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Coffee Consumption Increases the Antioxidant Capacity of Plasma and Has No Effect on the Lipid Profile or Vascular Function in Healthy Adults in a Randomized Controlled Trial[1,2]

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Abstract

Background: Coffee, a source of antioxidants, has controversial effects on cardiovascular health.

Objective: We evaluated the bioavailability of chlorogenic acids (CGAs) in 2 coffees and the effects of their consumption on the plasma antioxidant capacity (AC), the serum lipid profile, and the vascular function in healthy adults.

Methods: Thirty-eight men and 37 women with a mean ± SD age of 38.5 ± 9 y and body mass index of 24.1 ± 2.6 kg/m^2 were randomly assigned to 3 groups: a control group that did not consume coffee or a placebo and 2 groups that consumed 400 mL coffee/d for 8 wk containing a medium (MCCGA; 420 mg) or high (HCCGA; 780 mg) CGA content. Both were low in diterpenes (0.83 mg/d) and caffeine (193 mg/d). Plasma caffeic and ferulic acid concentrations were measured by GC, and the plasma AC was evaluated with use of the ferric-reducing antioxidant power method. The serum lipid profile, nitric oxide (NO) plasma metabolites, vascular endothelial function (flow-mediated dilation; FMD), and blood pressure (BP) were evaluated.

Results: After coffee consumption (1 h and 8 wk), caffeic and ferulic acid concentrations increased in the coffee-drinking groups, although the values of the 2 groups were significantly different ($P < 0.001$); caffeic and ferulic acid concentrations were undetectable in the control group. At 1 h after consumption, the plasma AC in the control group was significantly lower than the baseline value (−2%) and significantly increased in the MCCGA (6%) and HCCGA (5%) groups ($P < 0.05$). After 8 wk, no significant differences in the lipid, FMD, BP, or NO plasma metabolite values were observed between the groups.

Conclusions: Both coffees, which contained CGAs and were low in diterpenes and caffeine, provided bioavailable CGAs and had a positive acute effect on the plasma AC in healthy adults and no effect on blood lipids or vascular function. The group that did not drink coffee showed no improvement in serum lipid profile, FMD, BP, or NO plasma metabolites. This trial was registered at registroclinico.sld.cu as RPCEC00000168. *J Nutr* 2016;146:524–31.

Keywords: cardiovascular disease, chlorogenic acids, phenolic acids, oxidative stress, diterpenes, caffeine, flow-mediated dilation, cholesterol

Introduction

Coffee has been recognized as a natural source of antioxidants because of its phenolic compound content, of which chlorogenic acids (CGAs)[9] are the most prevalent (1). These compounds may play a key role in inhibiting LDL cholesterol lipid peroxidation and in modulating oxidative stress, thereby lowering the risk of atherosclerosis (2). After CGAs are acutely consumed, they appear not to be absorbed as such. Instead, CGAs have been shown to be hydrolyzed in the gastrointestinal tract, which results in the generation of end products such as caffeic and

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[9] Abbreviations used: AC, antioxidant capacity; BP, blood pressure; CGA, chlorogenic acid; CVD, cardiovascular disease; FMD, flow-mediated dilation; FRAP, ferric-reducing antioxidant power; HCCGA, high chlorogenic acid content; LOD, limit of detection; MCCGA, medium chlorogenic acid content; TC, total cholesterol.

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ferulic acids (3). In vitro studies have shown that the antioxidant capacity (AC) of coffee is high and comparable to that of red wine and green tea (4).

An imbalance of antioxidant defenses and the production of reactive oxygen species have been identified as one of the mechanisms underlying the development of cardiovascular diseases (CVDs). Reactive oxygen species generate oxidative stress and contribute to endothelial dysfunction, an event that has also been identified as a risk factor for CVD (5).

The effect of coffee consumption on lipid profiles and vascular function is unclear. Coffee intake has been associated with an increase in total cholesterol (TC) (6), which was attributed to the diterpene compounds (cafestol and kahweol) that are present in boiled coffee. These compounds are extracted because of the high temperatures and prolonged extraction time of this type of coffee preparation (7). Some studies have shown that the use of filters during the preparation of coffee could reduce the amount of diterpenes and the effect on TC (8, 9). In a study by Ahola et al. (8) that used a crossover design, 20 healthy volunteers randomly consumed strong boiled coffee or filtered boiled coffee for 4 wk; an increase in TC was observed only in the group that consumed unfiltered coffee. In a review, Rodrigues et al. (9) summarized the most relevant results regarding the effect of coffee consumption on various risk factors for CVD, lipid profiles, and other measures and also found a positive association between unfiltered coffee consumption and increased TC. Contrary to these reports, other studies have reported increased TC when subjects consumed filtered coffee (10, 11). In a parallel clinical trial of 120 nonsmoking healthy subjects, Strandhagen et al. (10) showed that consuming 600 mL filtered coffee/d for 4 wk significantly increased TC and that not consuming coffee significantly reduced TC. The available studies have limitations related to small sample sizes, the inclusion of only men or few women, and underrepresentation of specific age or ethnic groups. In addition, the amount of coffee consumed by the participants was difficult to determine in some of the studies, whereas others did not report either the coffee cup size or the coffee's chemical characteristics.

The reported relations between coffee consumption and endothelial function are contradictory. Some studies that evaluated the effect of the consumption of coffee with caffeine concentrations between 80 and 130 mg by healthy adults have reported a decrease in flow-mediated dilation (FMD) (12, 13); contrary to the studies cited previously, another study showed that in both healthy individuals and those with coronary arterial disease, FMD increased 1 h after the intake of 200 mg of pure caffeine (14). In contrast, Mubarak et al. (15) did not find any effect on FMD after the consumption of 400 mg of pure CGAs, which is equivalent to the amount found in 2 cups of coffee.

