.3	2905 ± 1782.7	750.2 ± 2450.2	0.0372*			
Cytokines								
MCP-1 (pg/mL)	"Benifuuki"	107.6 ± 55.6	105.1 ± 44.3	−4.7 ± 50.1	0.5173			
	"Yabukita"	108.5 ± 39.8	103.2 ± 41.8	−5.3 ± 29.1	0.1979	0.7361	0.7059	0.9549
	Barley infusion	106.7 ± 47.1	99.7 ± 35.9	−9.0 ± 39.3	0.1157			
TNF-α (pg/mL)	"Benifuuki"	0.7 ± 0.3	0.8 ± 0.3	0.1 ± 0.4	0.1108			
	"Yabukita"	0.8 ± 0.4	1.0 ± 0.5	0.2 ± 0.5	0.0214***	0.2736	0.1628	0.8964
	Barley infusion	1.1 ± 1.5	1.1 ± 0.8	0.1 ± 0.9	0.6703*			
Adiponectin (μg/mL)	"Benifuuki"	8.5 ± 3.9	9.0 ± 4.2	0.4 ± 1.0	0.0044**			
	"Yabukita"	9.2 ± 4.5	9.4 ± 4.9	0.2 ± 1.5	0.2859	0.9013	0.8767	0.9876
	Barley infusion	9.6 ± 3.9	9.9 ± 4.2	0.3 ± 1.3	0.1359			
High sensitivity CRP	"Benifuuki"	1091.6 ± 1144.3	1114.7 ± 1272.4	23.1 ± 1179.6	0.8917			
(μg/L)	"Yabukita"	940.6 ± 988.3	1091.2 ± 1316.4	150.6 ± 1371.1	0.4365	0.8894	0.1798	0.1662
	Barley infusion	1260.4 ± 1286.4	1334.7 ± 1290.2	74.3 ± 1393.6	0.7107			
A biomarker for advanced glycation end products								
Pentosidine	"Benifuuki"	106 ± 30.2	106.1 ± 29.4	0.4 ± 33.3	0.9329			
(pmol/mL)	"Yabukita"	113 ± 32.8	99.1 ± 29.6	−13.6 ± 36.9	0.0109*	0.0965	0.2723	0.1248
	Barley infusion	105 ± 26	100 ± 27.2	−4.6 ± 31.4	0.3107			
Complete blood count								
Hb (g/L)	"Benifuuki"	142.8 ± 14.0	140.6 ± 13.1	−2.2 ± 5.5	0.0080**			
	"Yabukita"	144.1 ± 13.3	144.1 ± 13.9	0.1 ± 6.1	0.9450	0.2792	0.6995	0.2366
	Barley infusion	144.0 ± 13.1	142.8 ± 13.3	−1.2 ± 7.1	0.2574			
Hct (/L)	"Benifuuki"	0.44 ± 0.04	0.43 ± 0.03	−0.01 ± 0.02	0.0001***			
	"Yabukita"	0.44 ± 0.03	0.44 ± 0.04	0.00 ± 0.02	0.5983	0.0674	0.2966	0.2541
	Barley infusion	0.44 ± 0.03	0.43 ± 0.04	−0.01 ± 0.02	0.0479*			
RBC (10^4/μL)	"Benifuuki"	473 ± 44	467 ± 39	−7 ± 18	0.0163*			
	"Yabukita"	475 ± 36	476 ± 36	2 ± 18	0.4562	0.1451	0.6333	0.0888
	Barley infusion	474 ± 37	472 ± 37	−2 ± 19	0.3805			
Plt (10^4/μL)	"Benifuuki"	26.9 ± 4.9	26.9 ± 5.4	0 ± 2.8	0.9713			
	"Yabukita"	26.6 ± 5.1	26 ± 4.8	−0.5 ± 2.2	0.1067	0.6668	0.8954	0.1042
	Barley infusion	25.6 ± 3.9	25.7 ± 4.6	0.1 ± 3.3	0.7579			
WBC (/μL)	"Benifuuki"	5884 ± 1712	5708 ± 1858	−176 ± 1114	0.2775			
	"Yabukita"	5916 ± 1618	5682 ± 1607	−233 ± 985	0.0968	0.8246	0.2592	0.5704
	Barley infusion	5863 ± 1454	5592 ± 1468	−271 ± 1232	0.1294			

