Original Article

APOE and *CETP TaqIB* polymorphisms influence metabolic responses to *Hibiscus sabdariffa* L. and *Gynostemma pentaphyllum* Makino tea consumption in hypercholesterolemic subjects

Nutjaree Jeenduang PhD[1], Boonnisa Sangkaew BSc[1], Pacharee Chantaracha BSc[1], Sirada Chanchareonsri BSc[1], Thunyaluk Plyduang MSc[2], Wanida Thitdee BSc[1], Cathaleeya Samae BSc[1], Wacharaporn Pitumanon BSc[1]

[1]*School of Allied Health Sciences, Walailak University, Nakhon Si Thammarat, Thailand*
[2]*Center for Scientific and Technological Equipments, Walailak University, Nakhon Si Thammarat, Thailand*

Background and Objectives: *Hibiscus sabdariffa* L. (HS) and *Gynostemma pentaphyllum Makino* (GP) have been used as traditional medicines to treat diabetes and hypercholesterolemia. Nevertheless, there is inter-individual variation in the metabolic responses to HS and GP consumption. This may be due to genetic factors. The aim of this study was to investigate the effects of HS and GP tea consumption on anthropometric data, fasting blood glucose (FBG), and lipid concentrations in hypercholesterolemia subjects with different genotypes of the *APOE* and *CETP TaqIB* polymorphisms. **Methods and Study Design:** Forty-eight subjects with hypercholesterolemia were given either HS or GP tea for 30 days. Anthropometric and biochemical variables were determined, and *APOE* and *CETP TaqIB* polymorphisms were analyzed using the polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP). **Results:** E4 (p=0.008) and homozygous B1B1 (p=0.010) carriers had significantly decreased HDL-C concentrations after HS consumption; in addition, B2 carriers who consumed HS showed significantly decreased triglyceride (TG) concentrations (p=0.039). Regarding GP consumption, non-E4 carriers had significantly decreased HDL-C (p=0.009) and FBG (p=0.042) concentrations. Furthermore, B2 carriers had significantly decreased total cholesterol (TC) (p=0.045), HDL-C (p=0.004), and FBG (p=0.026) concentrations. **Conclusions:** HS consumption may have beneficial effects with respect to TG concentrations in the B2 carriers, but it may adversely affect HDL-C concentrations in homozygous B1B1 and E4 carriers. In contrast, GP consumption may have favorable effects on TC and FBG concentrations but not on HDL-C concentrations for B2 and/or non-E4 carriers.

Key Words: ***APOE*, *CETP TaqIB*, *Gynostemma pentaphyllum* Makino, *Hibiscus sabdariffa* L., Polymorphisms**

INTRODUCTION

Dyslipidemia has been associated with an increased risk of cardiovascular disease (CVD). The etiology of dyslipidemia has been attributed to environmental factors such as a sedentary lifestyle, western diet, lack of exercise, smoking, alcohol consumption, and stress. Moreover, several genetic factors have been investigated to determine their link with dyslipidemia; for example, *APOA5, APOE, CETP, LPL,* and *LDLR*.[1] Dyslipidemia can be prevented in individuals by improving their lifestyle behavior, controlling their diet, or receiving pharmacological therapy. Early treatment of dyslipidemia can substantially reduce cardiovascular risk and the rate of morbidity and mortality.[2] Statins are the most common agent used to treat plasma lipid disorders; however, some patients have adverse side effects to this drug, such as the elevation of liver enzymes, gastrointestinal symptoms, predisposition to cholelithiasis, rhabdomyolysis, myopathy, and renal dysfunction.[3] Consequently, herbal therapy with fewer side effects may be an alternative approach to reduce hypercholesterolemia.

In Thailand, *Hibiscus sabdariffa* L (HS) and *Gynostemma pentaphyllum* Makino (GP) are consumed traditionally as a hot drink or beverage. Studies carried out in animals and humans have primarily demonstrated that HS and GP extracts have a low degree of toxicity.[4-10] HS has been used as a traditional medicine to treat hypertension,[11] inflammatory disease,[12] cancer,[13] kidney stones, urinary bladder stones, hypercholesterolemia, fungi, and bacterial infections.[14] In addition, GP is commonly used to treat a variety of diseases such as diabetes mellitus,

Corresponding Author: Dr Nutjaree Jeenduang, School of Allied Health Sciences, Walailak University, 222 Thaiburi, Thasala, Nakhon Si Thammarat 80161, Thailand.
Tel: +66 75672193; Fax: +66 75672106
Email: nutjaree.je@wu.ac.th
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cancer, gastritis, bronchitis, hypertension, andhypercholesterolemia.[15] Nevertheless, several studies have shown that there are variable effects on lipid profiles after HS and GP consumption.[16-22] These variations may be due to several environmental factors as well as genetic factors.