Within the past few years, epidemiological studies have associated coffee consumption with a low risk of CVD. Freedman et al. (16) explored the associations between coffee consumption and subsequent total and cause-specific mortality among 229,119 men and 173,141 women in the NIH-AARP diet and health study; these participants were aged 50–71 y at the start of the study. The results showed a significant inverse association between coffee consumption and death from all causes and specifically with death resulting from heart disease. Because coffee is among the most widely consumed beverages in the world, it is important to explore its antioxidants and their effects on cardiovascular health. This study evaluated the bioavailability of the chlorogenic and phenolic acids provided by 2 types of filtered coffee with different CGA contents, which was measured based on 2 major CGA metabolites (caffeic and ferulic acids). The effects of the CGAs on the plasma AC, lipid profiles, and vascular function in healthy adults were then evaluated.

Methods

Study design. The controlled clinical trial was single-blinded with respect to the analysis. The participants, who were apparently healthy adult volunteers who were habitual coffee drinkers and who met the inclusion criteria, were randomly assigned to 3 study groups: a control group (no coffee consumption, no placebo) and 2 groups that drank 1 of 2 types of coffee (400 mL/d) for 8 wk that differed with respect to CGA content. The sample size was calculated based on the reported minimum mean difference of plasma AC with use of ferric-reducing antioxidant power (FRAP) [22 ± 12 μmol Fe^{2+}/L (17)] and on changes in LDL cholesterol concentration [17.1 ± 18.8-mg/dL difference between a treated group (coffee consumption) and the control group (no coffee consumption, no placebo) (GM Agudelo-Ochoa, unpublished results, 2008)]. For a power of 0.8 and a significance level of 0.05, 25 subjects were selected per group for a total of 75 (**Figure 1**).

Subjects. The volunteer subjects consisted of 38 men and 37 women who met the following inclusion criteria: aged 20–60 y; BMI (in kg/m^2) between 18.5 and 29.9; regular coffee drinker (at least 3 cups/d; 1 cup = ~100 mL); nonsmoker; physical activity of <10 h/wk; no history and/or diagnosis of chronic disease (cardiovascular, gastric, or psychiatric disorder; dyslipidemia; diabetes; cancer; renal or liver alteration; hypothyroidism; insomnia; dysautonomia; or sleep disturbance); not currently consuming medication (lipid-lowering drugs, antioxidant dietary supplements, anticonvulsants, anti-inflammatory steroids, hypnotics, or

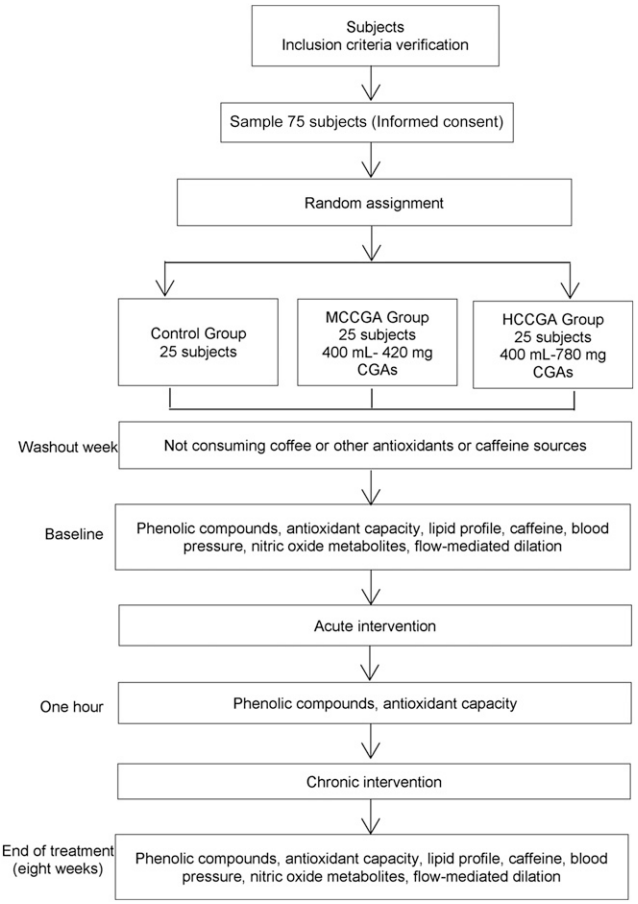

FIGURE 1 Study design. CGA, chlorogenic acid; HCCGA, high chlorogenic acid content; MCCGA, medium chlorogenic acid content.

caffeine); and a maximum alcohol consumption of 10 units/wk for women and 15 units/wk for men (1 unit = 10 g of ethanol). Pregnant or nursing women, highly competitive athletes, and vegetarians were excluded. The study was approved by the CES University Ethics Committee and registered with the International Clinical Trials Registry Platform (RPCEC00000168).

The volunteers provided informed consent and were randomly assigned to 3 groups (matched for age, sex, and BMI): the control group and the 2 groups in which the participants drank 400 mL coffee/d with 1 of 2 CGA contents: a medium CGA content (MCCGA; 420 mg) or high CGA content (HCCGA; 780 mg). Both coffees were low in the diterpenes kahweol (MCCGA: 0.89 mg/d; HCCGA: 0.92 mg/d) and cafestol (0.75 mg/d) and in caffeine (MCCGA: 188 mg/d; HCCGA: 197 mg/d). The total CGAs and phenolic acid content was defined as the sum of the individual contributing acids: 5-*O*-caffeoylquinic; 3-*O*-caffeoylquinic; 4-*O*-caffeoylquinic; caffeic; ferulic; 3,4-di-*O*-caffeoylquinic; 3,5-di-*O*-caffeoylquinic; and 4,5-di-*O*-caffeoylquinic. This content was known by the researchers after the results were analyzed.

