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The Divergent Effect of Coffee Polyphenol and Hydroxyhydroquinone Ingestion on Postprandial Hyperglycemia and Vascular Function in Healthy Men

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Abstract Epidemiological studies indicate that coffee consumption reduces the risk of diabetes and cardiovascular diseases. However, interventional studies have failed to clarify the beneficial effects of coffee consumption on blood glucose and the cardiovascular system. We previously demonstrated that 1) coffee polyphenol (CPP) consumption improved postprandial hyperglycemia and vascular endothelial function in humans, and 2) improvement in vascular endothelial function due to CPP was impaired by hydroxyhydroquinone (HHQ) in rats. This study aimed to elucidate the impact of concomitant consumption of HHQ, a prooxidant in coffee, on the beneficial effects of CPP consumption on postprandial blood glucose and vascular function in humans. We conducted a single-blind, randomized, placebo-controlled, crossover intervention study in healthy male adults, measuring blood and urine parameters and flow-mediated dilation after ingestion of a meal with CPP with or without HHQ up to 180 min postprandially. Ten healthy male adults consumed a test meal with either a placebo, control (CPP with HHQ), or active (CPP without HHQ) beverage. The CPP-including active (without HHQ) beverage significantly blunted the postprandial increase in blood glucose and decline in flow-mediated dilation but not the control (with HHQ) beverage, compared with the placebo beverage. The active beverage reduced blood oxidative stress biomarker response compared with the control beverage. In conclusion, these results demonstrate that concomitant ingestion of HHQ, which increases oxidative stress, interferes with the improvement of postprandial blood glucose and vascular endothelial function after CPP consumption in healthy humans.

Keywords: *adult men, coffee polyphenol, flow-mediated dilatation, hydroxyhydroquinone, oxidative stress, postprandial blood glucose*

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1. Introduction

Coffee is one of the most popular beverages worldwide and has been consumed for hundreds of years because of its flavor and physiological effects. Many studies have demonstrated the potential health benefits of daily coffee consumption. Previous epidemiological studies have reported that an increase in daily consumption of coffee was associated with a reduced risk of metabolic syndrome [1,2,3], diabetes [4], and death due to cardiovascular disease [5]. Similar findings were also observed with decaffeinated coffee consumption [6]. However, the active components mediating the decline in the risk of these diseases associated with daily coffee consumption have not been fully elucidated [7].

Coffee is also one of the major sources of dietary polyphenols. A study by Fukushima et al. showed that half of the daily polyphenol consumption in the Japanese population derives from coffee [8]. The health benefits of daily coffee consumption may be associated with its polyphenol content, which leads to a range of physiological effects [9]. Caffeic acid and its quinic acid ester (chlorogenic acid) have been identified as the most abundant polyphenols in coffee; a single cup of coffee contains 70–350 mg of chlorogenic acids [10].

Although the mechanism mediating the reduced risk of diabetes with daily coffee consumption has not been fully explained, our recent study demonstrated that coffee polyphenol (CPP) consumption lowered postprandial hyperglycemia, which was associated with improved vascular endothelial function and reduced oxidative stress in humans [11]. A recent study by Kubota et al. [12] demonstrated that an improvement in vascular endothelial function restores impaired glucose tolerance by reducing insulin resistance in skeletal muscle. Kawano et al. demonstrated that vascular endothelial function measured by flow-mediated dilation (FMD) was decreased after glucose ingestion, with a concomitant increase in oxidative stress markers [13]. Furthermore, ingestion of antioxidants reduced postprandial endothelial dysfunction

[14]. Therefore, the reduction in postprandial hyperglycemia after CPP consumption appears to be caused by improved vascular endothelial function, which may result from decreased oxidative stress after dietary consumption.

Coffee contains prooxidants that generate reactive oxygen species (ROS), such as benzenetriols, including hydroxyhydroquinone (HHQ; 1,2,4-trihydroxybenzene) [15,16]; therefore, it is conceivable that the prooxidants and antioxidants counteract each other so that coffee fails to elicit any beneficial action. Studies have revealed that the hypotensive effect of CPPs was significantly inhibited by the concomitant ingestion of HHQ in rodents [17] and in humans [18]. However, the acute effect of concomitant ingestion of HHQ on the beneficial actions of CPPs on blood glucose and vasodilation has not been fully elucidated. Postprandial hyperglycemia is a direct and independent risk factor for coronary heart disease [19], and endothelial dysfunction has been identified as an important contributing factor for coronary heart disease. Therefore, we focused on endothelial dysfunction and hyperglycemia after meal ingestion.

