European Journal of Clinical Nutrition (2010), 1–7

0954-3007/10 $32.00
www.nature.com/ejcn

ORIGINAL ARTICLE

Acute effects of coffee on endothelial function in healthy subjects

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Background/Objectives: Coffee is the most widely consumed beverage in the world, but its effect on the cardiovascular system has not been fully understood. Coffee contains caffeine and antioxidants, which may influence endothelial function, both of which have not yet been investigated. The objective of this study was to investigate the acute effects of coffee on endothelial function measured by brachial artery flow-mediated dilation (FMD).
Subjects/Methods: A total of 20 (10 males and 10 females) healthy non-obese subjects underwent a double-blind, crossover study. Subjects ingested one cup of caffeinated (CC) and one cup of decaffeinated (DC) Italian espresso coffee in random order at 5- to 7-day intervals.
Results: Following CC ingestion, FMD decreased progressively and significantly (mean ± s.e.m.: 0 min, 7.7 ± 0.6; 30 min, 6.3 ± 0.7; 60 min, 6.0 ± 0.8%; ANOVA (analysis of variance), $P<0.05$), but it did not significantly increase after DC ingestion (0 min, 6.9 ± 0.6; 30 min, 8.1 ± 0.9; 60 min, 8.5 ± 0.9%; $P=0.115$). Similarly, CC significantly increased both systolic and diastolic blood pressure; this effect was not observed after DC ingestion. Blood glucose concentrations remained unchanged after ingestion of both CC and DC, but insulin (0 min, 15.8 ± 0.9; 60 min, 15.0 ± 0.8 μU/ml; $P<0.05$) and C-peptide (0 min, 1.25 ± 0.09; 60 min, 1.18 ± 0.09 ng/ml; $P<0.01$) blood concentrations decreased significantly only after CC ingestion.
Conclusions: CC acutely induced unfavorable cardiovascular effects, especially on endothelial function. In the fasting state, insulin secretion is also likely reduced after CC ingestion. Future studies will determine whether CC has detrimental clinically relevant effects, especially in unhealthy subjects.
European Journal of Clinical Nutrition advance online publication, 3 February 2010; doi:10.1038/ejcn.2010.9

Keywords: coffee; endothelial function; FMD; insulin

Introduction

Coffee, a major source of caffeine (Frary *et al*., 2005), is the most widely consumed beverage in the world. It is manufactured under various formulations and preparations. Although not all components of coffee have been fully identified, some phenolic components, such as those of the family of chlorogenic acids, are well known to be abundant in coffee and possess high antioxidant capacity (Fujioka and Shibamoto, 2008). Information with regard to the metabolic and cardiovascular effects of coffee has been conflicting (Greenberg *et al*., 2006; Bonita *et al*., 2007; Klatsky *et al*., 2008). Short-term studies on the acute effects of coffee intake have generally reported detrimental cardiovascular and metabolic influences (Mahmud and Feely, 2001; Moisey *et al*., 2008; Riksen *et al*., 2009). In some instances,

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Contributors: SB contributed to the experimental design, performed the FMD measurements, interpreted data, drafted the manuscript and performed the statistical analysis. SV contributed to the experimental design, data interpretation and the writing of the manuscript. JAB contributed to the interpretation of the data, critical revision of the manuscript and final approval of the submitted manuscript. MD supervised the hormonal and other laboratory blood measurements and contributed to data interpretation. MRT, SB, AM and GP recruited participants and performed data collection and analysis. GC contributed to the experimental design, data interpretation, writing of the manuscript and trial coordination and had overall responsibility for the study.
Received 3 August 2009; revised 22 November 2009; accepted 11 December 2009

epidemiological studies have indicated that regular consumption of coffee is associated with lower risk of cardiovascular disease and type 2 diabetes (Silletta *et al*., 2007; Larsson *et al*., 2008; Odegaard *et al*., 2008; van Woudenbergh *et al*., 2008; Van Dam and Hu, 2008). A possible explanation for this 'coffee paradox' is related to both the caffeine and the antioxidant content in coffee, as the latter may be efficacious in the long term, whereas the former may have more immediate effects. Therefore, understanding the potential biological effects of coffee may have important public health implications.