(*continued on next page*)

Table 2 – (*continued*)

	Interventions	Week 0	Week 12	Δ value at week 12[a]	Paired *t* test	p-value of multiple comparison test[c]		
						Drinking tea habit		
					p-value[b]	*ALL*	*YES*	*NO*
Liver functions								
AST (U/L)	"Benifuuki"	24.8 ± 10.9	24.6 ± 9.6	–0.3 ± 6.8	0.7705			
	"Yabukita"	23.5 ± 7.9	23.1 ± 8.4	–0.4 ± 4.9	0.5300	0.8636	0.9341	0.4724
	Barley infusion	23.9 ± 7.6	24.8 ± 9.8	0.9 ± 9.8	0.5435			
ALT (U/L)	"Benifuuki"	29.3 ± 18.3	29.9 ± 19.9	0.6 ± 13.5	0.7438			
	"Yabukita"	28.2 ± 20.8	28.2 ± 22.9	0 ± 9.2	0.9879	0.8191	0.3425	0.6364
	Barley infusion	29.1 ± 13.4	28.6 ± 15.4	–0.6 ± 8.9	0.6537			
γGTP (U/L)	"Benifuuki"	53.9 ± 42.4	54.1 ± 44.4	0.1 ± 24.0	0.9716			
	"Yabukita"	36.5 ± 27.5	36.4 ± 24.4	–0.1 ± 18.1	0.9754	0.1010	0.0893	0.8395
	Barley infusion	40.5 ± 34.7	35.5 ± 22.5	–5 ± 17.8	0.0544			
Iron metabolism								
Fe (μmol/L)	"Benifuuki"	20.86 ± 7.94	18.37 ± 6.46	–2.49 ± 9.66	0.0780			
	"Yabukita"	20.51 ± 7.79	19.85 ± 6.81	–0.66 ± 7.60	0.5376	0.3642	0.9635	0.1557
	Barley infusion	20.19 ± 5.74	19.79 ± 6.32	–0.41 ± 7.34	0.6979			
Ferritin (ng/mL)	"Benifuuki"	134 ± 108	133.7 ± 117.2	0.2 ± 32.8	0.9637			
	"Yabukita"	155 ± 143	142.1 ± 123.3	–12.8 ± 40.4	0.0282*	0.5034	0.2659	0.9093
	Barley infusion	160 ± 138	151.8 ± 140.4	–8.1 ± 56.2	0.3205			

All data are mean ± standard deviation except the sex parameter. The γGTP and LAB levels were significantly different between the three groups at baseline. "Benifuuki" group, n = 49; "Yabukita" group, n = 51; barley infusion group, n = 49. The initial values were not significantly different between the groups except for the LAB values.

HbA1c is described by both the NGSP HbA1c (as %) and SI, IFCC-recommended units (as mmol/mol) in this manuscript.

[a] The value is the change from Week 0 to Week 12.

[b] A paired *t*-test was performed in each group to compare the data at baseline and after 12 weeks intervention (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$)

[c] p-values reflect overall comparison of the groups by Kruskal–Wallis test (p-values of only sex parameter reflect overall comparison of the groups by Pearson's chi-square test).

SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HbA1c, haemoglobin A1c; IRI, immunoreactive insulin; HOMA-IR, homeostatic model assessment of insulin resistance; sLOX-1, soluble lectin-like oxidized low-density lipoprotein receptor-1; LAB, ligands containing apolipoprotein B; MCP-1, monocyte chemoattractant protein-1; TNF-α, tumour necrosis factor α; CRP, C-reactive protein; Hct, haemoglobin count; RBC, red blood cell count; Plt, platelet count; WBC, white blood cell count; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ GTP, γ glutamyl transpeptidase.

