Acute dose-response effect of coffee-derived chlorogenic acids on the human vasculature in healthy volunteers: a randomized controlled trial

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ABSTRACT

Background: Epidemiological studies have reported lower risk of cardiovascular disease with moderate coffee consumption. In addition, emerging evidence indicates that consumption of coffee beverages enriched in chlorogenic acids (CGAs) may influence blood pressure and endothelial function, suggesting that the beneficial cardiovascular effect of coffee may relate to its CGA content.

Objectives: We conducted a double-blind randomized crossover trial to test the effect of acute consumption of a decaffeinated green coffee extract (DGCE), rich in CGAs, on endothelial function in healthy subjects.

Methods: We compared 3 different doses of DGCE (302, 604, and 906 mg, respectively) with a placebo. Endothelial function was defined as the percentage change in the internal diameter of the brachial artery in response to flow-mediated dilation (%FMD). In addition, we followed the plasma concentration-time profiles of 25 systemic CGA metabolites over 24 h after DGCE consumption and we explored the relation between systemic concentrations of CGAs and the effect on %FMD.

Results: The DGCE formulations containing different amounts of CGAs resulted in dose-proportional increases in overall total polyphenol concentrations. The systemic appearance of total CGAs was biphasic, in agreement with previous results suggesting 2 sites of absorption in the gastrointestinal tract. Compared with the placebo group, a significant FMD increase (>1%) was observed 8.5, 10, and 24 h after consumption of 302 mg DGCE (∼156.4 mg CGAs). The differences with placebo observed in the other 2 groups were not statistically significant. Evaluation of the relation between phenolic exposure and %FMD showed a positive tendency toward a larger effect at higher concentrations and different behavior of CGA metabolites depending on the conjugated chemical position.

Conclusions: We demonstrated an acute improvement in %FMD over time after ingestion of a DGCE, explained at least partly by the presence in the blood circulation of CGAs and their metabolites. This trial was registered at clinicaltrials.gov as NCT03520452. *Am J Clin Nutr* 2021;113:370–379.

Keywords: coffee, phenolic acids, human, endothelial function, flow-mediated dilation

Introduction

Coffee is a popular beverage consumed globally. In recent years, epidemiological studies have suggested a lower cardiovascular disease (CVD) risk with moderate coffee consumption (3–5 cups/d) (1–3). Most of the studies reported a J-shaped relation between coffee consumption and the risk of developing CVD, stroke, heart disease, or acute coronary syndromes (myocardial infarction or unstable angina). Heavy coffee consumers (>600 mL/d) had a higher risk of developing CVD than those who did not drink coffee, whereas moderate coffee consumers

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Supplemental Table 1 is available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

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Abbreviations used: CA3S, caffeic acid-3'-*O*-sulfate; CGA, chlorogenic acid; Cmax, maximum concentration; CVD, cardiovascular disease; DGCE, decaffeinated green coffee extract; DHCA, dihydrocaffeic acid; DHCA3S, dihydrocaffeic acid-3'-*O*-sulfate; DHFA, dihydroferulic acid; DHFA4G, dihydroferulic acid-4'-*O*-glucuronide; DHFA4S, dihydroferulic acid-4'-*O*-sulfate; DHiFA, dihydroisoferulic acid; ECG, electrocardiogram; FA4G, ferulic acid-4'-*O*-glucuronide; FMD, flow-mediated dilation; iAUC, incremental AUC; iFA3G, isoferulic acid-3'-*O*-glucuronide; mDHCoA, dihydro-m-coumaric acid; NONSG, neither sulfated nor glucuronidated; SG, sulfated or glucuronidated; Tmax, time of maximal response; UWA, The University of Western Australia; 5-CQA, 5-*O*-caffeoylquinic acid.

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(<300 mL/d) had a 30% lower risk than those who did not drink coffee (4).

Endothelial dysfunction plays an important role in the development and progression of CVD. Evidence suggests that endothelial dysfunction is an early and integral feature associated with several CVD risk factors (i.e., hyperglycemia, insulin resistance, hyperinsulinemia, hypertension, and dyslipidemia). Flow-mediated dilation (FMD) is a noninvasive technique widely used and recognized for assessing endothelium-dependent and largely NO-mediated arterial function (5–7). Every 1% increase in FMD has been associated, in several systematic reviews and meta-analyses, with a 10%–13% lower risk of cardiovascular events (8–12). The FMD test represents an important tool to improve our physiological insight and understanding of mechanisms involved in endothelial and vascular functions.

Few human intervention studies have assessed the effects of coffee or coffee components on endothelial function and associated biomarkers, with contradictory findings (13–20), and the mechanisms by which coffee may exert effects on the endothelium remain unclear. Some of the cardiovascular health benefits may be attributable to the presence of chlorogenic acids (CGAs) (21), one of the most abundant classes of compounds in coffee beverages (22). No previous study to our knowledge has undertaken serial assessment of FMD and the systemic concentration-time profile of coffee CGA metabolites over 24 h. Nor have studies systematically utilized different CGA doses and simultaneously assessed FMD in humans. A better understanding of the relation between pharmacodynamic measurements (FMD) and pharmacokinetics of coffee CGA metabolites could valuably inform the design of future studies.

The aim of the present study was to evaluate the acute effect of 3 different concentrations of CGAs from a decaffeinated green coffee extract (DGCE) on endothelial function in healthy subjects with suboptimal baseline FMD values. Our teams have established optimal FMD techniques (23–27) and analysis approaches (28) and developed related guidelines (7, 29). An additional objective was to explore the relation between improvement of endothelial function and systemic concentration of coffee CGA metabolites.