Apolipoprotein E (apoE) is a component of plasma chylomicrons, chylomicron remnants, very-low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), and high density lipoprotein (HDL).[23] ApoE acts as ligand for low density lipoprotein receptor (LDLR) and LDL-related protein (LRP).[23] The *APOE* gene is located on chromosome 19q13.2 and consists of 4 exons and 3 introns.[23] There are three common alleles (E2, E3, and E4) in the *APOE* gene, which code for six genotypes of E2/E2, E3/E3, E4/E4, E2/E3, E2/E4, and E3/E4.[23] The E4 allele is associated with higher concentrations of low density lipoprotein cholesterol (LDL-C) and total cholesterol (TC) compared with E3 allele, while the E2 allele is associated with lower concentrations of the same plasma lipoproteins and lipids.[24] In addition, the E4 allele is associated with lower concentrations of high density lipoprotein cholesterol (HDL-C).[24,25] It has been reported that the E4 allele is associated with an increased risk of CVD and Alzheimer's disease.[24,25]

The cholesteryl ester transfer protein (CETP) plays a key role in the metabolism of HDL.[26] CETP enables the transfer of cholesteryl esters from HDL to VLDL, IDL, and low density lipoprotein (LDL); the IDL and LDL are catabolized via the LDLR in the liver. The *CETP* gene is located on chromosome 16q21.[26] Several polymorphisms have been reported in this gene;[27] the most commonly studied of these is *TaqIB*, which is a silent base change affecting the 277th nucleotide in the first intron of the *CETP* gene.[27] The B2 allele is associated with increased HDL-C concentrations and decreased CETP concentrations and activity;[28] additionally, it is further associated with a lower CVD risk.[29] Several environmental factors such as smoking, alcohol consumption, obesity, and diet have been reported to modulate the effect of the *APOE* and *CETP TaqIB* polymorphisms on lipid concentrations.[30-34]

The aim of this study was to investigate the effects of HS and GP tea consumption on anthropometric data, fasting blood glucose (FBG), and lipid concentrations according to *APOE* and *CETP TaqIB* polymorphisms in hypercholesterolemic subjects.

SUBJECTS AND METHODS

Subjects

Participants were recruited from the personnel of Walailak University. Inclusion criteria were hypercholesterolemia subjects who were diagnosed as having fasting TC >5.17 mmol/L and/or LDL-C >3.36 mmol/L. Exclusion criteria were as follows: chronic disease such as diabetes, thyroid disease, liver disease, renal disease, cancer, and triglyceride (TG) >4.52 mmol/L, as well as the use of anti-hypertensive, diuretics, and lipid lowering drugs. Sixty-six participants were included in the study. Eighteen subjects were dropped because they did not meet the criteria (n=8), declined to participate (n=5), lost to attrition (n=3), and for other reasons (n=2). Thus, the final analysis included 48 participants (17 males and 31 females). The study protocol was explained in detail to all participants, who gave written informed consent at the beginning of the study. The study protocol was approved by the Ethics Committee of Walailak University (Protocol number 14/021).

Study design and dietary assessment

After recruitment, the participants were randomly allocated into one of two groups. Each group of participants was instructed to take HS or GP tea two times a day, one in the morning and another in the afternoon, between the main meals for 30 days. The tea sachet (3 g) was added to 240 mL of boiling water and drunk after a steeping time of 10 min. HS and GP tea were purchased directly from the herbal medicine company in Thailand; all tea products were approved by the Thai Food and Drug Administration (FDA). The participants were instructed to avoid drinking other types of tea during the study and their diet and behavior were kept unchanged. The dietary habits were assessed by a semi-quantitative food frequency questionnaire (SFFQ) at the beginning and at the end of the study. The food items in the SFFQ were based on local foods in the Thai Food Composition Table.[35] The nutritional energy based on the SFFQ was computed by the multiplying the consumption frequency of each food by the nutritive values of the food. Smoking, alcohol consumption, and exercise were also recorded by questionnaire.

Anthropometric measurements and biochemical analyses

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were recorded at the brachial artery using the Omron T8 with Intellisense (HEM757A4-C1) automatic blood pressure monitor after 20 minutes of rest. The height and weight of subjects were obtained when they were wearing light clothing and without shoes. Waist circumference (WC) was also measured. Body mass index (BMI) was calculated as weight (kg) divided by the square of the height (m^2). Venous blood was collected into blood tubes after 12 h fasting; FBG and lipid profiles were measured at baseline (day 0) and day 31 of the study period. The concentrations of TC, TG, HDL-C, and FBG were measured by standardized enzymatic technique on Konelab analyzer (KONELAB 20, Tokyo, Japan). LDL-C was calculated using the Friedewald formula.