Considering the findings set out above, we hypothesized that concomitant ingestion of HHQ may reduce the beneficial actions of CPP consumption on postprandial hyperglycemia and vascular endothelial function by increasing oxidative stress in humans. To test this hypothesis, we examined the acute effects of a single intake of CPPs with or without HHQ on postprandial blood glucose, peripheral endothelial function, and oxidative stress biomarkers, in a single-blind, randomized, placebo-controlled, crossover trial in healthy male adults.

2. Materials and Methods

2.1. Materials

Coffee polyphenol extract (CPE) was prepared for this study from roasted coffee beans (Vietnam Robusta, Brazil; L value = 25 for each) by hot water extraction. Chlorogenic acids predominantly include the following 9 compounds: 5-Caffeoylquinic acid (CQA), 3-CQA, 4-CQA, 3,4-diCQA, 3,5-diCQA, 4,5-diCQA, 3-Feruloylquinic acid (FQA), 4-FQA, and 5-FQA. The most abundant component is 5-CQA. In preparing an active beverage, we reduced the level of HHQ in the CPE by an absorption treatment using activated carbon filtration [20,21]. The polyphenol composition of CPE was measured by high-performance liquid chromatography. The composition of CQAs in CPE was 72.3% CQA (total 3-CQA, 4-CQA, and 5-CQA), 19.3% feruloylquinic acid (3-FQA, 4-FQA, and 5-FQA), and 8.4% dicaffeoylquinic acid (3,4-diCQA, 3,5-diCQA, and 4,5-diCQA) for the active beverage, and 85.8% CQA (total 3-CQA, 4-CQA, and 5-CQA), 9.6% feruloylquinic acid (3-FQA, 4-FQA, and 5-FQA), and 4.6% dicaffeoylquinic acid (3,4-diCQA, 3,5-diCQA, and 4,5-diCQA) for the control beverage, respectively.

2.2. Test Beverages

The beverages were taste and flavor-matched (coffee-flavored), and differed in polyphenol and HHQ content. Standard brewed coffee contains both chlorogenic acids and HHQ. It was determined, on the basis of our high-performance liquid chromatography analysis, that a cup of coffee typically contains 40–350 mg of chlorogenic acids and 0.1–1.7 mg HHQ. In preparing test beverages, we adjusted the chlorogenic acid content of the CPEs to approximately 300 mg per 185 mL. The control (CPE with HHQ) beverage (185 mL) contained 300 mg of chlorogenic acids and 0.7 mg of HHQ. The active (CPE without HHQ) beverage (185 mL) contained 306 mg of chlorogenic acids and 0.1 mg of HHQ. The placebo beverage did not contain chlorogenic acids or HHQ. The caffeine content of each beverage was 50, 65, and 56 mg for the placebo, control, and active beverages, respectively (Table 1).

Table 1. Composition of test beverages (185 mL)

	Placebo	Control	Active
Chlorogenic acids (mg)	N.D.	300	306
HHQ (mg)	N.D.	0.7	0.1
Caffeine (mg)	50	65	56

HHQ: hydroxyhydroquinone; N.D.: not detected.

2.3. Subjects

A statistical power analysis was performed for sample size estimation (n = 10) based on data from a preliminary pilot study (subjects, n = 10; significance, $\alpha = 0.05$, power, $1 - \beta = 0.96$). Thirty-three people were recruited for eligibility screening using an in-house mail system, and 10 subjects were enrolled in the study. All subjects completed the study, and therefore, a total of 10 healthy Japanese male subjects (BMI 21.5 ± 1.8), aged 25 to 43 (32.6 ± 6.7) years, were analyzed in this study (Figure 1). There were no smokers among the subjects. Subjects were not taking any medication or undergoing lifestyle interventions, and did not report any allergies, hypersensitivity to caffeine or coffee, or heavy alcohol use. The Human Ethics Committee of Kao Corporation approved the study protocol. All subjects provided written informed consent. The present study was conducted under the supervision of the chief investigator, in accordance with the Declaration of Helsinki.

2.4. Study Design

We examined the acute effects of meal ingestion with a single intake of the CPE-containing beverage on blood and urine parameters and endothelial function in a single-blind, randomized, placebo-controlled, crossover trial, with a washout period of at least 7 days.

The subjects consumed a meal provided as a lunch box (975 kcal, carbohydrate: fat: protein = 113.2: 38.1: 35.5 (g)) before 9 PM on the night preceding each study session; this was followed by fasting (only water was allowed). Subjects were not allowed to smoke. On the day of the study session, the subjects were asked to void urine after they woke up and to drink a bottle of water (300 mL), which was provided, before 8 AM. They presented to the test room at 8:30 AM, their height and body weight were measured, and participants were interviewed to assess their health status. A baseline urine sample was collected in an ice-cooled, light-shielding polystyrene bottle.