Methods

Design and study population

This human clinical trial (NCT03520452) was an acute, double-blind, randomized, placebo-controlled crossover study conducted at a single site: The Cardiovascular Research Group at The University of Western Australia (UWA). Twenty-one healthy subjects (5 women, 16 men) completed the study between April and November 2018. Inclusion criteria were men and postmenopausal women aged 45–65 y, healthy as determined by the medical questionnaire and physical examination, with a priori lower range endothelial function, defined as a brachial artery flow-mediated dilation (FMD) measurement < 7.5% (30, 31). All subjects had normal electrocardiogram (ECG) and were regular tea and/or coffee drinkers (≥1 cup/d). Exclusion criteria were food allergies, regular consumption of multivitamin tablets and other supplemental compounds 10 d before the study start and throughout the study, abnormal blood pressure defined as systolic < 90 or >140 mm Hg or diastolic < 60 or >90 mm Hg, regular consumption of cholesterol-lowering or antihypertensive medication, alcohol consumption of >280 g/wk, recent (in the past 3 mo) significant weight loss or gain (>6% of body weight) or BMI outside the 18–35 kg/m^2 range, inability to comply with the protocol, and current or past participation in another clinical trial during the last 2 wk.

After enrolment, subjects were randomly allocated by the investigator into 4 intervention sequences via computer-generated randomization. We performed randomized postprandial testing in these 4 treatments, with 4 periods (visits), in a crossover design. Individual study visits were separated by a washout period of ≥1 wk to ensure total plasma metabolites returned to baseline concentrations (32). All participants gave their written informed consent and the study was conducted in accordance with the Declaration of Helsinki.

Before the first study visit, a general health screening, including a screening FMD assessment, was performed. Subjects were then assigned at random to 1 of the 4 following sequences (A = Placebo, B = 302 mg, C = 604 mg, D = 906 mg DGCE) using a Williams Latin Square design without any stratification factors. The sequences were replicated according to the sample size, namely 10 subjects/sequence (A-B-D-C; B-C-A-D; C-D-B-A; D-A-C-B). The randomization was performed using MEDIDATA Balance.

To control polyphenol intake from the diet, participants were asked to follow a diet low in polyphenols in the days before the study visits. To that end, all volunteers received a list of suggested foods that contain low amounts of polyphenols, especially CGAs. Participants were asked to refrain from the consumption of coffee, tea, soda, alcohol, and whole-grain cereal (white bread allowed) from 1 d before each of the study days. Subjects were also asked to fast overnight before each study day, in accordance with our published guidelines (7, 29). During the night, and in the morning before the blood sampling, only water could be drunk. Upon arrival at the UWA laboratory, volunteers were asked to drink water (4 g/kg body weight) in order to have similar levels of hydration. Baseline FMD was assessed, blood was sampled, and subjects then received 1 of the 4 study interventions, as randomly assigned to them. Each dose of investigational product or matching placebo was dissolved in an opaque glass of 200 mL mineral water at room temperature with a lid immediately before administration. The participants were asked to consume the drinks in <5 min. After they received the treatments, FMD was assessed at 1, 3, 5, 7, 8.5, 10, 12, and 24 h, whereas blood was collected at 0.50, 1, 2, 3, 5, 6, 7, 8.5, 10, 12, and 24 h. No longer than 30 min after collection, blood was centrifuged at 3500 x *g* for 15 min at 4°C and plasma aliquots (1 mL) were prepared after each collection time point. During the visit days the participants stayed at the study center where standardized breakfast (between 1 and 2 h after the treatment drink), lunch (between 5 and 6 h after the treatment drink), and dinner (between 10 and 11 h after the treatment drink) were provided. The subjects were allowed to drink as much water as they wanted while they rested quietly and watched television.

The safety of the intervention was monitored throughout the study by the investigators who had to document the incidence, type, and severity of any nonserious or serious adverse events and their relation to product intake.

The study protocol was approved by The Bellberry Human Research Ethics Committee in accordance with the Australian

TABLE 1 Composition of the 4 intervention treatments[1]

	Placebo	Dose 1	Dose 2	Dose 3
DGCE, mg	0.0	302.0	604.1	906.1
Total CGA, mg	—	156.4	312.8	439.9
3CQA, mg	—	30.0	60.0	84.3
4CQA + 3FQA, mg	—	39.0	78.1	109.8
5CQA, mg	—	38.1	76.2	107.1
3,4diCQA, mg	—	12.5	25.0	35.2
3,5diCQA, mg	—	4.6	9.2	13.0
4,5diCQA, mg	—	9.8	19.6	27.5
4FQA, mg	—	9.3	18.7	26.3
5FQA, mg	—	12.1	24.2	34.0
Caffeic acid, mg	—	0.6	1.3	1.8
Ferulic acid, mg	—	0.3	0.7	0.9
Caffeine, mg	—	5.8	11.6	16.4
Maltodextrin DE 21, mg	887.8	887.8	887.8	887.8
Strawberry aroma, mg	76.0	76.0	76.0	76.0
Total mass, mg	983.1	1291.2	1599.2	1907.3
Calculated energy content, kcal/100 g	376	371	368	366

[1]CGA, chlorogenic acid; DE, dextrose equivalent; DGCE, decaffeinated green coffee extract; 3CQA, 3-*O*-caffeoylquinic acid; 3FQA, 3-*O*-feruloylquinic acid; 3,4diCQA, 3,4-*O*-dicaffeoylquinic acid; 3,5diCQA, 3,5-*O*-dicaffeoylquinic acid; 4CQA, 4-*O*-caffeoylquinic acid; 4FQA, 4-*O*-feruloylquinic acid; 4,5diCQA, 4,5-*O*-dicaffeoylquinic acid; 5CQA, 5-*O*-caffeoylquinic acid; 5FQA, 5-*O*-feruloylquinic acid.