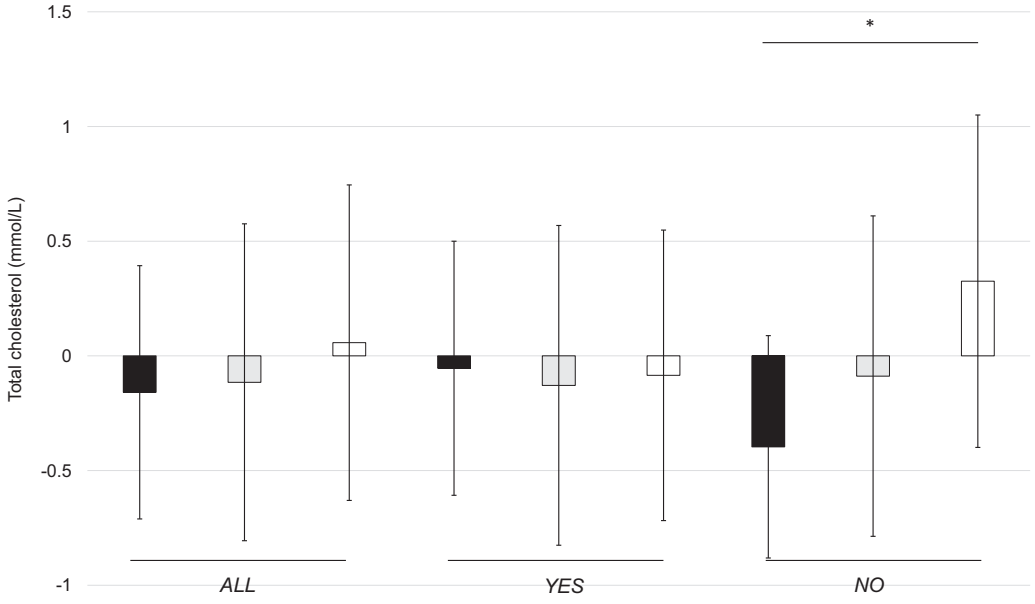

Fig. 3 – The change in the total cholesterol level from week 0 to week 12 (including sub-group analysis: the presence or absence of a habit of daily tea drinking). Dunn's multiple comparison test, mean ± SD. *ALL*, all participants; *YES*, participants with a habit of daily tea drinking; *NO*, participants without a habit of daily tea drinking. Black bars: "Benifuuki" green tea group; grey bars: "Yabukita" green tea group; white bars: barley infusion group.

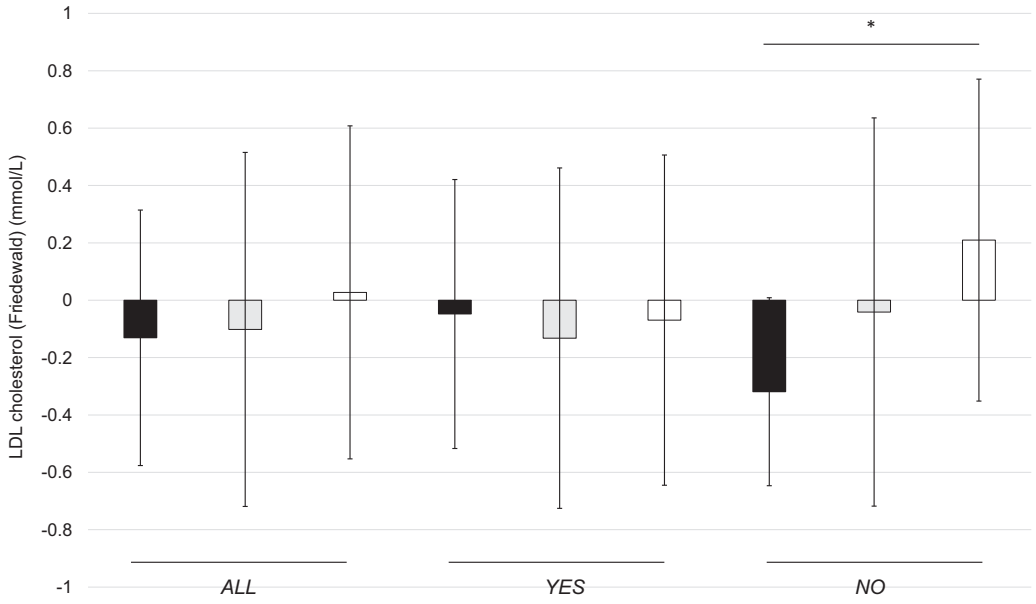

Fig. 4 – The change in the LDL cholesterol level from week 0 to week 12 (including sub-group analysis: the presence or absence of a habit of daily tea drinking). Dunn's multiple comparison test, mean ± SD. *ALL*, all participants; *YES*, participants with a habit of daily tea drinking; *NO*, participants without a habit of daily tea drinking. Black bars: "Benifuuki" green tea group; grey bars: "Yabukita" green tea group; white bars: barley infusion group.