DNA extraction and genotyping

The *APOE* and *CETP TaqIB* polymorphisms were analyzed by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis.[36,37] Genomic DNA was isolated from white blood cells using the Genomic DNA Mini kit (GeneAid Biotech Ltd., Taiwan). *APOE* genotype was determined by amplifying a 218 bp fragment of exon 4 of the gene by PCR followed by *Afl*III and *Hae*II digestion. The resulting DNA fragments were 145, 168, and 195 bp indicated for E3, E2, and E4 alleles, respectively. For *CETP TaqIB* genotyping, the amplification of 505 bp fragment of the intron 1 of this gene was carried out using PCR, followed by *TaqIB* digestion. The resulting DNA fragments were 415 and 90 bp for the B1 allele and an intact 505 bp fragment for the B2 allele.

Table 1. Allele and genotype frequencies of *APOE* and *CETP TaqIB* polymorphisms

	All	Men	Women	*p*-value†
n	48	17	31	
APOE genotypes				
E2E2	1 (2.10)	0 (0)	1 (3.23)	0.763
E2E3	7 (14.6)	3 (17.7)	4 (12.9)	
E3E3	22 (45.8)	8 (47.1)	14 (45.2)	
E3E4	16 (33.3)	6 (35.3)	10 (32.3)	
E4E4	2 (4.20)	0 (0)	2 (6.45)	
E2E4	0 (0)	0 (0)	0 (0)	
APOE alleles				
E2	9 (9.39)	3 (8.82)	6 (9.68)	
E3	67 (69.8)	25 (73.5)	42 (67.7)	
E4	20 (20.8)	6 (17.7)	14 (22.6)	
CETP TaqIB genotypes				
B1B1	19 (39.6)	7 (41.2)	12 (38.7)	0.947
B1B2	24 (50.0)	8 (47.1)	16 (51.6)	
B2B2	5 (10.4)	2 (11.8)	3 (9.68)	
CETP TaqIB alleles				
B1	62 (64.6)	22 (64.7)	40 (64.5)	
B2	34 (35.4)	12 (35.3)	22 (35.5)	

†Data were presented as n (%) and analyzed using Chi-square test.

Statistical analyses

Statistical analyses were performed using SPSS version 17.0 for Windows (SPSS Inc., Chicago, IL, United States). Differences in genotypic and allelic distributions between the groups were estimated using the Chi square ($\chi 2$) test. The normal distribution was tested by mean of Shapiro-Wilk test. Mean differences between genders and genotypes were assessed by independent t-test or Mann-Whitney U test. To evaluate the effects of the intervention, a paired t-test or Wilcoxon's Signed Rank test was performed. The interaction effect between the *APOE* and *CETP TaqIB* genotypes and tea consumption on metabolic parameters was tested by introducing corresponding interactions terms in the analysis of covariance (ANCOVA) model. A p-value <0.05 was considered statistically significant.

RESULTS

Anthropometric, biochemical, and nutritional characterization of the study population

The anthropometric and biochemical characteristics of the study subjects are summarized in Supplementary table 1. Among the 66 volunteers, 48 completed the study. Males had significantly increased in weight but decreased in HDL-C concentrations compared with females. There were no significant differences in the other parameters. The results of dietary nutrient intake and behavior of the study subjects are shown in Supplementary table 2. There were no significant differences in the total energy and nutrient intake at the beginning and the end of this study among the HS and GP consumption groups. The exercise, smoking, and alcohol consumption were similar throughout the study.

Frequency of the APOE and CETP TaqIB polymorphisms

The genotype and allele frequencies of *APOE* and *CETP TaqIB* polymorphisms in the study population are shown in Table 1. No deviation from the Hardy-Weinberg equilibrium was found in the distribution of genotypes (*APOE*: p=0.372; *CETP*: p=0.520). Additionally, no statistically significant gender differences for genotype frequencies of *APOE* (p=0.763) or *CETP TaqIB* (p=0.947) were observed in this study population.

Effects of APOE polymorphism on changes of anthropometric and biochemical characteristics after tea consumption

Table 2 shows the anthropometric and biochemical characteristics at baseline and after tea consumption in the subjects with different *APOE* genotypes. Due to the small number of homozygotes for the E4 alleles, the genotypes were referred to as E4 and non-E4 carriers for statistical analysis. In the GP group, LDL-C concentrations were significantly higher in E4 carriers compared with non-E4 carriers (p=0.043) at baseline. No significant differences in the other biochemical parameters were found at baseline between the E4 and non-E4 carriers in both HS and GP groups. After HS consumption, E4 carriers had significantly decreased HDL-C concentrations (p=0.008). In contrast, regarding the GP consumption, non-E4 carriers had significantly decreased HDL-C (p=0.009) and FBG (p=0.042) concentrations. However, there was no significant interaction between tea consumption and *APOE* genotypes on anthropometric and biochemical parameters.