National Health and Medical Research Council's National Statement on Ethical Conduct in Human Research.

Intervention

Four different treatments comprising different doses of CGAs were tested in the clinical trial. They were administered in the morning in fasting conditions, in the form of a beverage. **Table 1** reports the composition and CGA content of the 4 treatments.

Outcomes

The primary objective was to demonstrate the efficacy of 3 gradually increasing doses of CGAs from a DGCE in improving endothelial function as measured by %FMD in healthy subjects with suboptimal baseline FMD over 24 h. The secondary objectives were to explore the systemic concentration-time profiles of coffee CGA metabolites of the 3 treatments and to demonstrate the link between the improvement of the endothelial function and the presence of CGAs and phenolic acids in plasma.

%FMD

Brachial artery vasodilation function was assessed using high-resolution ultrasound according to previously published and internationally accepted guidelines (7, 29). A single trained ultrasonographer performed all measurements for a given subject, whereas the analysis was blinded. Endothelium-dependent vasodilation was assessed as the response to a 5-min period of forearm ischemia. Briefly, participants rested in a supine position in a quiet, temperature-controlled room (21–25°C). The left arm was comfortably rested on a foam support and ECG was monitored continuously. A 12- to 15-MHz transducer connected to a Terason (Terason, t3300) high-resolution vascular ultrasound machine was placed in position over the brachial artery 5–10 cm proximal to the antecubital crease, and location recorded to ensure the same location for all subsequent measurements. A baseline artery diameter recording was performed for 1 min. A pneumatic cuff was then placed around the left forearm and inflated to 200 mm Hg for 5 min to induce reactive hyperemia. The brachial artery image was continuously recorded for 4 min after cuff deflation to assess %FMD. Images were downloaded for retrospective analysis.

Analysis of scans was performed with semiautomated edge-detection software (28). This automatically and continuously calculated the brachial artery diameter, corresponding to the internal diameter. Responses were calculated as the maximum percentage change in brachial artery diameter from baseline after cuff deflation (auto-detected using a curve-fitting algorithm). The automated analysis was overseen by an experienced observer, blinded to the study treatments. Reproducibility studies have previously demonstrated an intrasubject variation coefficient of 6.7% (28).

Analysis of coffee CGA and phenolic acid metabolites in plasma samples

Coffee phenolic acids were extracted from human plasma samples in duplicate and analyzed using a validated LC–electrospray ionization–tandem MS method (33). The coffee phenolic compounds assessed in plasma were as follows: caffeic acid; ferulic acid; ferulic acid methyl ester; isoferulic acid; feruloylquinic acid; ferulic acid-4'-*O*-sulfate; ferulic acid-4'-*O*-glucuronide (FA4G); isoferulic acid-3'-*O*-glucuronide (iFA3G); isoferulic acid-3'-*O*-sulfate; caffeic acid-3'-*O*-sulfate (CA3S); caffeic acid-3'-*O*-glucuronide; caffeic acid-4'-*O*-sulfate; caffeic acid-4'-*O*-glucuronide; dihydrocaffeic acid (DHCA); dihydroferulic acid (DHFA); dihydroferulic acid methyl ester; dihydroferulic

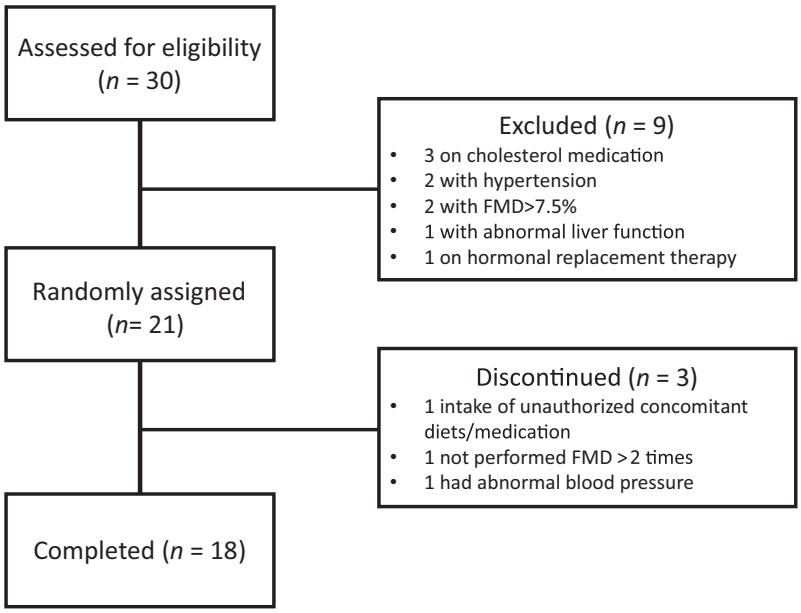

FIGURE 1 Flow diagram of the study participants. FMD, flow-mediated dilation.

acid-4'-*O*-sulfate (DHFA4S); dihydroferulic acid-4'-*O*-glucuronide (DHFA4G); dihydroisoferulic acid (DHiFA); dihydro-m-coumaric acid (mDHCoA); dihydroisoferulic acid-3'-*O*-sulfate; dihydroisoferulic acid-3'-*O*-glucuronide; dihydrocaffeic acid-3'-*O*-sulfate (DHCA3S); dihydrocaffeic acid-3'-*O*-glucuronide; dihydrocaffeic acid-4'-*O*-sulfate; and dihydrocaffeic acid-4'-*O*-glucuronide. The lower limit of quantification was 5 nmol/L, except for mDHCoA where it was 25 nmol/L.