Vascular endothelial function was evaluated using UNEXEF18GII (Unex Co. Ltd., Nagoya, Japan), followed by baseline blood sampling. The subjects then ingested a cookie-type clinical test meal [11,22] (Meal Test C®, Saraya Co., Ltd, Osaka, Japan; 592 kcal/115 g, carbohydrate: fat: protein = 75.0: 28.5: 8.0 (g)), with a single intake of either the placebo or CPE beverage containing CPE (300 or 306 mg chlorogenic acids/beverage, respectively), within 12 minutes. Blood samples were collected at 0.5, 1, 2, 3, and 4 hours after ingestion, and FMD was measured at 1, 2, 3, and 4 hours after ingestion. The timing and measurements were determined in a previous study by our group [11], showing that measurement of major outcomes peaked at 0.5 or 1 hour after meal ingestion, and returned to baseline level within 4 hours. Urine samples were pooled in another ice-cooled, light-shielding polystyrene bottle until 4 hour after meal ingestion. During each of the three study sessions, subjects remained in a room maintained at 25 ± 2°C with 50% humidity until the session was completed.

2.5. Flow-mediated Dilation

The FMD of the right brachial artery was evaluated by A- and B-mode ultrasonography using a 10-MHz linear-array transducer (UNEXEF18GII, UNEX Corp., Nagoya, Japan) as described in our previous study [11]. Briefly, the subjects were instructed to lie down for 10 minutes. The baseline diameter of the brachial artery was defined as its mean diameter 5 cm proximal to the elbow joint during 10 consecutive diastoles on an electrocardiogram before hyperemia. After this baseline diameter had been determined, forearm hyperemia was produced using sphygmomanometric cuff inflation to 180 mmHg for 5 minutes. After the cuff had been deflated, the maximum diameter of the brachial artery after hyperemia was measured for 120 seconds. The rate of change in diameter (%) determined using the maximum diameter at baseline and after hyperemia was defined as the FMD. The FMD of each test group determined at preload was designated FMD0, and measurements at 1, 2, 3, and 4 hours after loading were designated as FMD1, FMD2, FMD3, and FMD4, respectively.

To evaluate differences in FMD change between the two groups, the percent change from FMD0 to FMDt was calculated as 100 × (FMDt-FMD0)/FMD0. To evaluate the correlations between changes in FMD and metabolic blood parameters, the percent change in FMD in the three groups was calculated as follows: ΔFMDt% = 100 × (FMD0-FMDt)/FMD0.

2.6. Biochemical Measurements

Blood samples were collected at 0 (baseline), 0.5, 1, 2, 3, and 4 hours after meal ingestion. Blood glucose was determined by employing a blood glucose self-monitoring device (ACCU-CHECK COMFORT®, Roche Diagnostics Co., Tokyo, Japan) immediately after blood collection. After centrifugation at 3,000 × g for 15 min at ambient temperature, the serum samples were stored at -80°C until analysis. After adding protease inhibitor (Protease Inhibitor Cocktail powder, P2714-1BTL, Sigma, St. Louis, USA) and serine protease inhibitor (Pefabloc, Roche Diagnostics K.K., Basel, Switzerland), the blood samples were centrifuged at 2,900 × g for 10 min at ambient temperature. The plasma samples were stored at -80°C until analysis.