Effects of CETP TaqIB polymorphism on changes of anthropometric and biochemical characteristics after tea consumption

Table 3 shows the anthropometric and biochemical characteristics at baseline and after tea consumption in the subjects with different *CETP TaqIB* genotypes. Due to the small number of homozygotes for the B2 allele, the genotypes were referred to as B2 and non-B2 carriers for statistical analysis. In the HS group, FBG concentrations were significantly higher in B2 carriers than non-B2 carriers at baseline (p=0.041) and after intervention (p=0.048). In the GP group, BMI was significantly lower

Table 2. Characteristics at baseline and after *Hibiscus sabdariffa* L. or *Gynostemma pentaphyllum* Makino tea consumption according to *APOE* genotype

Variables	*Hibiscus sabdariffa* L. (n=24)		*p*-value†	*Gynostemma pentaphyllum* Makino (n=24)		*p*-value†	*p*-interaction§
	Non-E4 carriers (n=13)	E4 carriers (n=11)		Non-E4 carriers (n=17)	E4 carriers (n=7)		
Weight (kg)							
Baseline	63.5±8.78	62.4±14.5	0.816	64.2±8.35	71.4±23.9	0.611	0.757
Endpoint	63.9±8.88	62.9±14.8	0.486	64.9±7.38	71.1±23.5	0.518	
p-value‡	0.337	0.103		0.173	0.321		
Body mass index (kg/m^2)							
Baseline	24.4±1.93	23.7±4.04	0.607	25.5±3.30	27.1±9.09	0.427	0.370
Endpoint	24.6±2.01	23.9±4.12	0.629	22.5±9.01	26.9±8.96	0.286	
p-value‡	0.348	0.141		0.132	0.356		
Waist circumference (cm)							
Baseline	81.4±5.78	79.8±15.9	0.745	83.9±7.74	85.9±14.9	0.949	0.173
Endpoint	81.2±6.85	78.3±10.5	0.415	83.8±7.81	87.1±16.9	0.519	
p-value‡	0.881	0.645		0.445	0.356		
Systolic blood pressure (mmHg)							
Baseline	126±24.9	131±16.4	0.605	124±22.9	128±17.1	0.668	0.933
Endpoint	124±19.1	126±22.7	0.783	125±21.0	117±14.7	0.402	
p-value‡	0.611	0.235		0.496	0.078		
Diastolic blood pressure (mmHg)							
Baseline	82.9±15.9	86.7±14.7	0.494	82.8±14.5	85.6±9.74	0.652	0.763
Endpoint	81.9±12.1	82.9±13.7	0.885	82.5±18.6	80.0±15.5	0.772	
p-value‡	0.585	0.337		0.318	0.279		
Total cholesterol (mmol/L)							
Baseline	6.24±0.86	6.56±1.04	0.417	5.90±0.82	6.56±0.62	0.067	0.298
Endpoint	6.06±0.86	6.54±0.93	0.203	5.73±0.84	6.17±1.16	0.335	
p-value‡	0.176	0.900		0.358	0.148		
Triglyceride (mmol/L)							
Baseline	1.58±0.81	1.52±0.82	0.794	1.25±0.68	1.30±0.56	0.804	0.522
Endpoint	1.49±0.96	1.42±0.61	0.733	1.30±0.61	1.10±0.35	0.440	
p-value‡	0.606	0.413		0.831	0.353		
LDL-C (mmol/L)							
Baseline	4.15±0.90	4.33±1.02	0.643	3.77 ± 0.78	4.45±0.47	0.043*	0.618
Endpoint	4.06±0.76	4.44±0.77	0.250	3.68 ± 0.69	4.28±0.98	0.102	
p-value‡	0.566	0.409		0.502	0.493		

LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol.
Each value represents the mean±SD.
†Data were analyzed using Student's t-test or Mann-Whitney U test for the comparison between non E4 carriers and E4 carriers.
‡Data were analyzed using paired t-test or Wilcoxon's Signed Rank test for the comparison between baseline and endpoint.
§Data were analyzed using ANCOVA for interaction term.
*p-value <0.05.

Table 2. Characteristics at baseline and after *Hibiscus sabdariffa* L. or *Gynostemma pentaphyllum* Makino tea consumption according to *APOE* genotype (cont.)