Statistical analyses

The computer software R version 3.1.2 (R Foundation for Statistical Computing) was used to calculate the sample size and perform the statistical analyses. Given the exploratory nature of this study, no adjustments for multiplicity were applied. The power calculation was based on results from a previous study (13). The sample size for our study was estimated using the significant difference observed between high CGA coffee and control at 5 h in FMD response: 1.98 ± 1.91% (mean ± SEM). With 18 participants, the proposed study was able to detect an effect size (Δ/SD) of 0.96, which is more conservative than the reference article (effect size of 1.04). If FMD is related to polyphenol absorption, we would expect a bigger effect size beyond 5 h of exposure, so this number of participants represents a conservative estimate for power purposes.

The main analysis of the primary endpoint compared the change from baseline of FMD between the different doses and the control condition at each time point after the baseline. A difference of 1% was considered clinically relevant (7) and a P value <0.05 considered statistically significant. The carryover effect was assessed by adding in the model the fixed effects of sequence and sequence × dose interaction. A linear mixed model was applied to explain the change in FMD at peak with baseline, time point, dose, and the interaction between time point and dose as fixed effects. The within-subject variability was taken into account by declaring the subject ID as a random effect in the model. Changes from baseline FMD were calculated for each individual, at each time point, for each visit. The value at T0 was subtracted from each consequent FMD at peak for each specific visit. The incremental AUC (iAUC) was calculated by correction of the baseline value for each individual at each visit (meaning 1 iAUC per subject per dose). Once the iAUCs were calculated, comparisons of each dose against the placebo were performed. Pharmacokinetic/pharmacodynamic analyses were performed by looking into the dynamics of the FMD across time. Analyses on maximum concentration (Cmax) and time of maximal response (Tmax) were assessed in a similar fashion (linear mixed model) as the primary endpoint but instead of ΔFMD, Cmax and Tmax were assessed. The associations between FMD and the blood biomarkers were modeled by using a multiple regression in which FMD was explained by multiple regressors (the biomarkers). Similar modeling techniques as for the primary endpoints were applied (linear mixed model).

Results

Participants

Twenty-one subjects completed the study (**Figure 1**). Three subjects were excluded from analysis owing to major protocol deviations: intake of unauthorized concomitant diets/medication, abnormal blood pressure, and unable to perform FMD measurement more than twice during the study.

Table 2 presents demographic data and clinical measurements of the participants. Baseline measurements of plasma taken before the consumption of the coffee drinks on each study visit (data not shown) showed very low or undetectable concentrations of coffee phenolic compounds, indicating good compliance with dietary restrictions as specified in the study protocol. No serious adverse events were reported by the participants.

TABLE 2 Baseline characteristics and clinical measurements of study participants[1]

Characteristics	ITT	PP
n	21	18
Sex, %		
Female	23.8	16.7
Male	76.2	83.3
Race, %		
White	90.5	88.9
Others	9.5	11.1
Age, y	56.4 ± 5.5	56.2 ± 5.2
Height, cm	174 ± 10	176 ± 9
BMI, kg/m^2	27.1 ± 3.8	27.5 ± 3.7
Brachial pulse rate, *n*/min	62.2 ± 7.7	62.1 ± 8.0
Diastolic blood pressure, mm Hg	71.7 ± 6.6	72.3 ± 6.1
Systolic blood pressure, mm Hg	122.5 ± 8.9	122.7 ± 9.6

[1] Values are means ± SDs. ITT, intention to treat; PP, per protocol.

Plasma pharmacokinetics of CGAs and phenolic acids

The appearance of coffee phenolic acids in plasma after the consumption of the different DGCE doses was biphasic (**Figure 2**A). In the first phase (from 0 to 30 min), the plasma appearance of phenolic compounds followed the same pattern for the 3 treatments and reached Cmax of 0.96 ± 0.45 mol/L, 2.07 ± 1.80 mol/L, and 2.70 ± 0.96 mol/L for the 302-, 604-, and 906-mg DGCE doses, respectively. During the second phase of absorption (from 3 to 24 h), Tmax was between 6 and 11 h and Cmax was 1.17 ± 0.97 mol/L at 6 h, 2.25 ± 2.00 mol/L at 6 h, and 2.32 ± 1.55 mol/L at 11 h for the 302-, 604-, and 906-mg DGCE doses, respectively. The dose influenced the total plasma bioavailability of chlorogenic and phenolic compounds, as indicated by the area under the concentration-time curve from 0 to 24 h ($AUC_{0–24h}$) after ingestion of the different doses (Figure 2B).

Effects of coffee extracts on %FMD response

The interaction sequence × dose was not statistically significant (data not shown), indicating that there was no carryover effect, and was therefore removed from the statistical model. A biphasic increase in endothelium-dependent brachial artery vasodilation from baseline, measured as Δ %FMD, was observed in response to placebo with peaks at 3 and 10 h (**Figure 3**A, **Supplemental Table 1**). An increase in Δ %FMD was detected for the 302-mg DGCE dose with a peak at 10 h after consumption (Figure 3B) and significant differences relative to the placebo condition were observed at 8.5 h (1.13% ± 0.5%; $P = 0.02$), 10 h (1.03% ± 0.5%; $P < 0.04$), and 24 h (1.21% ± 0.5%; $P = 0.02$). The differences in Δ %FMD induced by the 604-mg and 906-mg DGCE doses relative to the placebo were not statistically significant (Figure 3C, D), although there was a trend for increased Δ %FMD at 12 h (604 mg, $P = 0.1$; 906 mg, $P = 0.06$). No statistically significant differences were observed for any of the other time points.

