Effects of Hydroxyhydroquinone-reduced Coffee on Blood Pressure in High-normotensives and Mild Hypertensives

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The influence of hydroxyhydroquinone (HHQ) on the antihypertensive effects of chlorogenic acids in coffee was evaluated in a double-blind, randomized controlled trial of high-normotensive and mild hypertensive adult men and women. The subjects were randomly assigned to either an active group that ingested HHQ-reduced coffee (0.05 mg/184 ml) containing chlorogenic acids (299 mg/184 ml) or a control group that ingested coffee containing HHQ (1.69 mg/184 ml) and chlorogenic acids (299 mg/184 ml), corresponding to commercially available products. Each subject was instructed to continuously ingest one can of test beverage (coffee) daily for 12 weeks. A linear mixed-model repeated-measures analysis of covariance of 38 high-normotensives and 60 mild hypertensives showed that systolic blood pressure (SBP) was significantly lower in the active group (n = 51) than in the control group (n = 47) throughout the intake period (Group effect; p = 0.031). A stratified analysis suggested that the antihypertensive effect was greater in the mild hypertensives (Group effect; p = 0.013 in SBP and p = 0.015 in diastolic blood pressure) than in the high-normotensives. Safety assessment revealed no adverse effects associated with the reduction of HHQ to a level lower than the amounts in commercially available coffee products. These results suggest that HHQ-reduced coffee containing chlorogenic acids can be safely ingested to improve hypertension.

Key words —— chlorogenic acid, hypertension, randomized controlled trial, hydroxyhydroquinone, human

INTRODUCTION

Epidemiological studies have shown that high blood pressure is associated with greater prevalence of, and mortality from, stroke.[1–7] Thus, hypertension is a major risk factor for stroke and coronary artery disease,[1] and also is a risk factor for metabolic syndrome, as defined by the Japanese Society of Internal Medicine in 2005.[8] The 2004 National Health and Nutrition Survey showed that 44.9% of men and 32.0% of women had a systolic blood pressure (SBP) of more than 140 mmHg and/or a diastolic blood pressure (DBP) of more than 90 mmHg,[9,10] compared with 40.6% of men and 31.9% of women in 2003, indicating a worsening of blood pressure status in the past 2 years.

To prevent and manage hypertension, the Japanese Society of Hypertension formulated the Guidelines for the Management of Hypertension 2004 in Japan (JSH2004).[11] The Guidelines specify lifestyle modification in the first treatment stage and antihypertensive therapy in the second treatment stage. In the first treatment stage, the emphasis is on improving eating and exercise habits and on smoking cessation. In particular, the Guidelines recommend restricted intake of foods that raise blood pressure and increased consumption of vegetables and fruits, which are considered to lower blood pressure.

In recent years, several studies have reported the antihypertensive effects of various dietary components.[12–14] Among these, chlorogenic acids (a representative component of green coffee bean extract), abundantly present in coffee beans, have antihypertensive effects in animal studies.[15,16] Studies in spontaneous hypertensive rats (SHR) have suggested that the mechanism of action is nitric oxide (NO)-mediated improvement in vascular endothelial function.[16] Green coffee beans contain 6.2–11.2% chlorogenic acids,[17] a generic term for compounds in which caffeic or ferulic acid is esterified with quinic acid. Chlorogenic acids in green cof-

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fee beans primarily are comprised of the following nine compounds: 5-caffeoylquinic acid (as the representative constituent), 3-caffeoylquinic acid, 4-caffeoylquinic acid, 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, 4,5-dicaffeoylquinic acid, 3-feruloylquinic acid, 4-feruloylquinic acid, and 5-feruloylquinic acid.[18)]

Many studies have reported the effects of commonly consumed coffee beverages prepared by extracting roasted coffee beans with hot water on blood pressure. Jee *et al.*, in a meta-analysis of 11 clinical trials, reported that coffee consumption was associated with a slight rise in blood pressure.[19)] On the other hand, a cohort study over a median follow-up of 33 years reported that coffee intake was not associated with blood pressure.[20)] Moreover, Funatsu *et al.* found that coffee intake lowered blood pressure in habitual alcohol drinkers with a randomized controlled crossover trial.[21)] Thus, no definite conclusion has been reached as to the association between coffee intake and blood pressure, and the antihypertensive effects of chlorogenic acids after coffee consumption remain controversial.

Recently, Suzuki *et al.* demonstrated that hydroxyhydroquinone (HHQ), which is produced by roasting green coffee beans, inhibits the antihypertensive effects of chlorogenic acids in SHR, and a reduction in HHQ attenuates hypertension.[22)] HHQ has been reported to be a source of reactive oxygen species,[23)] suggesting that HHQ-derived reactive oxygen species inhibit the antihypertensive effects of chlorogenic acids. It has been demonstrated that a 12-week continuous intake of HHQ-reduced coffee (184 ml) containing chlorogenic acids (299 mg/day) lowers hypertension in high-normotensives and mild hypertensives, compared with consumption of HHQ-reduced coffee (184 ml) containing no chlorogenic acids (0 mg/day), indicating the antihypertensive effect of chlorogenic acids present in coffee.[24)]

In this study, to reinforce the above evidence, a 12-week continuous intake intervention study in high-normotensives and mild hypertensives was conducted to determine the influence of HHQ in coffee on the antihypertensive effects of chlorogenic acids.