Variables	*Hibiscus sabdariffa* L. (n=24)		*p*-value†	*Gynostemma pentaphyllum* Makino (n=24)		*p*-value†	*p*-interaction§
	Non-E4 carriers (n=13)	E4 carriers (n=11)		Non-E4 carriers (n=17)	E4 carriers (n=7)		
HDL-C (mmol/L)							
Baseline	1.37±0.28	1.53±0.45	0.300	1.56±0.29	1.52±0.23	0.761	0.549
Endpoint	1.31±0.30	1.46±0.44	0.362	1.48±0.32	1.38±0.22	0.480	
p-value‡	0.147	0.008*		0.009*	0.073		
Fasting blood glucose(mmol/L)							
Baseline	5.12±0.44	5.11±0.38	1.000	5.28±0.53	5.23±0.33	0.832	0.646
Endpoint	4.95±0.47	4.83±0.36	0.497	5.13±0.55	5.08±0.39	0.814	
p-value‡	0.115	0.061		0.042*	0.340		

LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol.
Each value represents the mean±SD.
†Data were analyzed using Student's t-test or Mann-Whitney U test for the comparison between non E4 carriers and E4 carriers.
‡Data were analyzed using paired t-test or Wilcoxon's Signed Rank test for the comparison between baseline and endpoint.
§Data were analyzed using ANCOVA for interaction term.
*p-value < 0.05.

Table 3. Characteristics at baseline and after *Hibiscus sabdariffa* L. or *Gynostemma pentaphyllum* Makino tea consumption according to *CETP TaqIB* genotype

Variables	*Hibiscus sabdariffa* L. (n=24)		*p*-value†	*Gynostemma pentaphyllum* Makino (n=24)		*p*-value†	*p*-interaction§
	Non-B2 carriers (B1B1) (n=11)	B2 carriers (B1B2+B2B2) (n=13)		Non-B2 carriers (B1B1) (n=8)	B2 carriers (B1B2+B2B2) (n=16)		
Weight (kg)							
Baseline	59.6±8.11	65.9±13.3	0.183	77.8±19.2	60.6±6.49	0.004*	0.268
Endpoint	60.1±7.93	66.3±13.8	0.296	76.6±19.1	61.4±6.31	0.060	
p-value‡	0.284	0.147		0.208	0.321		
Body mass index (kg/m^2)							
Baseline	23.2±2.61	24.9±3.24	0.184	29.6±7.66	24.2±2.79	0.016*	0.462
Endpoint	23.4±2.51	25.0±3.44	0.209	29.1±7.60	21.2±8.69	0.039*	
p-value‡	0.290	0.163		0.203	0.155		

LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol.
Each value represents the mean±SD.
†Data were analyzed using Student's t-test or Mann-Whitney U test for the comparison between non B2 carriers and B2 carriers.
‡Data were analyzed using paired t-test or Wilcoxon's Signed Rank test for the comparison between baseline and endpoint.
§Data were analyzed using ANCOVA for interaction term.
*p-value< 0.05.

Table 3. Characteristics at baseline and after *Hibiscus sabdariffa* L. or *Gynostemma pentaphyllum* Makino tea consumption according to *CETP TaqIB* genotype (cont.)