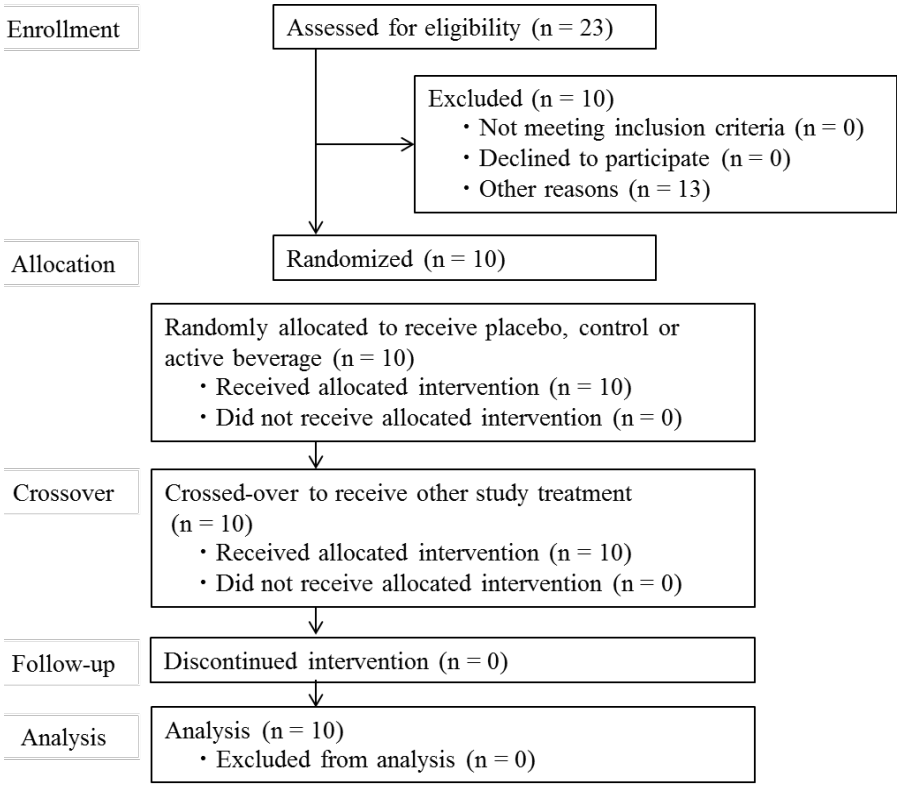

Figure 1. Flow and number of study participants from screening to study completion

Blood triglyceride (TG), non-esterified fatty acids (NEFA), total cholesterol (T-Chol), low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, lactate dehydrogenase, alanine aminotransferase, aspartate aminotransferase, and urinary creatinine levels were measured by automatic blood analyzer (ACCUTE TBA-40FR, TOSHIBA Co., Tokyo, Japan) using compatible reagents (Nittobo, Tokyo). Blood insulin (Mercodia, Sweden), and active glucagon-like peptide-1 (GLP-1; Millipore, Massachusetts) levels were analyzed by enzyme-linked immunosorbent assay. Blood hydrogen peroxide (H_2O_2) and diacron-reactive oxygen metabolites (d-ROMs) were measured using a BIOXYTECH H_2O_2-560^{TM} (OXIS International Inc., Tampa, FL) and Reactive Oxygen Metabolites Test (Wismerll, Tokyo), respectively.

Peak blood glucose clearance was calculated as Δglucose (30-60 min).

Urine samples were stored at -80°C until analysis. Urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG) was measured using a New 8-OHdG Check kit (NIKKEN SEIL Co., Ltd, Shizuoka).

2.7. Statistical Analyses

Numerical data are expressed as the means ± SEM. Blood parameter responses were assessed by calculating the incremental or decremental area under the curve (iAUC or dAUC) using the trapezoid rule. One-way analyses of variance (ANOVA) with the Bonferroni post hoc test for paired multiple comparison was used when comparing values among the three groups. Student's t-tests after a preliminary F-test of the homogeneity of within-group variance, or paired t-tests were used when comparing values between two groups. Two-way repeated ANOVA was used to compare changes over time and between the groups (STATVIEW for Windows Ver. 5.0, SAS Institute Inc., Cary, NC, USA). Differences were considered significant when the error probability was smaller than 0.05.

3. Results and Discussion

We aimed to test our hypothesis that concomitant ingestion of HHQ may reduce the beneficial actions of CPP consumption on postprandial hyperglycemia and vascular endothelial function by increasing oxidative stress in humans. The present study had three major findings.

HHQ reduces the improvement of postprandial blood glucose increase after CPP consumption in healthy human subjects.

The initial blood and urinary parameters measured in subjects were comparable between the three test beverage groups (Table 2).

Plasma glucose levels peaked 30 min after ingestion of the test meal with the placebo beverage, and then declined, reaching baseline after 180 min (Figure 2A). Peak blood glucose did not differ between the groups (Figure 2A). Interestingly, blood glucose levels at 60 min after meal ingestion were significantly ($P < 0.01$) lower after the active (CPE without HHQ) beverage than after the other test beverages (Figure 2A). Accordingly, peak blood glucose clearance from 30 to 60 min was significantly greater ($P < 0.01$) after the active beverage, but not after the control, compared to the placebo beverage (Figure 2B). The active beverage tended to lead to greater peak blood glucose clearance than the control beverage, although the difference was not statistically significant ($P > 0.05$, Figure 2B).

Insulin levels also peaked 30 min after meal ingestion, reaching baseline after 240 min (Figure 2C). Active GLP-1 (Figure 2D) levels peaked 30 min after meal consumption. TG levels increased steadily and peaked 180 min after ingestion during the 240-min test period (data not shown). The plasma concentration of NEFAs declined until 60 min after meal ingestion, and remained at a low level until the end of the test period (data not shown). No significant differences were observed in insulin levels (Figure 2C), active GLP-1 (Figure 2D), TG, or NEFAs (data not shown).