The week before the beginning of the study (washout period) and during the 8 wk of the intervention, all participants avoided consuming beverages with caffeine as well as beverages or foods that were naturally high in antioxidants (tea, dark chocolate, red wine, cocoa-derived products, herb infusions, berries, and soy) or that were enriched with antioxidants. They also avoided any antioxidant supplements, and the consumption of caffeine-containing drugs was forbidden. The participants were allowed to consume water, juices, milk, and other beverages without caffeine. Except for these restrictions, the volunteers continued their usual diets and lifestyles during the intervention. All of the volunteers received advice and support during the intervention to resolve any questions. During the intervention, the coffee drink was prepared and provided in the workplace; the volunteers were trained to prepare the drink as needed on weekends and received the necessary materials (coffee maker, filters, cups, and ground coffee). The research team monitored the participants for possible withdrawal symptoms and provided general recommendations for managing such symptoms. At the beginning and end of the intervention, the participants answered a semiquantitative survey on their consumption of antioxidant-containing foods aside from coffee; a change of 10% was permitted between the baseline and postintervention surveys.

Coffee beverage preparation and chemical characterization. Colombian Arabica coffee that was prepared by Colcafé S.A.S. was used. Until it was prepared, the coffee was stored in laminated packaging with a nitrogen-modified atmosphere. The coffee beverage was prepared by using the paper-filtered drip method (6 g/100 mL water). Standard HPLC procedures were used to chemically characterize the CGAs and to determine the caffeine content [CGAs: (18); caffeine: (19)]. Cafestol and kahweol were quantified according to Silvia et al. (20).

Anthropometric assessment. Each participant's weight, height, and waist circumference were measured at the beginning and end of the intervention after the evaluators were standardized by using internationally accepted equipment and techniques (21). BMIs and waist circumferences were calculated and classified according to WHO criteria (22).

Biochemical tests. Blood samples were drawn by puncturing the antecubital vein to obtain serum and plasma. For each participant, the plasma concentration of caffeic and ferulic acids, AC, lipid profile, and NO plasma metabolites were evaluated at baseline and 8 wk after coffee consumption (chronic time point). In the 3 groups, the plasma concentration of caffeic and ferulic acids and the AC were evaluated 1 h after the assigned intervention (acute time point). In the control group, the serum caffeine levels were evaluated with GC/MS at baseline and at weeks 4 and 8 (23). The plasma measurements at 8 wk were performed on samples that were taken 21 h after the last coffee consumption.

Quantification of plasma concentrations of caffeic and ferulic acid. Plasma was obtained by centrifugation at 1200 × *g* for 15 min at 4°C with use of 0.109 mol/L sodium citrate as an anticoagulant and then stored at −80°C until analysis. Enzymatic digestion was performed with glucuronidase and sulfatase (24); subsequent cleaning was performed via extraction in the solid phase. The obtained substances were derivatized with *N*-tert-butyldimethylsilyl-*N*-methyltrifluoroacetamide with 1% tert-butyldimethylchlorosilane and then analyzed by GC. Detection was performed with a quadrupole mass spectrometer. The reproducibility of the analytical system was determined (relative SD of 1.2% and 1.4% for the caffeic acid and ferulic acid, respectively). The recovery rate for this method was 97.58% for ferulic acid and 97.27% for caffeic acid; the analytical method was exact, and the concentration factor did not affect the variability of the results obtained. The limit of detection (LOD) and quantification was determined based on the SD of the response at low concentrations. The LOD was expressed based on the replicates of a low concentration that produced a signal-to-noise ratio >6:1 and a relative SD <20%. The LOD was 3 nM for caffeic acid and 5 nM for ferulic acid. The quantification was performed by using the external standard method.

Plasma antioxidant capacity. The FRAP method was applied to plasma samples immediately after they were obtained according to the method of Benzie and Szeto (25) with slight modifications. Briefly, the FRAP reaction (300 mM FRAP reagent, 40 mM ferric-tripyridyltriazine/HCl, and 20 mM $FeCl_3.6H_2O$) was monitored in a microwell plate assay

TABLE 1 Characteristics of the healthy adults assigned to the three study groups[1]

	Study group, *n* (%)				
	Control (*n* = 25)	MCCGA (*n* = 25)	HCCGA (*n* = 24)	Total	*P*[2]
Gender					0.91
Men	13 (52)	12 (48)	12 (50)	37	
Women	12 (48)	13 (52)	12 (50)	37	
Age, y					0.51
20–40	14 (56)	14 (56)	10 (42)	38	
41–60	11 (44)	11 (44)	14 (58)	36	
BMI,[3] kg/m^2					0.92
Normal	16 (64)	15 (60)	14 (58)	45	
Overweight	9 (36)	10 (40)	10 (42)	29	
Waist circumference,[4] cm					0.39
Adequate	24 (96)	24 (96)	21 (88)	69	
High	1 (4)	1 (4)	3 (12)	5	

[1] HCCGA, high chlorogenic acid content; MCCGA, medium chlorogenic acid content.
[2] Chi-square test.
[3] Normal BMI: 18.5–24.9; overweight: 25–29.9.
[4] Appropriate waist circumference: men: ≤94 cm; women: ≤80 cm.

TABLE 2 Characteristics of the 2 types of coffee used in the study[1]

	Medium CGA coffee		High CGA coffee	
Components	mg/100 mL[2]	mg/400 mL	mg/100 mL[2]	mg/400 mL
Caffeine	47 ± 1.4	188	49 ± 1	197
Total CGAs	105 ± 4.1	420	195 ± 6.9	780
Diterpenes				
Cafestol	0.19 ± 0.03	0.75	0.19 ± 0.01	0.75
Kahweol	0.22 ± 0.03	0.89	0.23 ± 0.02	0.92

[1] CGA, chlorogenic acid.
[2] Values are means ± SDs.

instead of cuvettes, and the Trolox calibration solutions (31.25–1000 μM) were prepared in PBS. The analyses were performed in triplicate, and the results were expressed in micromolar equivalent Trolox per liter of plasma.