Variables	*Hibiscus sabdariffa* L. (n=24)		*p*-value†	*Gynostemma pentaphyllum* Makino (n=24)		*p*-value†	*p*-interaction§
	Non-B2 carriers (B1B1) (n=11)	B2 carriers (B1B2+B2B2) (n=13)		Non-B2 carriers (B1B1) (n=8)	B2 carriers (B1B2+B2B2) (n=16)		
Waist circumference (cm)							
Baseline	76.2±6.16	84.5±13.5	0.052	91.9±12.4	80.8±6.26	0.018*	0.600
Endpoint	77.4±7.70	82.0±9.09	0.196	90.9±14.5	81.6±7.42	0.053	
p-value‡	0.325	0.194		0.577	0.813		
Systolic blood pressure (mmHg)							
Baseline	123±17.8	132±24.1	0.326	130±20.9	123±21.4	0.523	0.439
Endpoint	120±14.2	129±24.1	0.262	133±23.7	118±15.6	0.101	
p-value‡	0.496	0.287		0.643	0.174		
Diastolic blood pressure (mmHg)							
Baseline	78.7±8.32	89.8±18.3	0. 109	85.4±14.2	82.9±12.9	0.677	0.989
Endpoint	77.9±8.20	86.1±14.6	0.163	86.4±19.9	79.5±16.4	0.404	
p-value‡	0.789	0.313		0.331	0.274		
Total cholesterol (mmol/L)							
Baseline	6.45±0.90	6.34±1.01	0.770	5.73±0.85	6.27±0.75	0.123	0.490
Endpoint	6.41±0.68	6.18±1.08	0.554	5.80±1.06	5.91±0.90	0.784	
p-value‡	0.676	0.352		0.692	0.045*		
Triglyceride (mmol/L)							
Baseline	1.42±0.57	1.66±0.96	0. 865	1.23±0.64	1.29±0.66	0.806	0.103
Endpoint	1.55±0.83	1.37±0.80	0.459	1.13±0.38	1.30±0.62	0.491	
p-value‡	0.447	0.039*		0.554	0.959		
LDL-C (mmol/L)							
Baseline	4.45±0.87	4.05±0.99	0.317	3.60±0.83	4.15±0.68	0.101	0.314
Endpoint	4.42±0.60	4.08±0.89	0.294	3.79±1.03	3.89±0.72	0.776	
p-value‡	0.861	0.831		0.249	0.080		
HDL-C (mmol/L)							
Baseline	1.35±0.29	1.52±0.41	0.262	1.56±0.24	1.54±0.29	0.831	0.313
Endpoint	1.27±0.30	1.47±0.40	0.200	1.49±0.24	1.43±0.32	0.610	
p-value‡	0.010*	0.149		0.190	0.004*		
Fasting blood glucose (mmol/L)							
Baseline	4.94±0.35	5.26±0.40	0.041*	5.28±0.29	5.26±0.56	0.920	0.485
Endpoint	4.72±0.36	5.05±0.41	0.048*	5.15±0.32	5.10±0.58	0.815	
p-value‡	0.051	0.106		0.404	0.026*		

LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol.
Each value represents the mean±SD.
†Data were analyzed using Student's t-test or Mann-Whitney U test for the comparison between non B2 carriers and B2 carriers.
‡Data were analyzed using paired t-test or Wilcoxon's Signed Rank test for the comparison between baseline and endpoint.
§Data were analyzed using ANCOVA for interaction term.
*p-value< 0.05.

in B2 carriers than non-B2 carriers at both baseline (p =0.016) and after intervention (p=0.039). In addition, weight and WC were significantly lower in B2 carriers than non-B2 carriers in the GP group at baseline (p=0.004 and p=0.018, respectively). After HS consumption, subjects with the B1B1 genotype had significantly decreased HDL-C concentrations (p=0.010), whereas B2 carriers had significantly decreased TG concentrations (p=0.039). In contrast, regarding GP consumption, B2 carriers had significantly decreased TC (p=0.045), HDL-C (p=0.004), and FBG (p=0.026) concentrations. Nevertheless, there was no significant interaction between tea consumption and *CETP TaqIB* genotypes on anthropometric and biochemical parameters.

DISCUSSION

The effects of HS and GP consumption on the decreased TC, LDL-C, and FBG concentrations, as well as increased HDL-C concentrations, have been reported in several studies.[16-20] However, Kuriyan et al showed that the consumption of leaf extract (1 g/day) of HS in hypercholesterolemic subjects for 90 days did not appear to have a blood lipid lowering effect.[21] In addition, Mohagheghi et al reported that the consumption of calyx extract of HS in hypertensive subjects increased TC and HDL-C concentrations.[5] Moreover, Park et al reported that the consumption of GP extract or actiponin (450 mg/day) for 12 weeks in obesity subjects had significantly decreased HDL-C concentrations.[22] We suggest that the inconsistent effects of HS or GP consumption on lipid concentrations may be modulated by several factors including the concentrations or types of the administered HS and GP, the duration of the study, the number of subjects, environmental factors, and genetic factors. In the present study, we investigated the effects of HS and GP consumption on anthropometric data, FBG, and lipid concentrations in hypercholesterolemia subjects with different genotypes of the *APOE* and *CETP TaqIB* polymorphisms. To our knowledge, no human study examining the effect of HS and GP tea consumption on metabolic responses according to the *APOE* and *CETP TaqIB* polymorphisms has been performed.