The results demonstrate that postprandial hyperglycemia was significantly decreased by the CPP consumption, but not after CPP consumption associated with HHQ, in healthy male adults. The peak blood glucose clearance after meal ingestion was significantly increased after CPP consumption, while the effect of CPP on blood glucose clearance was diminished by concomitant consumption of HHQ.

Table 2. Initial blood and urinary parameters in the test groups

Parameter	Placebo	Control	Active	P
Glu (mg/dL)	98.2 ± 1.1	101.0 ± 1.4	100.8 ± 2.5	N.S.
TG (mg/dL)	122.4 ± 37.8	94.2 ± 18.9	86.7 ± 11.9	N.S.
T-Chol (mg/dL)	199.5 ± 30.0	194.6 ± 9.8	182.0 ± 6.7	N.S.
HDL-C (mg/dL)	60.9 ± 3.1	61.1 ± 3.3	59.2 ± 4.0	N.S.
LDL-C (mg/dL)	118.0 ± 10.2	114.4 ± 11.9	104.1 ± 8.0	N.S.
NEFA (Eq/dL)	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	N.S.
Insulin (mU/L)	4.3 ± 0.8	4.0 ± 0.7	3.4 ± 0.5	N.S.
Active GLP-1(pM)	2.2 ± 0.4	2.2 ± 0.4	2.1 ± 0.4	N.S.
LDH (U/L)	164.7 ± 7.8	166.0 ± 7.4	168.7 ± 10.5	N.S.
ALT (U/L)	25.5 ± 3.4	26.2 ± 3.4	7.5 ± 4.3	N.S.
AST (U/L)	23.3 ± 1.5	22.4 ± 1.7	24.8 ± 2.9	N.S.
d-ROMs (U.CARR)	344.9 ± 14.3	343.5 ± 16.3	347.6 ± 14.0	N.S.
Urinary 8-OHdG (ng/ mg Cre)	17.0 ± 5.1	17.0 ± 5.0	14.5 ± 4.8	N.S.

Data are expressed as means ± SEM (N =10).
Glu, glucose; TG, triglyceride; T-Chol, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NEFA, non-esterified fatty acids; GLP-1, glucagon-like peptide-1; LDH, lactate dehydrogenase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; d-ROMs, diacron reactive oxygen metabolites; 8-OHdG, 8-hydroxy-2'-deoxyguanosine; Cre, creatinine.
N.S.: not significant ($P > 0.05$) among the groups (ANOVA, Bonferroni post hoc test)