Lipid profile. Plasma containing lithium heparin, which was obtained by centrifuging blood samples at 1200 × *g* for 15 min at 4°C, was used. Plasma TC, HDL cholesterol, and TGs were enzymatically measured with use of cholesterol oxidase/peroxidase, cholesterol HDL direct, and TG glycerol phosphate oxidase/peroxidase commercial kits (BioSystems S.A.).The LDL cholesterol values were obtained by calculation (26).

Vascular function. Vascular function was assessed according to the following parameters:

- *FMD of the brachial artery:* In accordance with Bretón et al. (27), this measurement was performed in a temperature-controlled room (22°C) while the participants were fasting. A 7.5–13-MHz linear transducer connected to ultrasound equipment with a vascular program (General Electric Logic P5 and Toshiba Nemio XG) was used. To ensure the accuracy and reproducibility of the data, each volunteer was evaluated on the same equipment and by the same evaluator, who was previously trained and standardized.
- *Blood pressure (BP):* BP was obtained according to the standard techniques of the AHA and the European Cardiology Association. The subjects rested between 3 and 5 min. The cutoff for normal BP was 120/80 mm Hg (28).
- *NO metabolites:* These measurements were performed with a nitrate/nitrite colorimetric kit that is commercially available from Cayman Chemical Company. The quantification was performed from calibration curves, and values were expressed in μmol/L of plasma (29).

Statistical analysis. The age, sex, and BMI of the study population was described with use of measures of central tendency and dispersion for quantitative variables (data are expressed as the mean ± SD), whereas the qualitative variables were described through frequencies and percentages. A chi-square test was used to establish the comparability of independent variables between the study groups. The normality of the continuous variables in each group was evaluated with the Shapiro-Wilks test. To evaluate the differences in caffeic and ferulic acid concentrations between the treated groups, the Mann-Whitney U test was used. To evaluate the effect of the type of coffee consumed, a repeated-measures ANOVA that took into account 2 time points (baseline and 8 wk) and the 3 treatments (control, MCCGA, and HCCGA) was performed; to evaluate AC, 3 time points (basal, 1 h, and 8 wk) and the 3 treatments mentioned previously were taken into account. When an interaction was significant, Tukey's post hoc tests were applied. The normality assumptions of the model were checked, and transformations were applied (log for TGs and HDL cholesterol and square root for NO) to the data for variables that were not normally distributed. Systolic blood pressure and diastolic blood pressure were not normally distributed even after transformation and were therefore analyzed by using the nonparametric Kruskal-Wallis test. Significance was defined as $P < 0.05$, and 2-tailed tests were used. The statistical analysis was performed by using SPSS version 22 (IBM).

Results

Of the 75 volunteers who began the study, 74 completed it; 1 individual in the group that consumed the HCCGA coffee was excluded because of alterations in baseline lipids that required medication. **Table 1** shows the characteristics of the volunteers in each group at baseline. **Table 2** describes the characteristics of the 2 coffees used.

At baseline, the concentrations of caffeic and ferulic acid were below the detection threshold in all of the groups. The same situation was observed in the control group after 8 wk of intervention. Between the groups that drank coffee, the caffeic and ferulic acid concentrations were significantly higher in the MCCGA group at each of the 2 measurement time points (**Table 3**).

A significant time × treatment interaction was found for the plasma AC ($P < 0.001$). Repeated-measures ANOVA revealed that the AC was significantly lower than the baseline value in the control group at 1 h after coffee intake ($P < 0.05$). This result differs from what was observed in the groups that drank coffee; in these groups, the AC significantly increased ($P < 0.05$). At 8 wk, the AC decreased in the 3 groups, and the values did not significantly differ (**Table 4**).

There was not a significant time × intervention interaction effect for the lipid profile, FMD, or NO plasma metabolites ($P > 0.05$) (**Table 5**). There were no significant differences ($P > 0.05$) between pre- and posttreatment for systolic BP (control: 108 ± 7/106 ± 7; MCCGA: 108 ± 9/109 ± 11; HCCGA: 110 ± 10/109 ± 8 mm Hg) and diastolic BP (control: 75 ± 8/75 ± 5; MCCGA: 74 ± 7/75 ± 7; HCCGA: 75 ± 8 /76 ± 6 mm Hg).

In the control group, no caffeine was detected in the plasma at any of the 3 time points evaluated (baseline and weeks 4 and 8), confirming adherence to the intervention. No significant changes were found in the semiquantitative survey of the frequency of consumption of foods of interest between the beginning and end of the intervention.

Discussion

The results of various studies over the last decade have demonstrated the antioxidant effect of the CGAs provided by coffee. However, it has been suggested that the absorption of CGAs might be low because of their esterification (30). Several studies have concluded that CGAs in coffee are bioavailable (31, 32). Renouf et al. (24) provided healthy volunteers with a single dose

TABLE 3 Plasma caffeic and ferulic acid concentrations at baseline and at 1 h and 8 wk after the intervention in healthy adults[1]

	Control (n = 25)	MCCGA (n = 25)	HCCGA (n = 24)
Caffeic acid, nM			
Baseline	ND	ND	ND
1 h	ND	50.5 ± 6.9[a]	20.3 ± 3.3[b]
8 wk	ND	14.1 ± 0.8[a]	9.0 ± 1.5[b]
Ferulic acid, nM			
Baseline	ND	ND	ND
1 h	ND	201 ± 18.7[a]	137 ± 6.1[b]
8 wk	ND	44.6 ± 0.6[a]	34.3 ± 2.1[b]

[1] Values are means ± SDs. The 8-wk sample was obtained 21 h after the last consumption. Labeled means within a row without a common letter differ significantly (Mann-Whitney U test; $P < 0.001$). HCCGA, high chlorogenic acid content; MCCGA, medium chlorogenic acid content; ND, not detected (caffeic acid <3 nM and ferulic acid <5 nM).