MATERIALS AND METHODS

Subjects —— The subjects were either high-normotensive (SBP of 130–139 mmHg and/or DBP of 85–89 mmHg) or mild hypertensive (SBP of 140–159 mmHg and/or DBP of 90–99 mmHg), as specified by the JSH2004 Guidelines, men and women aged 20 to 65 years at the start of the study. Subjects had not received antihypertensive therapy. Individuals with any of the following conditions were excluded from the study: severe liver, kidney, cerebrovascular, or heart disease; endocrine disorders; metabolic disturbances; diabetes, or potential pregnancy. Heavy smokers (more than 21 cigarettes/day on average), heavy alcohol consumers (more than 30 g/day of alcohol on average), caffeine- or coffee-hypersensitive or -allergic persons, as well as those considered ineligible by the examining physician, also were excluded from the study.

Execution of the Study —— Execution of this study was entrusted to Soiken Inc. (Osaka, Japan), a contract research organization (CRO). Under proper management by the CRO, a double-blind, randomized controlled trial was conducted at Soiken Clinic (Osaka, Japan). Before the start of the study, the study protocol was approved by Soiken Inc. and by the Soiken Clinic Ethical Committee on Contract for the Clinical Trial. Written informed consent was obtained from all participants after they received a full written explanation of the content, nature, aim, and possible risks of the study, which was performed under the careful medical supervision of the principal investigator in accordance with the Helsinki Declaration (adopted in 1964, revised in 1975, 1983, 1989, 1996, and 2000, annotated in 2002 and 2004).

Test Beverages —— Commonly consumed, commercially available coffee products contain chlorogenic acids and HHQ. The chlorogenic acid content can range from 15 to 325 mg in one cup of coffee.[25)] High performance liquid chromatography (HPLC) analysis in our laboratory showed that the average cup of coffee contains 40–350 mg of chlorogenic acids and 0.1–1.7 mg of HHQ. Two test beverages were used in the study (Table 1). An active beverage was prepared by reducing the HHQ content of a commonly consumed, commercially available

Table 1. Compositions of Test Beverages

		Active beverage	Control beverage
Chlorogenic acids	(mg/184 ml)	299	299
Hydroxyhydroquinone	(mg/184 ml)	0.05	1.69
Caffeine	(mg/184 ml)	77	75
Energy	(kJ/184 ml)	29.3	37.7

coffee product with the adsorption method; the control beverage contained HHQ, and was commonly a coffee. Both the active and control beverages contained chlorogenic acids. The test beverages were prepared using a popular Japanese canned coffee drink (184 ml) and could not be distinguished from each other in appearance or taste. The active and control beverages contained, per 184 ml, 299 mg chlorogenic acids and, 0.05 and 1.69 mg HHQ (considered to influence the antihypertensive effect of chlorogenic acids), 77 and 75 mg caffeine, and 29.3 and 37.7 kJ, respectively.

Study Protocol —— In this study, a double-blind, randomized controlled trial was conducted. The observation period before the ingestion of the test beverage was 4 weeks, followed by a 12-week intake period, and a wash-out period of 4 weeks. The ingestion of the test beverage was started at 0 week (wk), and examinations were performed at −4, −2, −1, 0, 4, 8, 10, 12, and 16 wk. After the examination at −1 wk, subjects were randomized into 2 groups (Active and Control), stratified by blood pressure classification, according to the JSH2004 Guidelines, and sex. During the trial period, the subjects were given the following instructions: drink one can of the active or control beverage daily in the intake period; continue usual dietary habits without drinking and eating too much; and, no change in exercise and smoking habits. In addition, subjects were prohibited from ingesting blood pressure-influencing drugs, antihypertensive foods, and coffee other than the test beverage. On the day before examination, they were instructed to finish dinner by 9:00 p.m. and not to eat or drink anything but water until the end of examination. On the day of examination, they were prohibited from smoking until the end of the examination and were given the test beverage at the end of the examination during the intake period.

Examinations included SBP, DBP, pulse rate, body weight, height, hematological tests, biochemical blood tests, urine tests, and health history. We have estimated that minimal clinically important difference of SBP was 5–6 mmHg and standard deviation of it was 10 mmHg after 8 wk from our preliminary study. Therefore, the target enrollment numbers of high-normotensives and mild hypertensives were more than 50 subjects; the ratios of high-normotensives to mild hypertensives and of males to females were intended to be 1 : 1 so that the distribution of the subjects to be analyzed would not significantly deviate from that observed in a national blood pressure survey in Japan,[10] and examinations were performed every 2-weeks between 8 wk and 12 wk in order to decrease an estimation error of blood pressure.

Measurement of Blood Pressure and Pulse Rate and Subject Interview —— In accordance with the JSH2004 Guidelines, blood pressure was measured with a mercury manometer after the subject arrived at the clinic and sat quietly for at least 10 min. Blood pressure was measured by a skilled nurse several times at intervals, and the mean of two stable blood pressures (with an SBP difference of less than 5 mmHg) was used in subsequent analyses. Pulse rate was measured once at each clinic visit. The physician interviewed the subjects to evaluate their subjective symptoms and objective findings.

Hematological, Biochemical Blood, and Urine Tests —— Hematological, biochemical blood, and urine tests were performed at 0, 12, and 16 wk at the Kishimoto Clinical Laboratory Group (Hokkaido, Japan). On the day of clinic visit, a skilled nurse collected blood from the subject after at least 10 min of rest in the seated position. Hematological tests included white and red blood cell counts, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and blood platelet.