Several clinical studies have shown that the E4 allele plays a role in the development of atherosclerosis and CVD.[24] It is well known that the E4 allele is associated with higher concentrations of TC, LDL-C and TG;[24,38] our findings found that E4 carriers had significantly higher LDL-C concentrations than non-E4 carriers in the GP group at baseline. Because of the relatively small sample size in the present study, the effect of the *APOE* genotype on TC and TG concentrations in both HS and GP groups may fail to reach statistical significance. After HS consumption, we observed that E4 carriers had significantly decreased HDL-C concentrations, which is consistent with previous studies. Egert et al showed that E4 carriers had a significantly decreased concentration of serum HDL-C and apoA1 after Quercetin supplementation.[39] In addition, Minihane et al demonstrated that supplementation with fish oil in apoE4 individuals led to a significant increase in TC and a trend toward reduction in HDL-C relative to the common homozygous E3E3 profile.[40] In contrast, our findings shown that non-E4 carriers had significantly decreased HDL-C and FBG concentrations after GP consumption. Our results were similar to a previous study; Loktionov et al reported that the drinking of black tea by a subject with E3E3 homozygous revealed lowered HDL-C concentrations, while there was no response in E4-bearing subjects.[41]

The effects of HS and GP tea consumption on FBG, and lipid concentrations according to *CETP TaqIB* polymorphisms were also observed. It is generally acknowledged that the *TaqIB* polymorphism in the *CETP* gene influences the HDL-C values,[28] with individuals homozygous for the B1 allele having lower concentrations of HDL-C than carriers of at least 1 B2 allele. In the present study, we did not observe the significant difference in HDL-C concentrations between B2 and non-B2 carriers in both HS and GP groups at baseline and after intervention. Moreover, B2 carriers in the HS group showed significantly higher FBG concentrations than non-B2 carriers at baseline and after intervention. In contrast, B2 carriers in the GP group had a significantly lower weight, BMI, and WC than non-B2 carriers at baseline; these inconsistent results may be due to the small sample size in our study.

After HS consumption, we found that homozygous B1B1 and B2 carriers had significantly decreased HDL-C and TG concentrations, respectively, whereas GP consumption in non-E4 and B2 carriers showed significantly decreased HDL-C and FBG concentrations. Our results were inconsistent with other studies that have demonstrated a favorable effect on HDL-C concentrations according to *CETP TaqIB* alleles after dietary intervention. Li et al demonstrated the beneficial effects of the B2allele on HDL-C concentrations in men with higher intakes of total fat, animal fat, saturated fat, and monounsaturated fat.[34] Du et al demonstrated that the elevated HDL concentrations after high carbohydrate and low fat (HC/LF) diet in healthy Chinese Han youth were associated with the B2 allele,[42] whereas males with the B1B1 genotype are more susceptible to the influence of a HC/LF diet on their HDL-C concentrations.[42] In addition, Estévez-González et al showed that the consumption of skim milk enriched with olive oil increased the HDL-C and apolipoproteinA-I concentrations in children with hypercholesterolemia, this effect being more intense in carriers of the B1B1 genotype.[43] Finally, Gammon et al. revealed that B1B1 homozygotes had a significantly lower TAG:HDL-C ratio after kiwifruit intervention than after the control intervention.[44]

Although a significant decrease in lipid and FBG concentrations after HS or GP tea consumption according to *APOE* or *CETP TaqIB* genotypes was observed in our study, the significant interaction between tea consumption and these genetic polymorphisms on lipid and FBG concentrations was not demonstrated; however, this lack of power to detect a statistical significance may be due to the small sample size of the study. In addition, the mechanism underlying the reduction of lipid and FBG concentrations after HS and GP tea consumption according to *APOE* or *CETP TaqIB* polymorphisms is still unknown. However, we suggest that GP consumption could potentially reduce FBG concentrations in non-E4 and B2 carriers by improving insulin sensitivity. In previous studies,

the insulin sensitizer rosiglitazone only improved glucose tolerance in ApoE3 knock-in (KI) mice but not in ApoE4 KI mice.[45,46] *In vivo* study showed that the expression of APOE4 reduced insulin-receptor substrate 1 (IRS-1), PI3K expression, and the reduced Akt phosphorylation led to reduced liver insulin signaling, insulin concentrations, and high glucose content.[47] In addition, subjects with the B2B2 genotype had significantly lower fasting insulin (FINS) and homeostasis model assessment-insulin resistance (HOMA-IR) levels compared with subjects with the B1B1 genotype.[48] Moreover, it has been reported that the dammarane compounds in the GP can suppress protein tyrosine phosphatase 1B (PTP-1B) activity and increase glucose transporter 4 (GluT4) translocation resulting in enhanced glucose uptake and improved insulin sensitivity.[49,50] This suggests that non-E4 and B2 carriers who consume GP tea may be more susceptible to suppressed PTP-1B activity or enhanced GluT4 translocation by dammarane compounds, resulting in lower FBG concentrations.