3.3. *Ancillary analysis*

A subgroup analysis was performed between the two participant groups with (n = 100) and without (n = 49) a habit of daily tea drinking. In the group without a habit of daily tea drinking, the primary endpoints, serum total cholesterol levels, and LDL cholesterol levels significantly decreased at the end of the study in the "Benifuuki" group when compared with those in the barley infusion group (total cholesterol, "Benifuuki" vs. barley infusion = –0.40 ± 0.48 mmol/L vs. 0.33 ± 0.72 mmol/L, p = 0.0185; LDL cholesterol, "Benifuuki" vs. barley infusion = –0.32 ± 0.33 mmol/L vs. 0.21 ± 0.56 mmol/L, p = 0.0127) (Figs 3 and 4) (Table 3). No significant change in the triglyceride and HDL cholesterol levels at the start and the end of the study was observed between

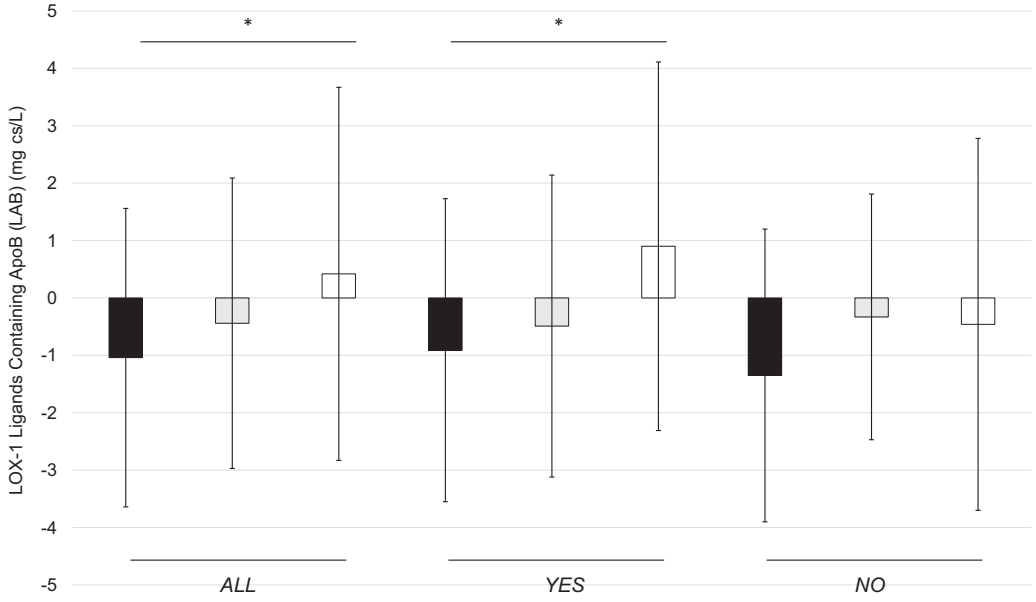

Fig. 5 – The change in LOX-1 ligands containing ApoB (LAB) level from week 0 to week 12 (including the sub-group analysis: the presence or absence of a habit of daily tea drinking). Dunn's multiple comparison test, mean ± SD. *ALL*, all participants; *YES*, participants with a habit of daily tea drinking; *NO*, participants without a habit of daily tea drinking. Black bars: "Benifuuki" green tea group; grey bars: "Yabukita" green tea group; white bars: barley infusion group.

Table 3 – Changes in the biochemical parameters after drinking "Benifuuki," "Yabukita," or barley infusion for 12 weeks with the p-value < 0.05 by Kruskal–Wallis test. Dunn's multiple comparison test, mean ± SD. *ALL*, all participants; *YES*, participants with a habit of daily tea drinking; *NO*, participants without a habit of daily tea drinking.