The iAUC for FMD response for all treatments showed a higher mean for all DGCE doses than for placebo (8.40% ± 30.9% × h, 23.3% ± 31.7% × h, 18.9% ± 33.3% × h, and 16.6% ± 35.4% × h for placebo, 302 mg, 604 mg, and 906 mg, respectively) (**Figure 4**). None of the doses were found to be statistically significantly different at $P < 0.05$ compared with placebo. However, when comparing iAUCs of the 302-mg dose to placebo, the *P* value was 0.08. Neither maximal response nor Tmax of the different doses were significantly different from those of the placebo treatment (Figure 4B, C).

Concentration–response relation between FMD and plasma CGAs and phenolic acids

Figure 5 shows the pooled relations between all FMD assessments and the time-matched systemic total polyphenol concentrations after intake of the different DGCE doses. The colored symbols indicate the dose groups and the horizontal dotted black line represents the no-effect from baseline and is included for reference. The solid line is a smooth through the data indicating the trend. This pooled graphical exploration supported the trend of a higher FMD at higher systemic total polyphenol concentrations, but also showed the wide variability observed in the data. Furthermore, the trend line through the data showed that the average change in FMD was ~1% for the largest part of the concentration range.

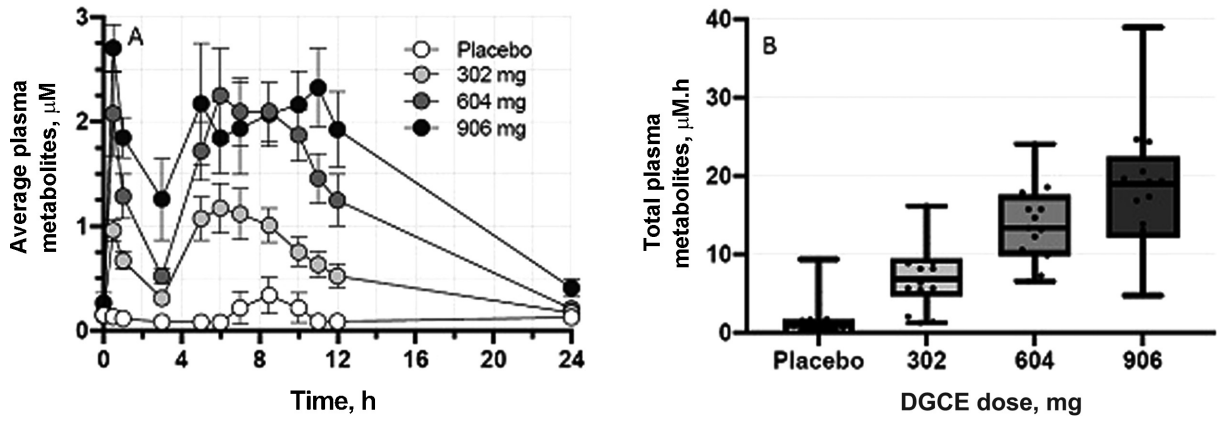

FIGURE 2 Plasma measurements of circulating CGA and phenolic acids after ingestion of placebo or DGCE treatments. (A) Appearance of metabolites in plasma over 24 h. (B) Total plasma metabolite exposure as AUC $_{0–24h}$. Values are means ± SEMs, $n = 21$.

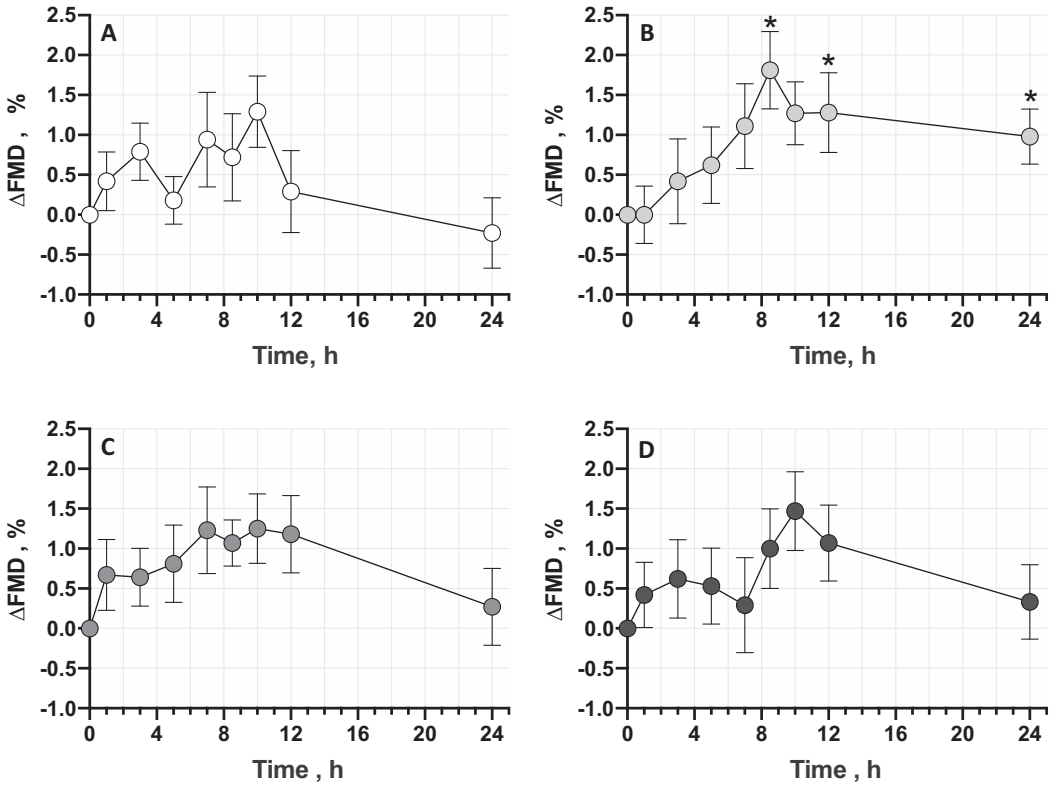

FIGURE 3 FMD change from baseline over time after ingestion of placebo (A), 302 mg DGCE (B), 604 mg DGCE (C), or 906 mg DGCE (D). Values are means ± SEMs, $n = 21$. Statistical analysis was performed using a linear mixed model including dose, time point, and the interaction between the 2 as fixed effects. This method takes into account the within-subject variability by declaring each subject as a random effect. FMD normality was assumed. Statistical significance ($^*P < 0.05$) was observed for comparisons between placebo and 302 mg DGCE, specifically at 8.5 h, 12 h, and 24 h after product intake. The differences estimated at those 3 time points were >1% FMD. DGCE, decaffeinated green coffee extract; FMD, flow-mediated dilation.