Furthermore, the effect of HS and GP consumption on decreased HDL-C concentrations according to *APOE* or *CETP TaqIB* genotypes in this study may be involved in the activities of enzymes in the reverse cholesterol transport (RCT) pathway, such as CETP, lecithin cholesterol acyltransferase (LCAT), hepatic lipase (HL), and/or lipoprotein lipase (LPL).[51] The interaction between *CETP TaqIB* or *APOE* polymorphisms and environmental factors (e.g. smoking, alcohol, diet, and exercise) were found to modulate the CETP, LCAT, HL, and/or LPL activities, which then led to variations on HDL-C concentrations.[52,53] Furthermore, we also observed a significantly decrease in TG and TC concentrations in B2 carriers after HS and GP consumption, respectively. The reduction of TG by HS consumption may result from the increased LPL activity or reducing VLDL production in the liver. Previous studies have shown that the HS extract is able to reduce VLDL cholesterol,[54,55] whereas the reduction of TC by GP consumption may be explained by a decrease incholesterol synthesis. *In vitro* studies have revealed that damulin A and B, two dammarane type saponins purified from the leaves of GP, were able to decrease cholesterol synthesis via the increased phosphorylation of AMP-activated protein kinase (AMPK).[50]

To the best of our knowledge, this is the first study to investigate the effects of HS and GP tea consumption on metabolic parameters according to *APOE* and *CETP* polymorphisms in hypercholesterolemic subjects. The limitations of our study result from a small sample size. Because two polymorphisms in two genes were studied, other variants in these genes or in other genes may be associated with the metabolic responses to HS or GP tea consumption. We recommend that further studies on a larger sample subdivided by gender are required to confirm the results. Moreover, the mechanism underlying the metabolic responses according to the *APOE* or *CETP TaqIB* genotypes should be elucidated by *in vitro* or *in vivo* study. The activities of CETP, LCAT, HL, and LPL, as well as insulin and HOMA-IR should be determined.

In conclusion, we indicate that HS consumption may have beneficial effects with respect to TG concentrations in the B2 carriers, but it may adversely affect HDL-C concentrations in E4 and homozygous B1B1 carriers. In contrast, GP consumption may have favorable effects on TC, FBG concentrations but not HDL-C concentrations for non-E4 and/or B2carriers. These findings may pave the way to personalized tea consumption to prevent CVD in hypercholesterolemic subjects.

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AUTHOR DISCLOSURES

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Supplementary table 1. Baseline characteristics of the subjects

Variables	All (n=48)	Men (n=17)	Women (n=31)	*p*-value†
Age (years)	42.5±8.64	41.1±9.25	43.2±8.35	0.080
Weight (kg)	64.7±12.9	68.7±12.3	62.5±13.0	0.035*
Body mass index (kg/m^2)	25.0±4.46	24.3±3.80	25.5±4.79	0.371
Waist circumference (cm)	82.6±10.8	84.5±12.9	81.5±9.42	0.437
Systolic blood pressure (mmHg)	127±20.9	130±19.6	125±21.9	0.450
Diastolic blood pressure (mmHg)	84.1±14.0	84.4±13.6	83.9±14.5	0.882
Total cholesterol (mmol/L)	6.24±0.88	6.20±0.75	6.26±0.96	0.803
Triglyceride (mmol/L)	1.41±0.73	1.78±0.98	1.21±0.45	0.074
LDL-C (mmol/L)	4.10±0.86	4.02±0.81	4.14±0.89	0.641
HDL-C (mmol/L)	1.49±0.32	1.36±0.30	1.57±0.32	0.030*
Fasting blood glucose (mmol/L)	5.19±0.44	5.18±0.39	5.19±0.48	0.821

LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol.
Each value represents the mean±SD.
†Data were analyzed using Student's t-test or Mann-Whitney U test for the comparison between genders.
*p-value <0.05.

Supplementary table 2. Dietary nutrition intake and behavior of the subjects

Variables	*Hibiscus sabdariffa* L. (n=24)		*p*-value†	*Gynostemmapentaphyllum* Makino (n=24)		*p*-value†
	Baseline	Endpoint		Baseline	Endpoint	
Total energy (kcal/day)	2303±242	2320±212	0.357	2424±193	2413±172	0.547
Protein (g/day)	103±11.1	105±11.4	0.323	105±14.9	103±15.2	0.139
Total fat (g/day)	50.1±8.52	50.1±9.00	0.953	54.2±6.81	52.9±7.72	0.092
Carbohydrates (g/day)	355±45.5	359±39.4	0.104	372±37.8	376±34.6	0.315
Smoking (n)	1	1	N/A	0	0	N/A
Alcohol consumption (n)	0	0	N/A	0	0	N/A
Exercise (days/wk)	2.42±2.06	2.75±2.44	0.349	1.50±1.98	1.38±1.97	0.317

Each value represents the mean±SD.
†Data were analyzed using paired t-test or Wilcoxon's Signed Rank test for the comparison between baseline and endpoint.
N/A, not applicable.