Biochemical blood tests included triglyceride, total cholesterol, high density lipoprotein (HDL) cholesterol, fasting blood sugar, total homocysteine, aspartate aminotransferase, alanine aminotransferase, γ-glutamyl transpeptidase, alkaline phosphatase, lactate dehydrogenase, total protein, albumin, uric acid, blood urea nitrogen, serum creatinine, sodium (Na), chlorine (Cl), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), unsaturated iron-binding capacity, and ferritin. Urine tests included determinations of Na, K, and urinary creatinine. Qualitative urine tests included pH, protein, sugar, occult blood, urobilinogen, and specific gravity. In addition, 24-hr urinary Na and K excretion were estimated using spot urine, according to the method developed by Kawasaki *et al.*[26]

Body Height and Weight —— The subject's height was measured at preliminary examinations before −4 wk. Body weight was measured at −4, −1, 0, 4, 8, 12, and 16 wk. Body mass index (BMI, kg/m^2) was calculated from body weight and height.

Statistical Analysis —— The primary endpoint was SBP and DBP improvement in high-normotensives and mild hypertensives throughout a 12-week intervention consisting of either

HHQ-reduced or HHQ-containing coffee with chlorogenic acids.

In general, intra- and inter-day blood pressure fluctuations are relatively large in high-normotensive and mild hypertensive subjects, making it inappropriate to use data collected at only one time point. Therefore, the mean blood pressure of the measurements obtained at −1 and 0 wk, immediately before the ingestion of the test beverage, was used as the initial SBP or DBP level (*i.e.*, baseline measurement). The subjects were classified as high-normotensives or mild hypertensives according to the baseline blood pressure measurements.

To evaluate improvements in SBP and DBP, a linear mixed-model (correlation structure: unstructured) repeated-measures analysis of covariance (ANCOVA) was used to assess the significance of changes in the actual value, with Group, week, Group*week interaction as fixed effects, the baseline values of blood pressure as covariates, and the measured values at 4, 8, 10, and 12 wk as variables. The extent of improvement at each time point was evaluated from the least-square means and standard errors obtained in the above analyses. Comparisons of the effect between the groups on blood pressure at the end (16 wk) of the wash-out period were evaluated by ANCOVA, adjusting with the baseline values as covariates.

For the evaluation of hematology, blood biochemistry, and urinalysis, a comparison between the groups at 12 wk was performed by ANCOVA adjusting with the values obtained at 0 wk (the baseline values) as covariates. For a comparison between the groups at the completion of test beverage ingestion (16 wk), ANCOVA was performed adjusting with the baseline values as covariates. The above statistical calculations were not applied to the non-quantitative items. Pulse rate, body weight, and BMI were evaluated using a linear mixed-model (correlation structure: unstructured) repeated-measures ANCOVA to assess the significance of changes in the actual value, with Group, week, Group*week interaction as fixed effects and the baseline values of each item as covariates, according to the statistical analysis method used for blood pressure. For all items, group differences at baseline were analyzed using a Student's *t*-test.

We also evaluated improvements in SBP and DBP with only mild hypertensives (stratified analysis) or all subjects just after randomization (intention-to-treat analysis). Because we were formerly interested in mild hypertensives with higher blood pressure than high-normotensives, intention-to-treat analysis was quite important for a clinical trial. It was assumed there was no deviation between distributions of the active and control groups in mild hypertensives due to stratified randomization with blood pressure classification.

The data are presented as the mean ± standard error of mean (SEM). All tests were two-sided, and $p < 0.05$ was regarded as significant. Statistical calculations were performed using SAS software release 8.2 (SAS Institute Inc., Cary, NC, U.S.A.). The method of data analysis was verified by an independent statistician.

RESULTS

Subject Characteristics

One hundred and twenty-two subjects who gave informed consent entered the study. Of these subjects before randomization, two subjects withdrew from the study because they could not continue to visit the clinic, one subject dropped out because of an inability to continue to ingest the test beverage, and another subject dropped out because it became apparent that there were signs of cerebrovascular disorder. 118 subjects were randomly assigned to either the active group ($n = 59$) or the control group ($n = 59$). Of the subjects after randomization, one subject dropped out because he could not continue to visit the clinic in the intake period. Of the subjects who completed the study, the following were excluded: two subjects who deviated from the protocol (one subject with a high level of triglyceride on the start day of the study, and another subject with excessive drinking), 16 subjects with an initial normal blood pressure, and one subject with moderately elevated blood pressure, because this study was conducted for high-normotensives and mild hypertensives. Finally, a total of 98 subjects were analyzed for the primary endpoint: an active group of 51 (22 men and 29 women) and a control group of 47 (19 men and 28 women). The active group consisted of 22 high-normotensives (10 men and 12 women) and 29 mild hypertensives (12 men and 17 women), and the control group consisted of 16 high-normotensives (5 men and 11 women) and 31 mild hypertensives (14 men and 17 women).

Table 2 shows the baseline values of age, height, SBP, DBP, pulse rate, body weight, BMI, hematological tests, biochemical blood tests, and urine tests of the subjects analyzed. The SBP and

Table 2. Demographic Characteristics of High-normotensives and Mild Hypertensives at Baseline