Attempts to identify the active moiety of the FMD effect

For most of our analyses, the systemic total polyphenol concentrations, as the sum of 25 measured systemic polyphenol metabolites, were used as the pharmacological amount responsible for the effect on FMD. In addition, concentration-time profiles of the most abundant metabolites were also graphically evaluated to inspect if a specific metabolite was a likely candidate for modification of FMD. This evaluation was performed by considering separately nonconjugated [neither sulfated nor glucuronidated (NONSG)] and conjugated [sulfated or glucuronidated (SG)] metabolites. As such, mDHCoA, DHFA, DHCA, and DHiFA were part of the first group, whereas DHCA3S, CA3S, DHFA4G, FA4G, DHFA4S, and iFA3G were included in the second group.

The sums of the NONSG metabolites and SG metabolites were calculated and graphically explored over time and as predictors for FMD response (**Figure 6**). The concentration-time profiles showed a different absorption pattern between NONSG and SG metabolites. The concentrations of NONSG metabolites were constant over the 24 h, whereas SG metabolites dominated the concentrations in the first absorption peak after dosing. Correlation plots between the effect on FMD and the concentrations of both groups showed that FMD response was associated to NONSG metabolites between 0–6 h (Figure 6A), whereas FMD response was more positively correlated to SG metabolites during the second peak of absorption (7–24 h) (Figure 6D).

Discussion

Our data showed that FMD was significantly increased at different time points after the acute ingestion of a DGCE (302 mg) containing 156.4 mg CGAs, in subjects with suboptimal endothelial function. A similar trend was observed for FMD with the higher DGCE doses (containing 312.8 and 439.9 mg CGAs, respectively) at 12 h, although the differences from the control group did not achieve statistical significance.

Our findings tend to be in accordance with those from a previous study, which evidenced an acute improvement of FMD, although at different time points (1 and 5 h, respectively), after consumption of a green coffee beverage containing 89 and 310 mg CGAs, respectively, as compared with a caffeinated control, in subjects with suboptimal endothelial function (13). Similar observations were also made in a crossover randomized double-blind controlled trial assessing the acute effect on FMD of pure 5-*O*-caffeoylquinic acid (5-CQA), the main CGA in coffee, at 2 different doses (450 and 900 mg) in healthy male subjects (13). A post hoc analysis in that study, which excluded subjects who had FMD values higher than a set inclusion criterion (i.e., 8.5%), indicated that, despite the reduced number of volunteers

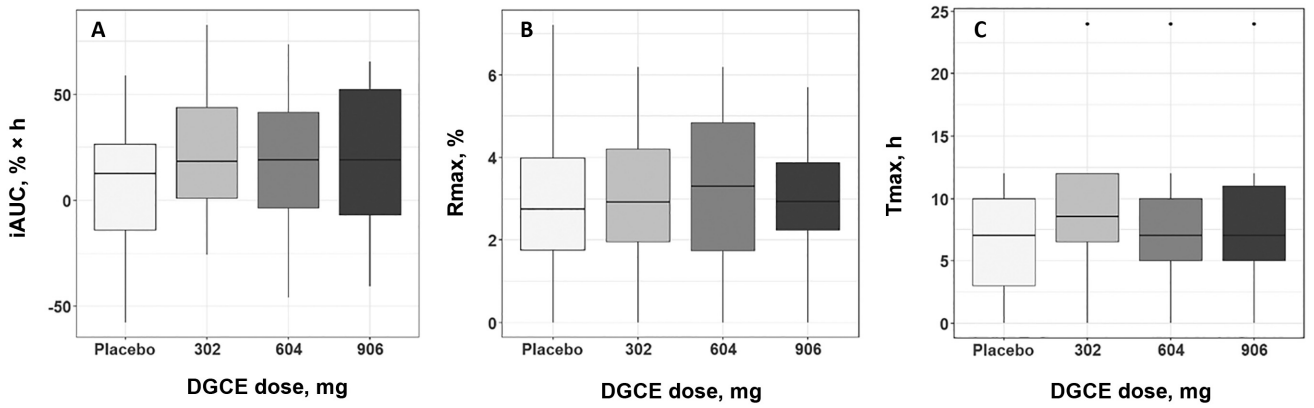

FIGURE 4 Boxplot of the iAUC (A), Rmax (B), and Tmax (C) for FMD values obtained after ingestion of placebo, 302 mg DGCE, 604 mg DGCE, or 906 mg DGCE. Values are reported as minimum, first quartile, median, third quartile, and maximum ($n = 21$). Statistical analysis was performed using a linear mixed model. This method takes into account the within-subject variability by declaring each subject as a random effect. FMD normality was assumed. None of the treatments showed significant differences ($P < 0.05$) compared with the placebo treatment. The dots appearing at the top of the graph pertain to people with Tmax peaking at 24 h. There is no "upper whisker" (vertical line) for the 302-mg dose because the 75th percentile and the maximum values are estimated to be equal. DGCE, decaffeinated green coffee extract; FMD, flow-mediated dilation; iAUC, incremental AUC; Rmax, maximal response; Tmax, time of maximal response.