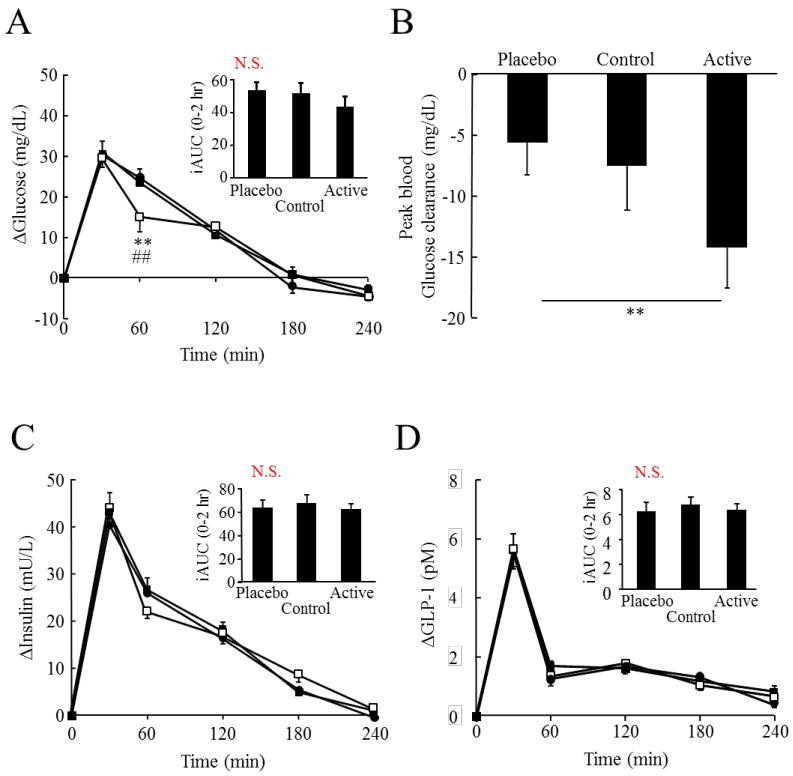

Figure 2. Postprandial change in blood parameters after consumption of test beverages. Blood (A) glucose, (B) peak blood glucose clearance, (C) insulin, and (D) active GLP-1 after consumption of the placebo (closed circles), control (CPE with HHQ, closed squares), and active (CPE without HHQ, open squares) beverages. Data are expressed as means ± SEM; n = 10 in each group. Two-way repeated ANOVA was used to compare changes over time and between the groups. Asterisks indicate the probability level of random differences between groups: **, $P < 0.01$ significantly different from the placebo group (ANOVA with Bonferroni post hoc test); ##, $P < 0.01$ significantly different from the control group. (A, C, D inset) Incremental area under the curve (iAUC) or decremental AUC (dAUC) of each blood variable for 2 hours after the meal plus test beverage ingestion. N.S., not significant ($P > 0.05$) among the groups (Bonferroni post hoc test). (B), Peak blood glucose clearance was calculated as Δglucose (30-60 min)

HHQ reduces the improvement of postprandial vascular endothelial function after CPP consumption in healthy human subjects.

The initial blood vessel diameters in the subjects using flow-mediated dilation were similar between the three test beverage groups (Table 3).

Table 3. The initial blood vessel diameters (mm) of subjects using flow-mediated dilation

Time	Placebo	Control	Active	P
0 min	4.06 ± 0.33	5.72 ± 1.12	4.07 ± 0.34	N.S.
60 min	3.95 ± 0.43	3.74 ± 1.22	4.16 ± 0.32	N.S.
120 min	4.15 ± 0.32	4.23 ± 0.98	3.95 ± 0.32	N.S.
180 min	3.99 ± 0.24	4.61 ± 0.89	4.13 ± 0.23	N.S.
240 min	4.05 ± 0.43	5.17 ± 1.11	4.03 ± 0.30	N.S.

Data were expressed as means ± SEM (N =10).
N.S.: not significant ($P > 0.05$) among the groups (ANOVA, Bonferroni post hoc test)

FMD levels decreased up to 60 min after meal ingestion with the placebo beverage and increased thereafter, although FMD did not reach baseline even after 240 min (Figure 3A).

Overall, postprandial FMD levels were higher (Group × Time interaction $P < 0.01$) in the active beverage group; FMD levels were comparable in the control (CPE with HHQ) beverage group and the placebo beverage group (Figure 3A). The postprandial decline (dAUC) in FMD levels was significantly ($P < 0.01$) less after consumption of the active beverage, but not after the control beverage compared with after the placebo beverage (Figure 3A). However, there was no significant difference in the postprandial FMD decline between the placebo and the control beverages (Figure 3A). Peak FMD decline (after 60 min) was significantly ($P < 0.01$) lower after consumption of the active beverage than after consumption of the placebo beverage, and reached the baseline after 240 min (Figure 3A).

The results clearly show that vascular endothelial function after meal ingestion was significantly improved by CPP consumption. However, the beneficial effect of CPPs on vascular endothelial function was significantly reduced by HHQ.

HHQ reduces the improvement of postprandial oxidative stress after CPP consumption in healthy human subjects.

Blood H_2O_2 levels peaked 120 min after meal consumption and did not reach baseline within 240 min (Figure 3C). Blood d-ROM levels peaked 30 min after meal ingestion, returning to baseline levels at 120 min, and then steadily increased thereafter (Figure 3B).

Overall, postprandial blood d-ROM responses (Group × Time interaction $P < 0.05$) were significantly ($P < 0.01$) lower after consumption of the active beverage than after consumption of the control beverage (Figure 3B). The lowest peak blood d-ROM levels were found among subjects consuming the active beverage and highest in those consuming the control beverage compared with subjects consuming the placebo beverage (Figure 3B). Postprandial blood H_2O_2 (Figure 3C) responses were comparable between the placebo and the control beverage

groups, and tended to be lower after consumption of the active beverage than after consumption of the placebo or control beverage. Postprandial blood H_2O_2 responses were blunt at 30 min after consumption of the meal in combination with the active beverage, and significantly ($P < 0.05$) lower than the H_2O_2 responses following control beverage consumption (Figure 3C). These results indicate that postprandial increase in oxidative stress was significantly reduced by the consumption of CPPs, but not after consumption of CPP in combination with HHQ.