Impaired endothelial function is involved in the pathogenesis of atherosclerosis and cardiovascular diseases, and is characterized by a reduction in the bioavailability of nitric oxide, a potent vasodilator and inhibitor of platelet adhesion and aggregation with antiinflammatory and antiproliferative properties (Fuchgott and Zawadzki, 1980; Deanfield *et al*., 2007). Endothelial function is measured *in vivo* by flow-mediated dilation (FMD) in the brachial artery, and has proven to be a strong predictor of cardiovascular events (Gokce *et al*., 2002; Widlansky *et al*., 2003; Yeboah *et al*., 2007; Rossi *et al*., 2008). FMD is influenced by many factors, including insulin resistance, diabetes, drugs and diet (Vogel *et al*., 2000; Wu and Meininger, 2002; Hamdy *et al*., 2003; Keogh *et al*., 2005; Shimabukuro *et al*., 2007). Inflammation and oxidative stress influence endothelial function and have a crucial role in atherogenesis (Libby *et al*., 2002). However, the data have been conflicting, showing that coffee consumption is either inversely (Lopez-Garcia *et al*., 2006) or positively (Zampelas *et al*., 2004) associated with C-reactive protein, interleukin-6 and tumor-necrosis factor-α.

Owing to the limited data on the effect of coffee on endothelial function, the aim of this study was to investigate the acute effects of caffeinated coffee (CC) vs decaffeinated coffee (DC), prepared as Italian espresso coffee, on FMD and some post-absorptive fasting measures of glucose metabolism in healthy subjects.

Subjects and methods

Subjects

A total of 20 non-obese healthy hospital employees voluntarily participated in the study after responding to an announcement in the medical center. There was no incentive provided to the participants. The study period was from November 2007 to February 2008. Inclusion criteria included ages 25–50 years and body mass index (body weight (kg)/height $(m)^2$) of 20–28 kg/m^2. Exclusion criteria included patients with any dyslipidemia, hypertension, diabetes, cardiovascular or systemic disease, any medication treatment, smoking of any tobacco products, pregnancy or lactation in the past 6 months, habitual daily consumption of greater than two cups of coffee or weekly ingestion of more than one commercial caffeinated beverage and abstaining from chocolate or other flavonoid-containing beverages up to the preceding day. The study protocol was approved by the ethics committee of the University Hospital Policlinico P. Giaccone of Palermo, Italy, and an approved informed consent form was signed by each subject. This study is registered as an International Standardized Randomized Controlled Trial (ISRCTN85096812).

Study design

The study followed a randomized, crossover, double-blind design with each subject receiving two different study treatments, in random order, and repeated on separate days at 5- to 7-day intervals. Anthropometric measurements, routine blood tests and an oral glucose (75 g) tolerance test were obtained in all subjects before participating in the study by MRT, SB, AM, AR and GP, who were blinded to study participant randomization. Subjects were tested in the morning after an overnight fast; women underwent measurements between the 7th and the 21st day from their menstrual cycle. FMD of the brachial artery was performed by the same operator (SB) who was blinded to the participant's mixture of coffee tested before, 30 min and 60 min after drinking a cup of Italian espresso CC or DC; ultrasound images were video recorded and analyzed by a trained reader who was blinded to the participant's mixture of coffee tested (SV). A venous blood sample was taken before and 60 min after coffee ingestion. Subjects had continuous electrocardiogram and blood pressure (10 min intervals) recorded for the duration of each test. Serum samples obtained at each time point of the study were frozen at $-80\,^{\circ}C$ for subsequent analysis.

Coffee testing

Fresh CC or DC was prepared using a commercial automatic machine (easy serving espresso; Italy) by a blinded study nurse. The blinding process involved a coffee envelope that was coded anonymously by the Morettino farm, only to be decoded by means of a code at the conclusion of the study. One cup of coffee consisted of 25 ml of espresso obtained with an average extraction time of 20 s from 7 g of a coffee mixture pressed in packet. Each packet of CC or DC contained a mixture of 65% Robusta (variety Canephora) and Arabica (A Morettino s.p.a., Palermo, Italy). The average caffeine content in 25 ml of CC and DC measured by chromatography–spectrophotometry (Chemical Laboratory, Camera di Commercio Industria Artigianato e Agricoltura, Trieste, Italy) was 130 mg and 5 mg, respectively. No addition of sugar or milk was permitted.