included in the analysis (19 instead of 24), the effect of 5-CQA (450 mg) showed a close to significant difference compared with control at 1 and 4 h ($P = 0.06$ and $P = 0.08$, respectively), with an effect size almost comparable with that of the positive control used in the study (epicatechin). Treatment with the higher dose of 5-CQA (900 mg), however, did not show any significant differences with the placebo treatment, although the absorption of CGAs was higher in the 900-mg treatment than in the 450-mg treatment. This nonlinear relation between coffee compounds and FMD, also observed in our present findings, was supported by several meta-analyses of randomized controlled trials which found that the relation between the doses of different types of polyphenols and FMD followed an inverted U-shape and that high doses of polyphenols could have smaller effects on endothelial function than lower doses (34, 35). This observation has also been made in individual studies; for instance, 1 study testing the effects of different doses of a blueberry drink on FMD showed a comparable inverted U-shape, whereby the vascular response increased with increasing amounts, then reached a plateau and finally decreased at higher intakes (36). Finally, a previous study testing the effect of purified CGA (5-CQA) at different doses (450 and 900 mg) did not show any significant effect on peak FMD response relative to control although a post hoc analysis found a significant effect on the continuous FMD assessment compared with control at 1 and 5 h, respectively (14).

Another relevant finding of our study relates to effects on postprandial FMD. In a previous experiment, a single dose of a green coffee extract containing 355 mg CGAs significantly attenuated the postprandial impairment of endothelial function

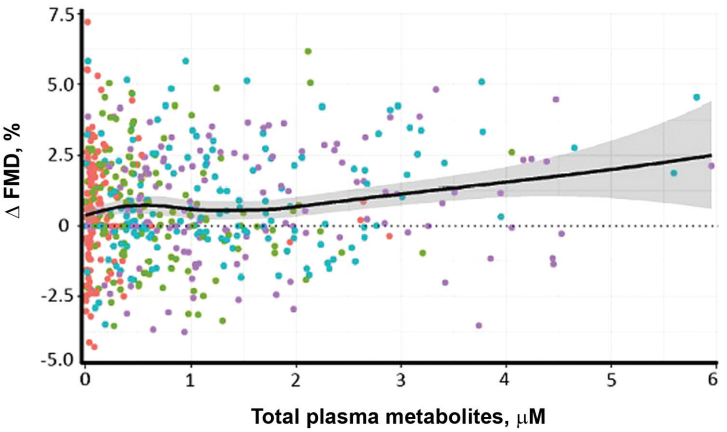

FIGURE 5 FMD change from baseline compared with total plasma circulating CGA and phenolic acid metabolites after ingestion of placebo, 302-mg, 604-mg, or 906-mg DGCE treatments. The colored symbols indicate the individual observations at different doses (red: placebo; green: 302-mg DGCE; blue: 604-mg DGCE; and violet: 906-mg DGCE treatments); $n = 21$. The solid black line is a trend line through the data (smooth), the dark gray bands represent 95% CIs showing the uncertainty in the smooth, and the dotted horizontal line is the no-difference from baseline amount. CGA, chlorogenic acid; DGCE, decaffeinated green coffee extract; FMD, flow-mediated dilation.

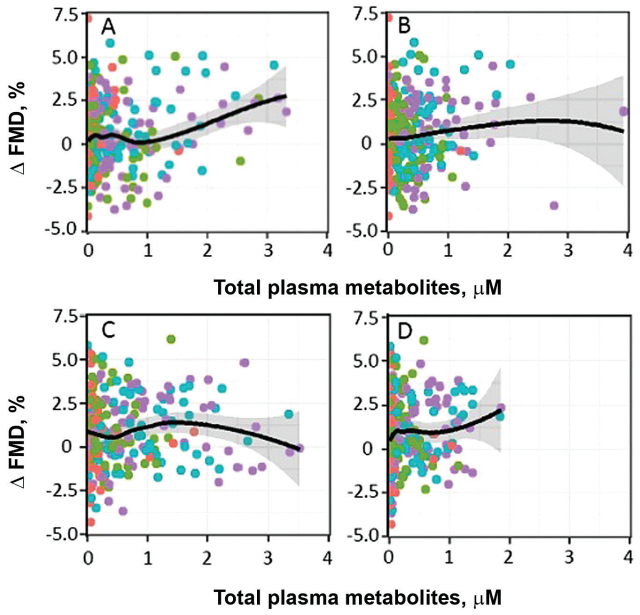

FIGURE 6 FMD change from baseline compared with total plasma circulating nonconjugated (A, C) or conjugated (B, D) metabolites for observation between 0 and 6 h (A, B), and 7 and 24 h (C, D) after ingestion of treatments. The colored symbols indicate the individual observations at different doses (red: placebo; green: 302-mg DGCE; blue: 604-mg DGCE; and violet: 906-mg DGCE treatments); $n = 21$. The solid black line is a trend line through the data (smooth), the dark gray bands represent 95% CIs showing the uncertainty in the smooth. DGCE, decaffeinated green coffee extract; FMD, flow-mediated dilation.

(measured by FMD) that typically occurs after a meal in healthy subjects (15). Similar results were seen with a single dose of a green coffee extract containing 600 mg CGAs (19) or after a single dose of a green coffee extract containing 306 mg CGAs (16). Another study conducted in 12 healthy subjects with a decaffeinated coffee containing 287 mg CGAs did not produce any significant effects, suggesting to the authors of that experiment that caffeine, rather than CGAs, may be the principal coffee constituent having an acute effect on FMD (17). These results are consistent with earlier findings that caffeine (200 mg) acutely improves FMD in subjects with or without coronary artery disease (37), but contradict other studies which found that decaffeinated coffee can improve FMD in a dose-dependent manner (38). By conducting a study that specifically explored the relation between CGAs and FMD over a daylong period of time, as compared with a placebo, we think that we have shown that coffee components other than caffeine such as CGAs were clearly and positively influencing FMD.