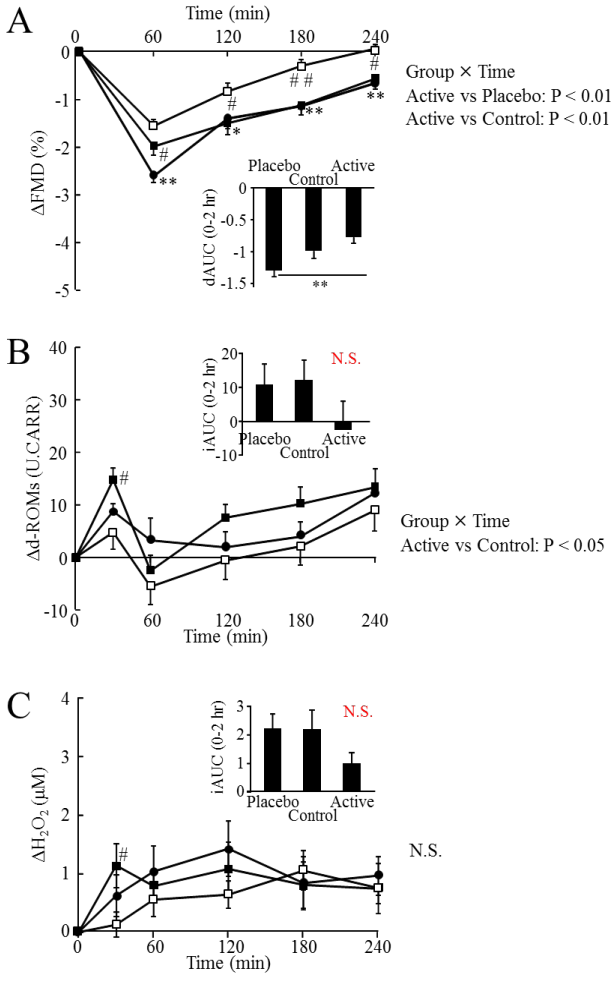

Figure 3. Postprandial change in endothelial function and blood d-ROM after consumption of the test beverages. (A) FMD, (B) d-ROM, and (C) blood H_2O_2 after consumption of the placebo (closed circles), control (CPE with HHQ, closed squares), and active (CPE without HHQ, open squares) beverages. (A, B, C inset) Decremental area under the curve (dAUC) or incremental AUC (iAUC) of each variable for 2 hours after meal plus test beverage ingestion. Data are expressed as means ± SEM; n = 10 in each group. Two-way repeated ANOVA was used to compare changes over time and between the groups. Asterisks indicate the probability level of random differences between groups: *, $P < 0.05$, **, $P < 0.01$ significantly different from the placebo group (Bonferroni post hoc test); #, $P < 0.05$, ##, $P < 0.01$ significantly different from the control group (Bonferroni post hoc test). N.S., not significant

Urinary 8-OHdG levels were lower after consumption of a CPE-containing (active or control) beverage than after placebo beverage consumption, and statistical significance was found between the placebo and the control groups (Figure 4). The results suggest that postprandial increase in oxidative DNA damage [23] was reduced by the consumption of CPPs, which was not affected by concomitant consumption of HHQ.

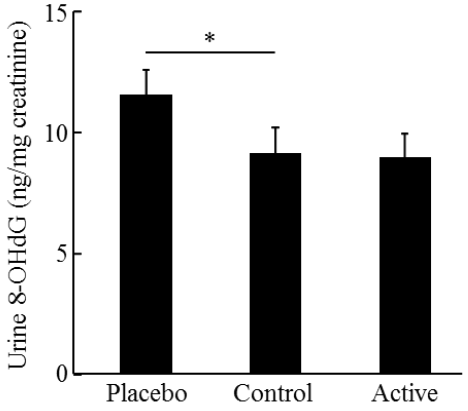

Figure 4. Postprandial change in urinary 8-OHdG after consumption of the test beverages. Urinary 8-OHdG for 4 hours after consumption of the placebo, control (CPE with HHQ), or active (CPE without HHQ) beverages. Data are expressed as means ± SEM; n = 10 in each group. Asterisks indicate the probability of random differences from the placebo group: * $P < 0.05$ significantly different between the groups (Bonferroni post hoc test)

These major findings support the hypothesis that, in healthy male adults, concomitant HHQ consumption reduces the beneficial effects of CPP consumption on postprandial hyperglycemia and vascular endothelial function by increasing postprandial oxidative stress.

Whereas postprandial insulin response was similar, postprandial hyperglycemia was significantly improved by CPP consumption, accompanied by improved vascular endothelial function. The increase in postprandial blood glucose levels was positively correlated with a decrease in postprandial vascular endothelial function. The results were consistent with our previous findings [11], suggesting that the improved blood glucose response, associated with comparable insulin levels, was due to improved vascular endothelial function after CPP ingestion. As demonstrated by Kubota et al. [12], vascular endothelial dysfunction caused skeletal muscular insulin resistance by reducing insulin delivery to the tissues. In turn, increased endothelial function significantly improved skeletal muscle insulin sensitivity and reduced blood glucose levels [12]. In the present study, peak blood glucose clearance after CPP consumption was reduced by concomitant HHQ consumption, which was associated with a decrease in postprandial vascular endothelial function. The results suggested that reduced vascular endothelial function after concomitant HHQ consumption impaired the beneficial effect of dietary CPPs on postprandial blood glucose control.