	Active $n = 51$	Control $n = 47$
N (female/male)	29/22	28/19
Age (y)	51.4 (1.3)	51.6 (1.3)
Height (cm)	161.5 (1.2)	160.8 (1.1)
SBP (mmHg)	139.8 (1.2)	140.6 (1.0)
DBP (mmHg)	88.2 (0.8)	88.2 (0.6)
Pulse rate (beat/min)	73.7 (1.1)	71.9 (1.2)
Body weight (kg)	64.5 (1.5)	62.7 (1.7)
BMI (kg/m^2)	24.7 (0.5)	24.2 (0.5)
Red blood cells ($\times 10^4/\mu$l)	463.4 (5.5)	454.2 (5.2)
White blood cells ($\times 10^3/\mu$l)	5.4 (0.2)	5.5 (0.3)
Hemoglobin (g/dl)	14.2 (0.2)	13.9 (0.2)
Hematocrit (%)	41.9 (0.5)	41.0 (0.6)
Mean corpuscular hemoglobin (pg)	30.8 (0.3)	30.6 (0.3)
Mean corpuscular hemoglobin concentration (%)	34.0 (0.1)	33.9 (0.2)
Mean corpuscular volume (fl)	90.6 (0.7)	90.3 (0.8)
Blood platelet ($\times 10^4/\mu$l)	22.0 (0.8)	20.8 (0.7)
Triglyceride (mg/dl)	131.6 (10.3)	116.8 (10.2)
Total cholesterol (mg/dl)	219.8 (5.8)	210.1 (3.8)
HDL cholesterol (mg/dl)	60.3 (1.8)	63.1 (2.1)
Fasting blood sugar (mg/dl)	93.4 (1.2)	92.5 (1.0)
Total homocysteine (nmol/ml)	10.9 (0.4)	11.1 (0.4)
Aspartate aminotransferase (IU/l)	22.7 (1.3)	20.8 (0.8)
Alanine aminotransferase (IU/l)	24.1 (2.3)	20.2 (2.1)
γ-glutamyl transpeptidase (IU/l)	36.7 (3.6)	31.9 (4.4)
Alkaline phosphatase (IU/l)	220.4 (9.0)	221.1 (9.4)
Lactate dehydrogenase (IU/l)	183.8 (4.6)	180.4 (3.7)
Total protein (g/dl)	7.3 (0.04)	7.3 (0.04)
Albumin (g/dl)	4.6 (0.03)	4.6 (0.03)
Uric acid (mg/dl)	5.2 (0.2)	5.2 (0.2)
Blood urea nitrogen (mg/dl)	13.9 (0.5)	14.5 (0.5)
Serum creatinine (mg/dl)	0.9 (0.02)	0.9 (0.02)
Na (mEq/l)	142.6 (0.2)	142.5 (0.2)
Cl (mEq/l)	102.7 (0.3)	102.9 (0.3)
Ca (mg/dl)	9.2 (0.05)	9.2 (0.04)
Mg (mg/dl)	2.4 (0.02)	2.4 (0.02)
Fe (μg/dl)	100.4 (4.5)	101.4 (5.9)
K (mEq/l)	4.1 (0.05)	4.1 (0.05)
Unsaturated iron-binding capacity (μg/dl)	234.3 (8.3)	232.0 (9.0)
Ferritin (ng/ml)	123.7 (14.2)	123.8 (21.5)
Urinary Na (mEq/l)	150.6 (7.8)	158.9 (8.9)
Urinary K (mEq/l)	36.5 (2.6)	37.5 (2.5)
Urinary creatinine (mg/dl)	143.3 (9.8)	134.2 (9.8)
Presumptive urinary Na excretion (mEq/day)	197.4 (7.9)	200.2 (6.8)
Presumptive urinary K excretion (mEq/day)	41.1 (1.3)	42.1 (1.1)

Mean (SEM). SBP: systolic blood pressure, DBP: diastolic blood pressure, BMI: body mass index, HDL: high density lipoprotein. There were no significant differences between Active and Control groups at baseline of any items by student's t-test.

DBP in the active group were 139.8 ± 1.2 and 88.2 ± 0.8 mmHg, respectively, while the SBP and DBP in the control group were 140.6 ± 1.0 and 88.2 ± 0.6 mmHg, respectively. There were no significant differences between the groups at baseline of any items. The compliance with test beverage consumption was greater than 95% in both groups, with no differences between the groups. Table 3