	Interventions	Week 0	Week 12	Δ value at week 12	p-value[a]	p-value[b]
ALL						
Total cholesterol (mmol/L)	"Benifuuki"	6.39 ± 0.73	6.24 ± 0.68	−0.16 ± 0.55	0.0469*	
	"Yabukita"	6.27 ± 0.74	6.16 ± 0.79	−0.12 ± 0.69	0.2397	1.0000
	Barley infusion	6.20 ± 0.70	6.26 ± 0.78	0.06 ± 0.69	0.5610	0.7701
LDL cholesterol (Friedewald) (mmol/L)	"Benifuuki"	4.05 ± 0.55	3.92 ± 0.62	−0.13 ± 0.45	0.0450*	
	"Yabukita"	4.06 ± 0.74	3.96 ± 0.75	−0.10 ± 0.62	0.2443	1.0000
	Barley infusion	3.95 ± 0.55	3.97 ± 0.63	0.03 ± 0.58	0.7421	0.7747
LAB (mg cs/L)	"Benifuuki"	5.05 ± 1.81	4.01 ± 1.68	−1.04 ± 2.60	0.0070**	
	"Yabukita"	4.61 ± 1.76	4.18 ± 1.97	−0.44 ± 2.53	0.2253	0.9407
	Barley infusion	4.23 ± 2.09	4.66 ± 2.19	0.42 ± 3.25	0.3653	0.0405*
YES						
Total cholesterol (mmol/L)	"Benifuuki"	6.35 ± 0.71	6.30 ± 0.72	−0.05 ± 0.55	0.5735	
	"Yabukita"	6.34 ± 0.75	6.21 ± 0.76	−0.13 ± 0.70	0.2901	1.0000
	Barley infusion	6.31 ± 0.75	6.23 ± 0.76	−0.08 ± 0.63	0.4542	1.0000
LDL cholesterol (Friedewald) (mmol/L)	"Benifuuki"	4.10 ± 0.55	4.05 ± 0.61	−0.05 ± 0.47	0.5540	
	"Yabukita"	4.15 ± 0.79	4.02 ± 0.77	−0.13 ± 0.59	0.2030	1.0000
	Barley infusion	4.02 ± 0.60	3.95 ± 0.64	−0.07 ± 0.58	0.5007	0.8185
LAB (mg cs/L)	"Benifuuki"	4.89 ± 1.9	3.97 ± 1.54	−0.91 ± 2.64	0.0524	
	"Yabukita"	4.53 ± 1.94	4.04 ± 1.79	−0.49 ± 2.63	0.2860	1.0000
	Barley infusion	4.11 ± 2.11	5.01 ± 2.15	0.90 ± 3.21	0.1238	0.0200*
NO						
Total cholesterol (mmol/L)	"Benifuuki"	6.49 ± 0.79	6.09 ± 0.58	−0.40 ± 0.48	0.0068**	
	"Yabukita"	6.14 ± 0.73	6.05 ± 0.84	−0.09 ± 0.70	0.6097	0.8003
	Barley infusion	5.99 ± 0.55	6.32 ± 0.83	0.33 ± 0.72	0.0826	0.0185*
LDL cholesterol (Friedewald) (mmol/L)	"Benifuuki"	3.93 ± 0.56	3.61 ± 0.54	−0.32 ± 0.33	0.0021**	
	"Yabukita"	3.88 ± 0.60	3.84 ± 0.72	−0.04 ± 0.68	0.8056	0.3579
	Barley infusion	3.82 ± 0.44	4.03 ± 0.62	0.21 ± 0.56	0.1431	0.0127*
LAB (mg cs/L)	"Benifuuki"	5.43 ± 1.57	4.08 ± 2.02	−1.35 ± 2.55	0.0603	
	"Yabukita"	4.78 ± 1.36	4.45 ± 2.32	−0.33 ± 2.14	0.5807	1.0000
	Barley infusion	4.47 ± 2.09	4.01 ± 2.19	−0.46 ± 3.24	0.5625	1.0000

[a] Paired *t* test (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001).
[b] Dunn's multiple comparison test (vs Benifuuki) (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001).
Number of participants = "Benifuuki"/"Yabukita"/barley infusion; *ALL* = 49/51/49, *YES* = 34/34/32, *NO* = 15/17/17.

the three groups. However, in the participants with a habit of daily tea drinking, the LAB level significantly decreased in the "Benifuuki" group compared with that in the barley infusion group.

3.4. *Test beverage-associated adverse events*

No adverse effects were observed in any of the participants in the three study groups in terms of glucose metabolism, including the fasting plasma glucose and the HbA1c level. Similarly, peripheral blood analysis, liver functions, and ferrous metabolism confirmed the safety of all three test beverages.