A study by Johnston et al. [24] showed that chlorogenic acid ingestion did not affect postprandial blood glucose and insulin responses in humans following the ingestion of 25 g of glucose. Furthermore, Ochiai et al. [25] observed no differences in blood glucose and insulin level changes after ingestion of 75 g of glucose, with or without CPPs. Our previous [11] and present studies demonstrate that CPP consumption reduced blood glucose levels after meal ingestion in humans. One possible explanation for the discrepancy in these results may be the macronutrient composition (592 kcal, carbohydrate: fat: protein = 75.0: 28.5: 8.0 (g)) of the test meal used in our study. Moreover, the major carbohydrates contained in the test meal in the present study were starch (from wheat flour) and maltose,

resulting in a slower increase in blood glucose levels. When insulin sensitivity is improved in the tissues, increased insulin release and slower increase in glucose levels may cause more glucose uptake by the tissues. However, further studies are required to investigate the underlying mechanism for the improvement in blood glucose levels after CPP ingestion.

The underlying mechanism for the postprandial decrease in vascular endothelial function by concomitant HHQ ingestion with CPPs requires further investigation. One possible explanation is that increased production of ROS may be involved in the decrease in FMD levels after polyphenol ingestion. A number of studies have suggested that excess ROS reacts with nitric oxide, an endothelium-derived relaxing factor, resulting in endothelial function impairment [26]. Our previous study [11] and the present study both demonstrated that CPP consumption significantly reduced the increase in d-ROM, an indicator of ROS production, and improved postprandial decrease in FMD levels. In contrast, concomitant HHQ consumption diminished the decrease in the oxidative stress markers and impaired the improvement of vascular endothelial function by CPP consumption. Concomitant HHQ consumption seems likely to impair postprandial vascular endothelial function, in part, by increasing oxidative stress in blood vessels after CPP ingestion.

There was a minor difference in the CPP composition of the test beverages. The active beverage contained fewer caffeoylquinic acids and more feruloylquinic and dicaffeoylquinic acids compared to the control beverage. In our previous study [27], CQA strongly reproduced the vasodilatory effect of the CPE, and HHQ strongly interfered with the restoration of endothelial function by CQA. Accordingly, CQA is likely to be the main active component in CPPs. In this study, the active beverage improved the vascular function more than the control, despite its lower CQA content, compared to the control beverage. Therefore, we consider that the effects observed between the two groups on blood glucose and vascular function in this study were due to the difference in HHQ content rather than that in CPP composition.

Notwithstanding the significance of the current findings, it is important to consider the limitations of the present study. The major weakness of this study was the small sample size. Accordingly, the effect of CPPs on postprandial oxidative stress and its association with postprandial hyperglycemia improvement and endothelial function remain unclear. Further studies with a larger sample size are warranted to elucidate the effects of HHQ and CPPs on oxidative stress and their involvement in improved blood glucose and vascular endothelial function. A further limitation of this study was that FMD measured vascular endothelial function of relatively large blood vessels, but not of capillaries that contribute to insulin delivery and sensitivity in skeletal muscles. Therefore, further studies are required to examine the effects of dietary HHQ on capillary function and skeletal muscular insulin sensitivity in humans. Finally, the long-term influence of coffee consumption on acute postprandial response remains uncertain. Further studies are needed to clarify whether long-term consumption of HHQ-free coffee improves or prevents hyperglycemia and endothelial dysfunction in humans.

4. Conclusions

In conclusion, CPP consumption improved postprandial hyperglycemia and vascular endothelial function associated with decreased oxidative stress in healthy humans. Concomitant HHQ ingestion with CPPs impairs the polyphenol-induced improvement of postprandial blood glucose response. This effect may be because HHQ increases oxidative stress, and thereby vascular endothelial function, after the polyphenol consumption. HHQ (1,2,4-trihydroxybenzene) is one of the major prooxidants in coffee. Therefore, reduction of prooxidants in coffee may have beneficial implications in postprandial glycemic control and vascular maintenance. Further studies are in progress to investigate the underlying mechanisms of action in association with decreased insulin sensitivity and increased oxidative stress.

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Statement of Competing Interests

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Abbreviations

CPP; coffee polyphenol
CPE; coffee polyphenol extract
d-ROM; diacron-reactive oxygen metabolite
FMD; flow mediated dilation
HHQ; hydroxyhydroquinone
8-OHdG; 8-hydroxy-2'-deoxyguanosine
ROS; reactive oxygen species

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