Table 3. Demographic Characteristics of Mild Hypertensives at Baseline

	Active $n = 29$	Control $n = 31$
N (female/male)	17/12	17/14
Age (y)	53.6 (1.7)	51.0 (1.7)
Height (cm)	160.7 (1.6)	161.7 (1.5)
SBP (mmHg)	144.7 (1.4)	143.5 (1.1)
DBP (mmHg)	90.5 (1.1)	89.9 (0.7)
Pulse rate (beat/min)	74.3 (1.7)	72.4 (1.4)
Body weight (kg)	65.2 (1.8)	63.2 (2.4)
BMI (kg/m^2)	25.2 (0.6)	24.0 (0.7)
Red blood cells ($\times 10^4/\mu$l)	459.8 (7.1)	456.2 (7.1)
White blood cells ($\times 10^3/\mu$l)	5.3 (0.3)	5.3 (0.3)
Hemoglobin (g/dl)	14.0 (0.3)	14.0 (0.3)
Hematocrit (%)	41.4 (0.6)	41.2 (0.7)
Mean corpuscular hemoglobin (pg)	30.5 (0.4)	30.7 (0.4)
Mean corpuscular hemoglobin concentration (%)	33.8 (0.2)	34.0 (0.2)
Mean corpuscular volume (fl)	90.3 (0.9)	90.3 (1.0)
Blood platelet ($\times 10^4/\mu$l)	23.1 (1.0)	20.5 (1.0)
Triglyceride (mg/dl)	142.1 (14.3)	113.0 (12.6)
Total cholesterol (mg/dl)	223.2 (7.9)	205.0 (4.3) #
HDL cholesterol (mg/dl)	61.4 (2.5)	64.0 (2.9)
Fasting blood sugar (mg/dl)	93.5 (1.7)	91.4 (1.1)
Total homocysteine (nmol/ml)	11.4 (0.5)	11.2 (0.5)
Aspartate aminotransferase (IU/l)	25.0 (2.1)	21.6 (1.0)
Alanine aminotransferase (IU/l)	27.4 (3.8)	22.1 (3.0)
γ-glutamyl transpeptidase (IU/l)	39.9 (5.2)	35.7 (6.1)
Alkaline phosphatase (IU/l)	212.9 (12.4)	217.5 (12.1)
Lactate dehydrogenase (IU/l)	189.3 (6.5)	181.7 (4.7)
Total protein (g/dl)	7.4 (0.06)	7.3 (0.05)
Albumin (g/dl)	4.6 (0.04)	4.6 (0.03)
Uric acid (mg/dl)	5.3 (0.3)	5.4 (0.2)
Blood urea nitrogen (mg/dl)	14.2 (0.5)	14.6 (0.7)
Serum creatinine (mg/dl)	0.9 (0.02)	0.9 (0.02)
Na (mEq/l)	142.5 (0.2)	142.5 (0.3)
Cl (mEq/l)	102.4 (0.4)	102.7 (0.3)
Ca (mg/dl)	9.3 (0.08)	9.2 (0.06)
Mg (mg/dl)	2.4 (0.03)	2.4 (0.02)
Fe (μg/dl)	104.2 (6.4)	104.7 (7.9)
K (mEq/l)	4.1 (0.08)	4.1 (0.06)
Unsaturated iron-binding capacity (μg/dl)	233.7 (12.4)	232.8 (11.8)
Ferritin (ng/ml)	117.5 (16.0)	145.1 (30.6)
Urinary Na (mEq/l)	143.1 (10.7)	145.6 (10.8)
Urinary K (mEq/l)	33.2 (3.2)	36.3 (3.5)
Urinary creatinine (mg/dl)	134.6 (14.0)	135.0 (12.7)
Presumptive urinary Na excretion (mEq/day)	198.6 (10.2)	192.2 (6.5)
Presumptive urinary K excretion (mEq/day)	40.5 (1.7)	41.4 (1.2)

Mean (SEM). SBP: systolic blood pressure, DBP: diastolic blood pressure, BMI: body mass index, HDL: high density lipoprotein. #; $p < 0.05$ between Active and Control groups at baseline by student's t-test.

also shows demographic characteristics of only mild hypertensives at baseline. There were no significant differences between the groups at baseline of any items except total cholesterol.

Changes in SBP and DBP

Table 4 shows the SBP and DBP from 4 wk to 12 wk (the intake period) in high-normotensives and mild hypertensives. At 4, 8, 10, and 12 wk the es-

Table 4. Changes in Blood Pressure in High-normotensives and Mild Hypertensives

		Active (mmHg)		Control (mmHg)		Estimated difference
		Mean (SEM)	(min, max)	Mean (SEM)	(min, max)	(mmHg, 95% CI)
SBP	4 wk	135.2 (1.4)	(111, 158)	140.0 (1.4)	(123, 169)	−4.2, (−7.2 : −1.3) ##
	8 wk	136.0 (1.4)	(115, 159)	138.4 (1.5)	(119, 161)	−1.8, (−5.2 : 1.6)
	10 wk	137.5 (1.5)	(112, 162)	140.8 (1.3)	(119, 158)	−2.6, (−6.2 : 0.9)
	12 wk	139.1 (1.5)	(112, 169)	143.0 (1.6)	(117, 172)	−3.4, (−7.3 : 0.5)
Group	*p*	*0.031*				
DBP	4 wk	85.3 (0.9)	(65, 101)	86.7 (0.9)	(71, 98)	−1.3, (−3.4 : 0.7)
	8 wk	86.3 (0.8)	(74, 100)	87.6 (1.0)	(70, 98)	−1.2, (−3.3 : 1.0)
	10 wk	87.0 (1.0)	(69, 100)	88.9 (1.0)	(75, 103)	−1.8, (−4.3 : 0.7)
	12 wk	88.4 (0.9)	(74, 108)	89.8 (1.0)	(72, 104)	−1.4, (−3.8 : 1.1)
Group	*p*	*0.092*				

SBP: systolic blood pressure, DBP: diastolic blood pressure, min: minimam, max: maximum, CI: confidence interval. *Group* represents p value of group effect by repeated measures ANCOVA (covariate = baseline, variable = 4 wk, 8 wk, 10 wk, 12 wk). Estimated difference: the estimated difference between Active and Control groups at each week and 95% CI by a linear mixed-model. ##: $p < 0.01$ between Active and Control groups using adjusted least-square means and standard errors. n; Active = 51, Control = 47. Statistical multiplicity between SBP and DBP was not adjusted.