TABLE 4 Antioxidant capacity of plasma at baseline, 1 h, and 8 wk after the intervention in healthy adults[1]

				P		
	Control (n = 25)	MCCGA (n = 25)	HCCGA (n = 24)	Treatment	Time	Interaction
FRAP, μmol ET/L						
Baseline	562 ± 70.8	584 ± 63.6	563 ± 78.5	0.11	<0.001	<0.001
1 h	553 ± 71.6*[b]	618 ± 59.9*[a]	590 ± 81.3*[a,b]			
8 wk	519 ± 80.3*[a]	552 ± 59.3*[a]	533 ± 71.0*[a]			

[1] Values are means ± SDs. Labeled means in a row without a common letter differ significantly (repeated-measures ANOVA; $P < 0.01$). *Differences within the same group relative to baseline (repeated-measures ANOVA; $P < 0.05$). ET, equivalent Trolox; FRAP, ferric-reducing antioxidant power; HCCGA, high chlorogenic acid content; MCCGA, medium chlorogenic acid content.

of 335 mg of soluble CGAs in coffee and showed the presence of 2 groups of metabolites, the maximum concentrations of which were achieved between 1–12 h after coffee consumption. In our study, caffeic and ferulic acids were present in the groups that drank coffee and were not detectable in the control group, both after 1 h as well as 8 wk after the start of the intervention. These findings are consistent with previous results. It is important to note that at both the acute and chronic time points the 2 metabolites were present at significantly higher concentrations in the group assigned to drink the MCCGA coffee. Similar results were found in the study conducted by Stalmach et al. (33), in which reduced absorption in the small intestine was found to be associated with ingestion of the highest dose of some metabolites, probably because of saturation of the absorption sites.

Coffee is now recognized as a source of potent antioxidants, principally CGAs, that could help improve the redox state of plasma and prevent the oxidation of LDL (34). Natella et al. (35) reported that in healthy volunteers, the AC was higher after the consumption of coffee instead of tea; however, other studies of healthy adults have not found any effects of coffee on plasma AC (36). In our study, 1 h after the 2 types of coffee had been consumed, the plasma AC of the volunteers was significantly increased over the baseline value, in contrast to the plasma AC of the participants in the control group, which declined over the same period. Similar results were reported by Corrêa et al. (37), who found an increase in the AC in subjects who had consumed 2 types of filtered coffee that were roasted to different degrees and that had different CGA contents for 4 wk. An increase in plasma AC provides greater protection against free radicals, which helps reduce key events in the development of CVD. The results of our study regarding the bioavailability of the CGAs in coffee and their effects on the AC in healthy adults suggest an acute positive effect that tends to decrease as the metabolites are

TABLE 5 Markers of serum lipid profile and vascular function in healthy adults at baseline and 8 wk after the intervention[1]

				P[2]		
	Control (n = 25)	MCCGA (n = 25)	HCCGA (n = 24)	Treatment	Time	Interaction
Cholesterol, mg/dL						
Baseline	196 ± 32.7	196 ± 33.4	211 ± 31.0	0.26	0.02	0.69
8 wk	203 ± 38.2	201 ± 35.9	214 ± 32.1			
LDL cholesterol, mg/dL						
Baseline	104 ± 21.7	101 ± 22.5	118 ± 24.7	0.04	0.29	0.58
8 wk	107 ± 26.0	103 ± 23.8	118 ± 23.2			
HDL cholesterol, mg/dL						
Baseline	50.7 ± 13.9	52.9 ± 12.8	50.6 ± 10.3	0.84	0.16	0.26
8 wk	52.4 ± 12.5	53.2 ± 13.5	51.0 ± 10.9			
TGs, mg/dL						
Baseline	114 ± 53.6	118 ± 56.0	121 ± 51.7	0.74	0.09	0.37
8 wk	125 ± 101.8	134 ± 65.1	122 ± 51.2			
Arterial index						
Baseline	4.1 ± 1.0	3.9 ± 0.9	4.3 ± 0.9	0.33	0.42	0.53
8 wk	4.0 ± 1.1	4.0 ± 1.1	4.3 ± 0.9			
FMD, %						
Baseline	13.2 ± 11.1	13.6 ± 12.3	21.3 ± 11.0	0.26	0.13	0.50
8 wk	18.4 ± 18.1	19.5 ± 18.1	21.1 ± 21.2			
NO metabolites, μmol/L						
Baseline	3.0 ± 1.3	3.3 ± 1.0	3.0 ± 1.1	0.20	0.49	0.27
8 wk	2.7 ± 1.7	4.0 ± 3.6	4.3 ± 3.5			

[1] Values are means ± SDs. FMD, flow-mediated dilation; HCCGA, high chlorogenic acid content; MCCGA, medium chlorogenic acid content.
[2] Repeated-measures ANOVA.

eliminated by the body. The fact that the plasma AC increased 1 h after the consumption of the 2 coffee beverages and significantly increased after the consumption of the MCCGA coffee suggests that other substances with antioxidant properties may have been generated during the roasting process, which differed for the 2 coffees. This result illustrates the need to characterize this matrix in more detail so that other substances and the synergy between them can be identified.