4. Discussion

Green tea is a highly consumed beverage in Japan. Increased consumption has been reported to be associated with decreased all-cause mortality and mortality due to cardiovascular diseases (Kuriyama et al., 2006). Green tea may also affect lipid metabolism, as shown by several studies revealing its cholesterol- and triglyceride-lowering effects (Muramatsu et al., 1986; Wu et al., 2012). Catechins contained in green tea have been implicated in the underlying mechanism of these effects. Furthermore, Ikeda et al. (1992) showed that green tea catechins inhibited the intestinal absorption of cholesterol in rats by suppressing the micellization of cholesterol, and these catechins suppressed postprandial hypertriglyceridaemia by delaying the lymphatic transport of dietary fat (Ikeda et al., 2005). It was reported that the EGCG concentration in blood plasma can be determined after green tea administration (Nakagawa & Miyazawa, 1997), that EGCG improves cholesterol metabolism through the up-regulation of the LDL receptor, and that EGCG reduces extracellular apoB levels (Goto, Saito, Morikawa, Kanamaru, & Nagaoka, 2012).

The green tea catechins displaying these effects are mainly EC, EGC, EGCG, and ECG, and EGCG is the most potent. In stark contrast to barley infusion, both "Benifuuki" and "Yabukita" contain abundant EGCG. In addition, "Benifuuki" contains EGCG″3Me, which is not present in "Yabukita." EGCG″3Me displays higher intestinal absorption efficiency than any other catechin (Maeda-Yamamoto et al., 2007). Moreover, it has a unique anti-allergic effect that is not observed with the other catechins (Masuda, Maeda-Yamamoto, Usui, & Fujisawa, 2014). It was reported that compared with the effects observed for "Yabukita," "Benifuuki" significantly inhibited the expression of genes involved in the synthesis of cholesterol and

significantly upregulated the hepatic mRNA expression of CYP7A1, which converts cholesterol into bile acids (Suzuki et al., 2013). "Benifuuki" is speculated to exert a stronger cholesterol-lowering effect than any other tea through the effects of EGCG″3Me.

The present study has demonstrated that "Benifuuki" significantly decreased total cholesterol and LDL cholesterol levels during a 12-week period when compared with those at baseline. Unexpectedly, "Yabukita," which contains abundant catechins, did not significantly decrease the total cholesterol and LDL cholesterol levels, an effect similar to that observed for the non-catechin-containing barley infusion. Although a significant decrease in the total cholesterol and LDL cholesterol levels was found in the "Benifuuki" group, no statistically significant difference in these serum levels was found between the 3 beverage test groups. According to a meta-analysis (Kim et al., 2011) that examined the effect of green tea on lipid metabolism, green tea lowers total cholesterol and LDL cholesterol levels. However, the LDL cholesterol-lowering effect of the green tea depends on the study design, the study period, the amount of catechin, the population, etc. Two-thirds of the present study subjects had a habit of drinking green tea, as do many Japanese people. There is a possibility that this had an influence on the results of the present study, which did not include a wash-out period. Therefore, we separated the subjects based on tea habit to evaluate the effect of catechin more clearly. In the group without a tea habit, green tea, as expected, had a significant effect on the reduction of total and LDL cholesterol levels in comparison with the effect of barley infusion.

Several reports describe the relationship between cholesterol and risk of cardiovascular disease. The Framingham Heart Study, for example, identified hypercholesterolaemia as a risk factor for ischaemic heart disease (Kannel et al., 1961). Past studies have revealed a relationship between the rise in cholesterol levels and an increase in coronary heart disease mortality (Leren, 1970; Verschuren et al., 1995). Dyslipidaemia, especially a high LDL cholesterol level, is a significant risk factor for coronary heart disease, a typical atherosclerotic disease (Liu & Li, 2015).

Given the above findings, we hypothesize that "Benifuuki" reduces the risk for cardiovascular disease through its total cholesterol- and LDL cholesterol-lowering effects, and its effects on LAB, soluble LOX-1, and the LOX-index, which were used as parameters for atherosclerosis. Our study showed that compared with barley infusion, "Benifuuki" significantly decreased LAB levels irrespective of a habit of daily tea drinking. In addition, "Benifuuki" significantly reduced LAB levels compared with those at baseline. On the other hand, the sLOX-1 levels and LOX-index were not significantly different among the 3 groups.