Table 5. Changes in Blood Pressure in Mild Hypertensives

		Active (mmHg)		Control (mmHg)		Estimated difference
		Mean (SEM)	(min, max)	Mean (SEM)	(min, max)	(mmHg, 95% CI)
SBP	4 wk	139.2 (1.9)	(121, 158)	143.1 (1.7)	(125, 169)	−4.8, (−8.8 : −0.8) #
	8 wk	139.0 (1.9)	(120, 159)	141.8 (1.5)	(130, 161)	−3.7, (−8.1 : 0.7)
	10 wk	140.7 (1.9)	(121, 162)	143.7 (1.2)	(131, 157)	−4.0, (−8.3 : 0.4)
	12 wk	140.9 (2.3)	(112, 169)	145.7 (1.8)	(129, 172)	−5.7, (−10.8 : −0.6) #
Group	*p*	*0.013*				
DBP	4 wk	86.9 (1.1)	(70, 101)	88.4 (1.1)	(71, 98)	−1.9, (−4.4 : 0.6)
	8 wk	87.6 (1.2)	(79, 100)	89.4 (1.2)	(70, 98)	−2.3, (−5.3 : 0.7)
	10 wk	87.2 (1.3)	(70, 100)	90.6 (1.2)	(78, 103)	−4.0, (−7.0 : −0.9) #
	12 wk	88.9 (1.3)	(75, 108)	91.3 (1.2)	(79, 104)	−2.8, (−6.0 : 0.3)
Group	*p*	*0.015*				

SBP: systolic blood pressure, DBP: diastolic blood pressure, min: minimam, max:maximum, CI: confidence interval. *Group* represents p value of group effect by repeated measures ANCOVA (covariate = baseline, variable = 4 wk, 8 wk, 10 wk, 12 wk). Estimated difference: the estimated difference between Active and Control groups at each week and 95% CI by a linear mixed-model. #: $p < 0.05$ between Active and Control groups using adjusted least-square means and standard errors. n; Active = 29, Control = 31. Statistical multiplicity between SBP and DBP was not adjusted.

timated differences in SBP between the active and control groups were as follows: −4.2 mmHg [95% confidence interval (CI), −7.2 to −1.3], −1.8 mmHg [95% CI, −5.2 to 1.6], −2.6 mmHg [95% CI, −6.2 to 0.9], and −3.4 mmHg [95% CI, −7.3 to 0.5], respectively. Thus, significant differences were noted throughout the intake period between the active and control groups (Group effect; $p = 0.031$). The active group exhibited a significantly lower SBP than the control group at 4 wk ($p < 0.01$), using the least-squares mean and standard error at each time point.

The estimated differences between the active and control groups in DBP at 4, 8, 10, and 12 wk were as follows: −1.3 mmHg [95% CI, −3.4 to 0.7], −1.2 mmHg [95% CI, −3.3 to 1.0], −1.8 mmHg [95% CI, −4.3 to 0.7], and −1.4 mmHg [95% CI, −3.8 to 1.1], respectively. Thus, no significant differences were noted throughout the intake period between the active and control groups (Group effect; $p = 0.092$). No significant differences between the groups were noted in SBP or DBP during the wash-out period (16 wk data not shown).

A stratified analysis of only mild hypertensives in both groups showed that the estimated differences between the active and control groups in SBP at 4, 8, 10, and 12 wk were as follows: −4.8 mmHg [95% CI, −8.8 to −0.8], −3.7 mmHg [95% CI, −8.1 to 0.7], −4.0 mmHg [95% CI, −8.3 to 0.4], and −5.7 mmHg [95% CI, −10.8 to −0.6], respectively (Table 5). Thus, significant differences in SBP

were noted between the active and control groups throughout the intake period (Group effect; p = 0.013) in mild hypertensives. In addition, testing using the least-squares mean and standard error at each time point showed that the active group exhibited significantly lower values at 4 and 12 wk than the control group ($p < 0.05$).

The estimated differences between the active and control groups in DBP at 4, 8, 10, and 12 wk were: −1.9 mmHg [95% CI, −4.4 to 0.6], −2.3 mmHg [95% CI, −5.3 to 0.7], −4.0 mmHg [95% CI, −7.0 to −0.9], and −2.8 mmHg [95% CI, −6.0 to 0.3], respectively. Thus, significant differences in DBP were noted between the active and control groups throughout the intake period (Group effect; p = 0.015). Testing using the least-squares mean and standard error at each time point showed that the active group exhibited a significantly lower value at 10 wk than the control group ($p < 0.05$). No significant differences between the groups were noted in either SBP or DBP during the wash-out period (16 wk data not shown).

Intention-to-treat analysis of all subject just after randomization in both groups (n = 118, the active group = 59, the control group = 59) showed that the active and control groups in SBP at baseline, 4, 8, 10, and 12 wk were as follows: the active group, 138.1 ± 1.2 mmHg, 133.6 ± 1.6 mmHg, 135.1 ± 1.3 mmHg, 136.4 ± 1.4 mmHg, and 137.4 ± 1.5 mmHg; and, the control group, 138.4 ± 1.1 mmHg, 138 ± 1.5 mmHg, 137.6 ± 1.5 mmHg, 139.6 ± 1.4 mmHg, and 141.7 ± 1.6 mmHg, respectively. For both groups the DBP at baseline, 4, 8, 10, and 12 wk were as follows: the active group, 87 ± 0.8 mmHg, 83.9 ± 1.0 mmHg, 85.4 ± 0.8 mmHg, 85.9 ± 1.0 mmHg, and 87.3 ± 0.9 mmHg; and, the control group, 86.9 ± 0.7 mmHg, 86.1 ± 0.9 mmHg, 87.1 ± 0.9 mmHg, 88.2 ± 1.0 mmHg, and 89.1 ± 1.0 mmHg, respectively. Significant differences between the groups were noted in SBP and DBP throughout the intake period (Group effect; p = 0.014, p = 0.018, respectively).