An important finding of this study was the neutral effect of coffee consumption on the components of the lipid profile, as reported by other authors (10, 38). The results of studies that used unfiltered coffee have consistently shown the negative effect of the beverage on plasma TC, an effect that has been attributed to the diterpenes, specifically cafestol (39–41). Studies that evaluated the effect of filtered coffee on lipids have reported conflicting results; some studies have found the positive effects of reduced TC and LDL cholesterol concentrations and increased HDL cholesterol concentrations (42, 43). In contrast, other studies have reported an increase of TC after coffee intake and a decrease when coffee is not consumed (10, 38, 44). Naidoo et al. (45) showed that the low content of cafestol in beverages prepared with filters is not a risk for increased TC. Finally, the results of two meta-analyses of the effect of coffee on blood lipids attributed the negative effect to the diterpenes provided by unfiltered coffee (7, 46).

The 400 mL of filtered coffee with medium and high CGA content provided 0.89 mg and 0.92 mg of kahweol, respectively; both provided 0.75 mg of cafestol. Among the volunteers of our study, consumption of these beverages did not negatively affect the lipid profile. In contrast with our results, in a crossover clinical trial of healthy volunteers, Corrêa et al. (11) found an increase in both TC as well as the HDL cholesterol concentration after the consumption for 4 wk of 482-mL light- or medium-roasted filtered coffee/d containing antioxidants. Compared with our study, the light- and medium-roasted coffee of the Corrêa et al. study provided 21 and 25 times more cafestol, respectively. Another important difference between our study and that of Corrêa et al. was the intake of caffeine; in our study the mean intake was 192 mg/d, whereas in the Corrêa et al. study it was 763 mg/d.

The effect of coffee on endothelial function has primarily been evaluated acutely, and the effect of chronic coffee consumption, to our knowledge, has been rarely studied. In our study, the FMD values of the individuals who did not drink coffee did not improve, and the FMD values of the individuals who drank coffee showed no deterioration, contrary to previous reports that evaluated the effect of acute coffee consumption on this variable (12, 13). The authors attributed the unfavorable effect on the endothelium to caffeine, which decreases the levels of cyclic guanosine monophosphate, the second messenger of L-arginine/NO (13). The positive or neutral effect of coffee on the vascular endothelium could potentially be explained by the action of CGAs, which may offset the possible negative effects of caffeine (12). In our study, the total caffeine intake from the 2 types of coffee was considered safe (<300 mg/d) (47).

Various investigations have found that the consumption of antioxidants, such as flavanols, may increase NO concentrations (48); however, only a few studies, to our knowledge, have been published regarding the effect of CGAs in coffee on NO concentrations. In this study, the concentration of NO plasma metabolites at 8 wk did not significantly differ between the groups in terms of the effect over time or the treatment × time interaction. Similar to these results, other authors have found no differences in NO concentrations (15, 49).

In our study of normotensive habitual coffee drinkers, those who drank coffee did not exhibit an increase in BP at 8 wk; in the control group, BP also did not decrease. Consistent with these results, a study of normotensive volunteers who drank between 200 and 600 mL of coffee for 6 wk did not report noteworthy changes in BP (50). A possible explanation for these results is the duration of consumption; the individuals who drank coffee over this period may have developed a partial tolerance to caffeine, a substance that is related to the increase in BP (49). Substances in coffee, such as potassium, magnesium, and CGAs, have been postulated to exert a protective effect on the vascular system (51). More specifically, CGAs have been shown to inhibit the activity of angiotensin-converting enzymes (49). Finally, the relation between regular coffee consumption and BP can be described as a J–shaped curve, where moderate intake of this beverage may have a protective effect on BP, whereas high coffee consumption could increase the risk of hypertension (52).

Given the chemical complexity of coffee beverages, a limitation of this study was that other CGA metabolites as well as other components responsible for coffee's total AC were not measured. A strength of this study is that we used an amount of coffee and method of preparation similar to those used by the study population; thus, a recommendation to consume coffee in accordance with the amounts and preparation method used in this study is a feasible way to achieve the desirable antioxidant effects without affecting the lipid profile or vascular function.

In conclusion, the results of this study demonstrated acute absorption of the antioxidants in coffee after individuals consumed coffee beverages with either a medium or high CGA content. The results also revealed a positive acute effect on plasma AC and that chronic consumption of 2 drinks with antioxidants, low diterpene concentrations, and a concentration of caffeine within the recommended range had a neutral effect on blood lipids and vascular function. Finally, it is important to note that this study showed that ceasing to drink coffee did not improve serum lipid profile, FMD, BP, or NO plasma metabolites.

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GMA-O, CMV-R, MD-R, and KM-D designed the study, conducted the research, and performed the analyses; MN-C and MMQ-O designed, processed, and chemically analyzed the 2 types of coffee; ICP-Z and OJL-G conducted and analyzed the data of the clinical and laboratory tests; and OJL-G optimized the experimental conditions of the chemical tests. All authors read and approved the final manuscript.

References

1. Clifford MN. Chlorogenic acids and other cinnamates—nature, occurrence, dietary burden, absorption and metabolism. J Sci Food Agric 2000;80:1033–43.
2. Hoelzl C, Knasmüller S, Wagner KH, Elbling L, Huber W, Kager N, Ferk F, Ehrlich V, Nersesyan A, Neubauer O, et al. Instant coffee with high chlorogenic acid levels protects humans against oxidative damage of macromolecules. Mol Nutr Food Res 2010;54:1722–33.
3. Nardini M, Cirillo E, Natella F, Scaccini C. Absorption of phenolic acids in humans after coffee consumption. J Agric Food Chem 2002; 50:5735–41.
4. Brezová V, Šlebodová A, Staško A. Coffee as a source of antioxidants: an EPR study. Food Chem 2009;114:859–68.
5. Fearon IM, Faux SP. Oxidative stress and cardiovascular disease: novel tools give (free) radical insight. J Mol Cell Cardiol 2009;47: 372–81.