The impact we observed on endothelial function is not necessarily restricted to coffee CGAs. Similar acute effects on FMD have been observed for purified compounds or extracts from other polyphenol-containing foods such as cocoa, tea, grape, apple, and blueberry (36, 39–42). For instance, 2 h after ingestion of 176–185 mg cocoa flavanols, FMD was increased from 4.5% to 6.9% (43). Likewise, women (not men) receiving a single dose of *trans*-resveratrol (300 mg) had an acute improvement of FMD from 4.2% to 7.1% (44). Consumption of a single dose of anthocyanins (160 mg) acutely improved FMD by 1.3% and 1.1% at 2 and 6 h, respectively (45). More recent literature has provided better understanding of the mechanisms responsible for the pharmacological action of polyphenols on endothelial function (46–48). Such mechanisms are likely diverse, but FMD reflects the vasodilatory response resulting from a brief and substantive increase in shear stress, due in large part to increased NO bioactivity (6). Differences in biological activity among the CGA metabolites could at least in part explain the lack of effect observed at higher DGCE doses. Indeed, it has been found that various relative amounts of metabolites are produced after ingestion of different doses of coffee or coffee extracts (13). These metabolites may in turn be expected to exert different levels of activity on the bioavailability of NO.

The increase in the FMD values observed in the control or placebo group is consistent with a diurnal pattern of endothelial function previously observed over the day (49). Several research groups have documented this diurnal variation in FMD, where FMD is generally reduced in the morning as compared with the rest of the day (49–51), but no mathematical functions for the diurnal variation have been reported.

In our study, the DGCE treatment was effective in increasing FMD at a dose of 302 mg (156.4 mg CGAs). A 200-mL cup of brew coffee typically contains between 70 and 350 mg CGAs, whereas soluble coffee powder (2 g/cup) would provide a beverage with 70–220 mg CGAs (52). The 156.4-mg dose is therefore the amount contained within 1 or 2 cups of coffee (depending on the degree of roasting), which is a reasonable daily consumption. However, we cannot rule out the possibility that lower doses are also effective.

One potential limitation of our study is that we tested the effects of DGCE in healthy subjects with a suboptimal level of endothelial function. These were healthy adults within the age range 45–65 y who were included in the study if they had an FMD value <7.5% at screening. The true prevalence of peripheral endothelial dysfunction worldwide is not known, and normative data are only starting to emerge (30, 31). Furthermore, a historical problem with the FMD literature has been poor compliance with accepted guidelines (7, 29), which directly affects reproducibility of the technique (23) and should be borne in mind when interpreting previous studies. In the present study, we followed a standardized and recommended methodological approach, which increases the confidence we have in the findings. We also adopted multiple complementary approaches to the presentation and analysis of FMD. Our primary analysis, as displayed in Figure 3, related to the FMD% comparisons between conditions and across the multiple time points of assessment, illustrating which time points differed from the placebo condition. In Figure 5, we presented all data points, under all conditions, as a continuous relation to circulating CGAs and phenolic acids. In addition to these approaches, we presented a summary of the FMD responses within each condition, in the form of the iAUC for FMD responses (Figure 4A). Because the iAUC summarizes the collective FMD response under each condition, it complements and reinforces the differences observed in Figure 3 and is also consistent with the approach we adopted to summarize the plasma changes in circulating CGAs and phenolic acids (Figure 2B). Unfortunately, we did not assess FMD at time points beyond 24 h in the current experiment. At 24 h, the difference in FMD% between the 302-mg treatment and placebo was significant, at $P = 0.02$ (Figure 3B), but the iAUC for FMD did not quite

achieve significance (Figure 4A). It is interesting to speculate that a more extended time point (e.g., 48 h) may have uncovered some prolonged benefits, particularly given that the trend for placebo was downward, whereas the 302-mg treatment remained elevated at 24 h.

Finally, our study was an acute experiment and it has not yet been formally shown that coffee CGAs can improve fasting FMD chronically. Agudelo-Ochoa et al. (53) were the first to evaluate the effect of coffee consumption (400 mL/d with 420 or 780 mg CGAs) over 8 wk on FMD in 74 healthy subjects. No significant differences were observed between the groups in FMD and other measured parameters (e.g., lipid profile, blood pressure). Evaluation in a healthy population (baseline FMD values >10%) and high SD in the measurement of FMD (23) could have affected the study outcome. A more recent study found a significant improvement of FMD after consumption of a green coffee extract containing 300 mg CGAs for 2 wk (18). Therefore, properly designed and governed, methodologically robust human clinical trials will have to be undertaken to demonstrate whether the consumption of coffee CGAs for several weeks can significantly and meaningfully improve vascular function and health in different populations.

In conclusion, this is the first time, to our knowledge, that FMD response to a DGCE with high content in CGAs has been measured over a daylong period, and that a significant and meaningful acute improvement could be demonstrated at several time points along this period. Moreover, although we did not identify the biologically active moiety within the circulating coffee CGA metabolites, we showed, using a pooled evaluation, a positive tendency toward a larger effect on FMD at higher systemic total polyphenol concentrations, which suggests that the positive effects are likely due to compounds other than caffeine. In addition, our data provide evidence that nonconjugated and conjugated metabolites may have a different time-dependent effect on FMD. Additional research will have to investigate and demonstrate the sustainability over time of these effects.

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Data Availability

Data described in the article, code book, and analytic code will be made available upon request pending approval from the trial sponsor.

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