Changes in Body Weight, BMI, Pulse Rate, Hematological, Biochemical Blood, and Urine Tests

Table 6 shows the changes in body weight, BMI, and pulse rate from 4 wk to 12 wk (the intake period) in high-normotensives and mild hypertensives. No significant differences between the groups were noted in body weight, BMI, or pulse rate throughout the intake period. No significant differences between the groups were noted in these items during the wash-out period (16 wk data not shown). Table 7 shows 12 wk data at the end of the intake period in hematological, biochemical blood, and urine tests. No significant differences between the groups were noted in these items during the intake period. 16 wk data at the wash-out period was not shown, but significant differences between the groups were noted in lactate dehydrogenase, uric acid, and K (Group effect; p = 0.038, p = 0.031, and p = 0.050, respectively). The values of lactate dehydrogenase, uric acid, and K in the active and control groups at 16 wk were as follows: the active group, 182.3 ± 4.2 IU/l, 4.6 ± 0.2 mg/dl, and 4.0 ± 0.04 mEq/l; and, the control group, 191.3 ± 5.7 IU/l, 4.3 ± 0.2 mg/dl, and 4.1 ± 0.05 mEq/l, respectively. No significant differences between the groups were noted in any other items during the wash-out period. No clinically problematic findings were noted in urinary pH, protein, sugar, occult blood, urobilinogen, or specific gravity (data not shown).

Table 6. Change in Pulse Rate and Body Composition

		Active	Control
		Mean (SEM)	Mean (SEM)
Pulse rate (beat/min)	4 wk	73.5 (1.3)	71.6 (1.2)
	8 wk	72.1 (1.3)	71.4 (1.5)
	10 wk	72.5 (1.3)	69.4 (1.2)
	12 wk	72.2 (1.1)	70.0 (1.1)
Group[a)]	*p*	*0.393*	
Body weight (kg)	4 wk	64.5 (1.4)	62.9 (1.7)
	8 wk	65.1 (1.4)	63.5 (1.8)
	12 wk	65.4 (1.5)	64.1 (1.8)
Group[b)]	*p*	*0.070*	
BMI (kg/m^2)	4 wk	24.7 (0.5)	24.3 (0.5)
	8 wk	24.9 (0.4)	24.5 (0.5)
	12 wk	25.0 (0.5)	24.7 (0.5)
Group[b)]	*p*	*0.067*	

BMI: body mass index. *Group*[a)] represents p value of group effect by repeated measures ANCOVA (covariate = baseline, variable = 4 wk, 8 wk, 10 wk, 12 wk). *Group*[b)] represents p value of group effect by repeated measures ANCOVA (covariate = baseline, variable = 4 wk, 8 wk, 12 wk). n; Active = 51, Control = 47.

Adverse Events

The interview with the subjects did not reveal any adverse effects of variable amounts of HHQ in coffee. No clinically problematic changes in individual subjects were noted in SBP, DBP, or other laboratory data.

Table 7. Changes in Blood and Urinary Parameters at 12 Weeks

	Active $n = 51$	Control $n = 47$	*Group* p
Red blood cells ($\times 10^4/\mu l$)	466.0 (5.8)	460.0 (5.7)	0.472
White blood cells ($\times 10^3/\mu l$)	5.1 (0.2)	5.2 (0.2)	0.716
Hemoglobin (g/dl)	14.4 (0.2)	14.2 (0.2)	0.334
Hematocrit (%)	43.3 (0.5)	42.7 (0.6)	0.329
Mean corpuscular hemoglobin (pg)	31.0 (0.3)	30.9 (0.3)	0.565
Mean corpuscular hemoglobin concentration (%)	33.3 (0.1)	33.2 (0.1)	0.830
Mean corpuscular volume (fl)	93.1 (0.7)	93.0 (0.8)	0.686
Blood platelet ($\times 10^4/\mu l$)	23.4 (0.8)	22.1 (0.8)	0.880
Triglyceride (mg/dl)	150.5 (15.0)	119.5 (10.0)	0.187
Total cholesterol (mg/dl)	224.3 (5.2)	219.5 (4.2)	0.552
HDL cholesterol (mg/dl)	59.5 (2.0)	63.9 (2.4)	0.298
Fasting blood sugar (mg/dl)	94.4 (1.1)	94.7 (1.3)	0.401
Total homocysteine (nmol/ml)	13.1 (0.5)	13.2 (0.6)	0.937
Aspartate aminotransferase (IU/l)	24.4 (1.3)	24.4 (2.1)	0.267
Alanine aminotransferase (IU/l)	27.3 (2.6)	25.8 (3.1)	0.275
γ-glutamyl transpeptidase (IU/l)	40.8 (3.9)	37.7 (7.0)	0.369
Alkaline phosphatase (IU/l)	226.8 (9.6)	233.0 (12.8)	0.388
Lactate dehydrogenase (IU/l)	185.2 (4.7)	182.4 (3.7)	0.992
Total protein (g/dl)	7.4 (0.04)	7.4 (0.05)	0.455
Albumin (g/dl)	4.5 (0.03)	4.5 (0.02)	0.770
Uric acid (mg/dl)	5.3 (0.2)	5.3 (0.2)	0.918
Blood urea nitrogen (mg/dl)	14.7 (0.4)	15.2 (0.5)	0.745
Serum creatinine (mg/dl)	0.9 (0.02)	0.9 (0.02)	0.392
Na (mEq/l)	143.5 (0.3)	143.5 (0.2)	0.822
Cl (mEq/l)	102.7 (0.2)	102.8 (0.3)	0.842
Ca (mg/dl)	9.2 (0.04)	9.2 (0.04)	0.372
Mg (mg/dl)	2.3 (0.02)	2.3 (0.02)	0.945
Fe (μg/dl)	111.2 (5.0)	103.5 (6.5)	0.298
K (mEq/l)	4.0 (0.05)	4.0 (0.05)	0.983
Unsaturated iron-binding capacity (μg/dl)	220.1 (7.7)	231.2 (10.2)	0.163
Ferritin (ng/ml)	130.3 (14.7)	122.3 (19.8)	0.185
Urinary Na (mEq/l)	127.2 (6.7)	114.8 (6.6)	0.103
Urinary K (mEq/l)	36.9 (2.7)	34.8 (3.4)	0.602
Urinary creatinine (mg/dl)	117.8 (7.1)	106.7 (9.2)	0.452
Presumptive urinary Na excretion (mEq/day)	195.1 (6.9)	195.0 (6.6)	0.865
Presumptive urinary K excretion (mEq/day)	45.1 (1.6)	45.4 (1.5)	0.976

Mean (SEM). HDL: high density lipoprotein. *Group* represents p value of group effect by ANCOVA (covariate = base line value, variable = 12 wk).