6. Thelle DS, Arnesen E, Førde OH. The Tromsø heart study. Does coffee raise serum cholesterol? N Engl J Med 1983;308:1454–7.
7. Jee SH, He J, Appel LJ, Whelton PK, Suh I, Klag MJ. Coffee consumption and serum lipids: a meta-analysis of randomized controlled clinical trials. Am J Epidemiol 2001;153:353–62.
8. Ahola I, Jauhiainen M, Aro A. The hypercholesterolaemic factor in boiled coffee is retained by a paper filter. J Intern Med 1991;230: 293–7.
9. Rodrigues IM, Klein LC. Boiled or filtered coffee? Effects of coffee and caffeine on cholesterol, fibrinogen and C-reactive protein. Toxicol Rev 2006;25:55–69.
10. Strandhagen E, Thelle DS. Filtered coffee raises serum cholesterol: results from a controlled study. Eur J Clin Nutr 2003;57:1164–8.
11. Corrêa TA, Rogero MM, Mioto BM, Tarasoutchi D, Tuda VL, César LA, Torres EA. Paper-filtered coffee increases cholesterol and inflammation biomarkers independent of roasting degree: a clinical trial. Nutrition 2013;29:977–81.
12. Buscemi S, Verga S, Batsis JA, Donatelli M, Tranchina MR, Belmonte S, Mattina A, Re A, Cerasola G. Acute effects of coffee on endothelial function in healthy subjects. Eur J Clin Nutr 2010; 64:483–9.
13. Papamichael CM, Aznaouridis KA, Karatzis EN, Karatzi KN, Stamatelopoulos KS, Vamvakou G, Lekakis JP, Mavrikakis ME. Effect of coffee on endothelial function in healthy subjects: the role of caffeine. Clin Sci 2005;109:55–60.
14. Shechter M, Shalmon G, Scheinowitz M, Koren-Morag N, Feinberg MS, Harats D, Sela BA, Sharabi Y, Chouraqui P. Impact of acute caffeine ingestion on endothelial function in subjects with and without coronary artery disease. Am J Cardiol 2011;107: 1255–61.
15. Mubarak A, Bondonno CP, Liu AH, Considine MJ, Rich L, Mas E, Croft KD, Hodgson JM. Acute effects of chlorogenic acid on nitric oxide status, endothelial function, and blood pressure in healthy volunteers: a randomized trial. J Agric Food Chem 2012; 60:9130–6.
16. Freedman ND, Park Y, Abnet CC, Hollenbeck AR, Sinha R. Association of coffee drinking with total and cause-specific mortality. N Engl J Med 2012;366:1891–904.
17. Moura-Nunes N, Perrone D, Farah A, Donangelo CM. The increase in human plasma antioxidant capacity after acute coffee intake is not associated with endogenous non-enzymatic antioxidant components. Int J Food Sci Nutr 2009;60(Suppl 6):173–81.
18. Analysis of coffee and coffee products - Determination of chlorogenic acids content in roasted coffee and extracted coffee - HPLC method. DIN 10767:2014-07.
19. Coffee and coffee products -Determination of the caffeine content using high performance liquid chromatography (HPLC)-Reference method. ISO 20481:2008.
20. Silva JA, Borges N, Santos A, Alves A. Method validation for cafestol and kahweol quantification in coffee brews by HPLC-DAD. Food Anal Methods 2012;5:1404–10.
21. Lohman TJ, Roache AF, Martorell R. Anthropometric standardization reference manual. Champaign (IL): Human Kinetics Publishing House Books; 1992.
22. World Health Organization. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. World Health Organ Tech Rep Ser 2000;894:i–xii.
23. Routh JI, Shane NA, Arredondo EG, Paul WD. Determination of caffeine in serum and urine. Clin Chem 1969;15:661–8.
24. Renouf M, Guy PA, Marmet C, Fraering AL, Longet K, Moulin J, Enslen M, Barron D, Dionisi F, Cavin C, et al. Measurement of caffeic and ferulic acid equivalents in plasma after coffee consumption: small intestine and colon are key sites for coffee metabolism. Mol Nutr Food Res 2010;54:760–6.
25. Benzie IF, Szeto YT. Total antioxidant capacity of teas by the ferric reducing/antioxidant power assay. J Agric Food Chem 1999;47: 633–6.
26. Tietz NW. 1995 Clinical guide to laboratory tests. Philadelphia: Saunders; 1995.
27. Bretón G, Gómez L, Muñoz A. Métodos de diagnóstico no invasivo de enfermedades vasculares. Bogotá. [Methods for noninvasive diagnosis of vascular diseases. Bogotá.] Guadalupe LTDA 2006;2006:403–4 (in Spanish).
28. Pickering TG, Hall JE, Appel LJ, Falkner BE, Graves J, Hill MN, Jones DW, Kurtz T, Sheps SG, Roccella EJ. Recommendations for blood pressure measurement in humans and experimental animals: part 1: blood pressure measurement in humans: a statement for professionals from the Subcommittee of Professional and Public Education of the American Heart Association Council on High Blood Pressure Research. Circulation 2005;111:697–716.
29. Romitelli F, Santini SA, Chierici E, Pitocco D, Tavazzi B, Amorini AM, Lazzarino G, Di Stasio E. Comparison of nitrite/nitrate concentration in human plasma and serum samples measured by the enzymatic batch Griess assay, ion-pairing HPLC and ion-trap GC-MS: the importance of a correct removal of proteins in the Griess assay. J Chromatogr B Analyt Technol Biomed Life Sci 2007;851:257–67.
30. Manach C, Williamson G, Morand C, Scalbert A, Rémésy C. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. Am J Clin Nutr 2005; 81(1, Suppl)230S–42S.
31. Monteiro M, Farah A, Perrone D, Trugo LC, Donangelo C. Chlorogenic acid compounds from coffee are differentially absorbed and metabolized in humans. J Nutr 2007;137:2196–201.
32. Renouf M, Marmet C, Giuffrida F, Lepage M, Barron D, Beaumont M, Williamson G, Dionisi F. Dose-response plasma appearance of coffee chlorogenic and phenolic acids in adults. Mol Nutr Food Res 2014;58:301–9.
33. Stalmach A, Williamson G, Crozier A. Impact of dose on the bioavailability of coffee chlorogenic acids in humans. Food Funct 2014;5: 1727–37.
34. Mnatsakanyan M, Goodie TA, Conlan XA, Francis PS, McDermott GP, Barnett NW, Shock D, Gritti F, Guiochon G, Shalliker RA. High performance liquid chromatography with two simultaneous on-line antioxidant assays: evaluation and comparison of espresso coffees. Talanta 2010;81:837–42.
35. Natella F, Nardini M, Giannetti I, Dattilo C, Scaccini C. Coffee drinking influences plasma antioxidant capacity in humans. J Agric Food Chem 2002;50:6211–6.
36. Mursu J, Voutilainen S, Nurmi T, Alfthan G, Virtanen JK, Rissanen TH, Happonen P, Nyyssönen K, Kaikkonen J, Salonen R, et al. The effects of coffee consumption on lipid peroxidation and plasma total homocysteine concentrations: a clinical trial. Free Radic Biol Med 2005;38:527–34.
37. Corrêa TA, Monteiro MP, Mendes TM, de Oliveira DM, Rogero MM, Benites CI, Vinagre CG, Mioto BM, Tarasoutchi D, Tuda VL, et al. Medium light and medium roast paper-filtered coffee increased antioxidant capacity in healthy volunteers: results of a randomized trial. Plant Foods Hum Nutr 2012;67:277–82.
38. Christensen B, Mosdol A, Retterstol L, Landaas S, Thelle DS. Abstention from filtered coffee reduces the concentrations of plasma homocysteine and serum cholesterol—a randomized controlled trial. Am J Clin Nutr 2001;74:302–7.
39. Urgert R, Meyboom S, Kuilman M, Rexwinkel H, Vissers MN, Klerk M, Katan MB. Comparison of effect of cafetière and filtered coffee on serum concentrations of liver aminotransferases and lipids: six month randomised controlled trial. BMJ 1996;313:1362–6.
40. Grubben MJ, Boers GH, Blom HJ, Broekhuizen R, de Jong R, van Rijt L, de Ruijter E, Swinkels DW, Nagengast FM, Katan MB. Unfiltered coffee increases plasma homocysteine concentrations in healthy volunteers: a randomized trial. Am J Clin Nutr 2000;71:480–4.
41. de Roos B, Katan MB. Possible mechanisms underlying the cholesterol-raising effect of the coffee diterpene cafestol. Curr Opin Lipidol 1999;10:41–5.
42. Yukawa GS, Mune M, Otani H, Tone Y, Liang XM, Iwahashi H, Sakamoto W. Effects of coffee consumption on oxidative susceptibility of low-density lipoproteins and serum lipid levels in humans. Biochemistry 2004;69:70–4.
43. Kempf K, Herder C, Erlund I, Kolb H, Martin S, Carstensen M, Koenig W, Sundvall J, Bidel S, Kuha S. Effects of coffee consumption on subclinical inflammation and other risk factors for type 2 diabetes: a clinical trial. Am J Clin Nutr 2010;91:950–7.
44. Fried RE, Levine DM, Kwiterovich PO, Diamond EL, Wilder LB, Moy TF, Pearson TA. The effect of filtered-coffee consumption on plasma lipid levels. Results of a randomized clinical trial. JAMA 1992;267:811–5.
45. Naidoo N, Chen C, Rebello SA, Speer K, Tai ES, Lee J, Buchmann S, Koelling-Speer I, van Dam RM. Cholesterol-raising diterpenes in types of coffee commonly consumed in Singapore, Indonesia and India and associations with blood lipids: a survey and cross sectional study. Nutr J 2011;10:48.