LOX-1 is an endothelial receptor for oxidized LDL, and the binding of oxidized LDL to LOX-1 promotes endothelial dysfunction; thus, LOX-1 functions as an aggravating factor at every stage of atherosclerosis upon binding of oxidized LDL (Sawamura et al., 1997; Yoshimoto et al., 2011). It was reported that LAB was higher in arteriosclerosis mice and was significantly reduced by anti-oxidation treatment (Kakutani, Ueda, Naruko, Masaki, & Sawamura, 2001; Oka, Yasuhara, Suzumura, Tanaka, & Sawamura, 2006). These reports suggest that LAB accelerates atherosclerosis and that its plasma concentration may help predict the development of future atherosclerosis. To the best of our knowledge, the present study is the first to show that "Benifuuki," which contains EGCG″3Me, significantly reduces the LAB level, suggesting that EGCG″3Me reduces the LAB level through a mechanism different from that of other catechins.

The serum sLOX-1 level, together with the serum LOX-1 level, is considered to reflect the progression of sclerosis and inflammation of the arteries (Sawamura, Wakabayashi, & Okamura, 2015). In the present study, a significant increase in the sLOX-1 levels was observed in all 3 groups, but no significant difference was observed between the 3 groups. The sLOX-1 levels significantly increased in each group before and after the intervention; there were no differences between the groups in this study. It was reported that there is a strong seasonal effect, with cholesterol levels being higher in winter than in summer (Robinson, Hinohara, Bevan, & Takahashi, 1993). While there has been no report of seasonal changes in sLOX-1 levels to date, it may be a possibility since it is a lipid metabolism-related parameter. The LOX-index reflects the interaction between sLOX-1 and LAB and is considered a biomarker for cardiovascular diseases. However, there was no difference in the LOX-index between the groups in the present study. Furthermore, Okamura et al. (2013) investigated carotid intima-media thickness (IMT) and found that in US Caucasians, a higher LAB level was significantly associated with increased IMT.

High LAB and LOX-index levels, apart from high LDL cholesterol levels, are suggested to be risk factors for atherosclerosis. Our present study demonstrated that "Benifuuki" not only significantly reduced the LDL cholesterol level but also significantly decreased that of LAB and of oxidized LDL, suggesting a role of "Benifuuki" in delaying the ageing process. We believe that this is due to catechins derived from green tea suppressing the micellization of cholesterol in the intestinal tract, and EGCG″3Me inhibiting the synthesis of cholesterol and promoting excretion of cholesterol to bile acid through excellent absorption efficiency of EGCG″3Me from the intestinal tract. Among the 3 tea extracts, although the difference was not significant, "Benifuuki" showed the strongest LOX-index-lowering effect, suggesting that "Benifuuki" might prevent cardiovascular events. If we had used a larger amount (e.g., two or three times) of tea catechins in the present study, a more clear effect of tea catechins on lipid metabolism would have likely been obtained. However, we also recognized that it would not be easy for a person without a tea drinking habit to take high doses of tea catechins daily.

A limitation of our study is that polyphenols contained in chocolate, wine, and certain other foods were not evaluated in the Food Frequency Questionnaire because only the consumption of polyphenols present in tea and coffee were restricted. Another limitation of our study is that we could blind all test beverages in terms of appearance but could not blind all test beverages in terms of taste. Since patients with heart disease and cerebrovascular disorders were not included in the present study, the study results may not be extrapolated to high-risk group patients with atherosclerotic disease. There might be a bias because some participants could not continue the study. Six participants out of one hundred fifty-five participants were excluded after their initial allocation. Since green

tea consumption was assessed based on self-administered questionnaires, some misclassification of the consumption status could have affected the estimation of the effect of the respective test beverage. However, this misclassification seems likely to have been non-differential and would tend to result in an underestimation of the impact of green tea consumption. In addition, as mentioned previously, a washout period was not included in the study design.

5. Conclusions

"Benifuuki" significantly decreased serum LAB levels when compared with barley infusion. Furthermore, in the participant group without a habit of daily green tea consumption, "Benifuuki" significantly reduced serum total cholesterol and LDL cholesterol levels when compared with barley infusion. Thus, consumption of "Benifuuki" green tea may help prevent cardiovascular diseases by lowering serum LDL cholesterol and LAB levels.

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Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.jff.2016.05.004.

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