DISCUSSION

There is much evidence concerning the antihypertensive effect of HHQ-reduced coffee and its mechanism. In a study of SHR, Suzuki *et al.* showed that HHQ-reduced coffee had a blood pressure-lowering effect.[22] It was revealed that the antihypertensive effect of the representative component of chlorogenic acids, 5-caffeoylquinic acid, is associated with the production of NO in the vascular endothelium in an animal study concomitantly using a NO synthase inhibitor (N^G-nitro-L-arginine methyl ester). Also, the ingestion of 5-caffeoylquinic acid improved vascular endothelium-dependent vasodilation along with increasing NO bioavailability by decreasing NADPH-dependent superoxide anion (O_2^-) production in an experiment using SHR.[16] Moreover, the addition of HHQ attenuated, in a dose-dependent manner, the antihypertensive effect of green coffee extract (major component : chlorogenic acids), or 5-caffeoylquinic acid. Meanwhile, ingestion of HHQ alone did not influence blood pressure in these experiments.[22] *In vivo* and *in vitro* studies have reported that HHQ gener-

ates O_2^-.[23,27]

In a previous study, it was evident from clinical trials with high-normotensives and mild hypertensives that continuous ingestion for 12 weeks of the chlorogenic acids in HHQ-reduced coffee lowered blood pressure (the active beverage: 299 mg/day of chlorogenic acids, 0.05 mg/day of HHQ, the placebo beverage: 0 mg/day of chlorogenic acids, 0.02 mg/day of HHQ).[24] It was also suggested that the chlorogenic acids in HHQ-reduced coffee improved vascular endothelial function of subjects.

In this study, we investigated the influence of the HHQ content in chlorogenic acids-containing coffee on the antihypertensive effects in high-normotensive and mild hypertensive subjects. SBP was significantly lower, by 1.8–4.2 mmHg, in the active than in the control group. DBP was lower by 1.2–1.8 mmHg in the active compared with the control group. These results suggest that the lowering of HHQ in coffee containing chlorogenic acids improves blood pressure in high-normotensives and mild hypertensives. The antihypertensive alteration seemed almost saturated at 4 wk.

A stratified analysis of only the mild hypertensives showed that SBP was significantly lower, by 3.7–5.7 mmHg, in the active compared with the control group. DBP was also significantly lower, by 1.9–4.0 mmHg, in the active compared with the control group. These results suggest that the antihypertensive effect was greater in the mild hypertensives than in the high-normotensives.

The above observations suggest the following: the ingestion of chlorogenic acids in coffee improves NO bioavailability, enhancing vascular endothelial function and thereby improving blood pressure. Concomitantly, the generation of O_2^- arising from HHQ in coffee inhibits the improvement of NO bioavailability by chlorogenic acids, therefore the antihypertensive effect of chlorogenic acids attenuates. Reducing the HHQ content in coffee can educe the antihypertensive effect of chlorogenic acids in coffee. However, the influence on O_2^- in humans is unexplained in this study.

The antihypertensive effect of HHQ-reduced coffee is weaker than those of drugs such as Angiotensin-Converting Enzyme (ACE) inhibitors or angiotensin receptor blockers.[28,29] However, an epidemiological study by Stamler *et al.* found that a mean decrease in SBP of 2 mmHg reduced mortality from stroke by an estimated 6%,[30] suggesting that even a slight improvement in blood pressure has a significant preventive effect at the population level. HHQ-reduced coffee, as a food, can probably be ingested in many populations. Thus, the intake of HHQ-reduced coffee is useful from the viewpoint of preventive medicine, and is suited as a population strategy.[31]

Significant differences between the groups were noted in safety assessment parameters, such as lactate dehydrogenase, uric acid, and K, at 16 wk. However, changes in these three parameters were within the normal ranges. Therefore, it was speculated that the significant group differences were not due to discontinuation of the active beverage. Otherwise, the active group showed no clinically problematic changes in pulse rate, body weight, hematological tests, biochemical blood tests, or urine tests, compared with the control group that ingested a commercially available coffee product. In addition, the interview with the subjects did not reveal any adverse effects due to the HHQ content in coffee. For example, no side-effects such as dry cough and headache were noted, which are known to be caused by ACE inhibitors.[32] These findings suggest that HHQ-reduced coffee may have fewer negative side effects than antihypertensive drugs.

In summary, the 12-week continuous ingestion of HHQ-reduced coffee with chlorogenic acids significantly lowered SBP in the active group consisting of high-normotensive and mild hypertensive subjects, in comparison with the control group that ingested a commercially available coffee. The results of the present study suggest that HHQ-reduced coffee improves blood pressure in individuals with slightly elevated blood pressure, and can be safely ingested, making its ingestion a useful means of diet therapy in everyday life.

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