46. Cai L, Ma D, Zhang Y, Liu Z, Wang P. The effect of coffee consumption on serum lipids: a meta-analysis of randomized controlled trials. Eur J Clin Nutr 2012;66:872–7.
47. EFSA NDA Panel(EFSA Panel on Dietetic Products, nutrition and allergies). Scientific opinion on the safety of caffeine. EFSA Journal 2015; 13:4102.
48. Bondonno CP, Yang X, Croft KD, Considine MJ, Ward NC, Rich L, Puddey IB, Swinny E, Mubarak A, Hodgson JM. Flavonoid-rich apples and nitrate-rich spinach augment nitric oxide status and improve endothelial function in healthy men and women: A randomized controlled trial. Free Radic Biol Med 2012;52:95–102.
49. Zhao Y, Wang J, Ballevre O, Luo H, Zhang W. Antihypertensive effects and mechanisms of chlorogenic acids. Hypertens Res 2012; 35:370–4.
50. Agudelo G, Duque M, Velásquez C, Cardona O, Posada M, Pineda V, Orozco J, Suárez L, Echavarría E. Efecto del consumo de diferentes dosis de café filtrado sobre los niveles plasmáticos de homocisteína y presión arterial en un grupo de voluntarios sanos. [Effect of consumption of different doses of filtered coffee on plasma homocysteine levels and blood pressure in a group of healthy volunteers.] Rev Colomb Cardiol 2008;15:65–74 (in Spanish).
51. Noordzij M, Uiterwaal CS, Arends LR, Kok FJ, Grobbee DE, Geleijnse JM. Blood pressure response to chronic intake of coffee and caffeine: a meta-analysis of randomized controlled trials. J Hypertens 2005;23:921–8.
52. Zhang Z, Hu G, Caballero B, Appel L, Chen L. Habitual coffee consumption and risk of hypertension : a systematic review and meta-analysis of prospective observational studies. Am J Clin Nutr 2011;93: 1212–9.