Measurements

Body composition and fat distribution. Fat mass (percentage body weight) was estimated as previously described (Verga *et al*., 1994) by means of bioelectrical impedance analysis (BIA-103, RJL, Detroit, MI, USA; Akern, Florence, Italy). Body

circumference was obtained at the umbilicus (waist circumference) and at the most prominent buttock level (hip circumference).

Assessment of endothelial function. Endothelium-dependent reactivity in the macrocirculation, measured by FMD of the brachial artery, was determined using high-resolution vascular ultrasound (Sonoline G50; Siemens, Erlangen, Germany) with a 10 MHz linear array transducer. The transducer was held at the same position throughout the test by a stereotactic clamp with micrometer adjustment (EDI Progetti e Sviluppo, Pisa, Italy) to ensure image consistency. Reactive hyperemia was produced by inflating a sphygmomanometer cuff 2 cm below the antecubital fossa to occlude the artery for 5 min at ~220–250 mm Hg and then deflating it. A video processing system computed the brachial artery diameter in real time by analyzing B-mode ultrasound images (FMD Studio, Institute of Physiology CNR, Pisa, Italy). Briefly, the device captures the analog video signal from the ultrasound equipment. An edge detection algorithm, based on the localization of gray level discontinuities, automatically locates the two walls of the vessel. The diameter is obtained with subpixel precision and temporal resolution of 25 sample(s). The brachial artery diameters were displayed on a graphical interface over a time scale of 9 min. Baseline vessel size was considered the mean of the measures obtained during the first minute. The FMD was calculated as the maximum percentage of increase of the brachial artery diameter over baseline. These procedures are described in detail elsewhere (Corretti *et al*., 2002; Barac *et al*., 2007; Deanfield *et al*., 2007; Buscemi *et al*., 2009a, b). The intra-observer coefficient of variation for FMD was 2.9% in our laboratory.

Laboratory analysis. Basal lipid measurements and uric acid were ascertained using common clinical chemistry methods (IL test cholesterol; IL test high-density lipoprotein cholesterol; IL test triglycerides; IL test uric acid; Instrumentation Laboratory, Milano, Italy). Low-density lipoprotein cholesterol concentration was calculated according to the Friedewald formula (Friedewald, 1972). Plasma glucose concentrations were measured using the glucose oxidase method (Instrumentation Laboratory). Blood concentrations of insulin were measured by radioimmunoassay (Insik-5, DiaSorin, Saluggia, Italy). Blood concentrations of C-peptide were measured by electrochemiluminescence (ECLIA; Roche Diagnostics, Monza, Italy). The homeostasis model assessment of insulin resistance was calculated according to Matthews *et al*. (1985).

Statistical analysis

All data are presented as means ± standard error of the means. Basal pairwise comparisons between the two treatments (CC vs DC) were tested for statistical significance using the paired Student's *t*-test. An overall 3×2 ANOVA (analysis of variance) for repeated measures was performed to evaluate the composite effect of the two different (CC and DC) ingested coffees over time (three periods: baseline, and 30 and 60 min) on the parameters of interest. ANOVA for repeated measures was also carried out separately to detect significant changes in variables over time within the two sessions; Bonferroni's *t*-test was performed for individual differences between two time points (paired) when appropriate. A two-tailed $P<0.05$ was considered significant. All analyses were performed using Systat (Windows version 11.0; San Jose, CA, USA).

Table 1 Characteristics of study participants

	Mean ± s.e.m.	*Range*
Sex (male/female)	10/10	
Age (years)	31 ± 2	25–49
Body weight (kg)	68.7 ± 3.0	51.3–92.6
BMI (kg/m^2)	23.9 ± 0.7	20.1–28.0
Waist circumference (cm)	83.1 ± 2.6	66–98
Hip circumference (cm)	91.6 ± 1.4	80–102
Blood glucose (mg per 100 ml)		
Basal	88 ± 2	74–107
2 h After glucose oral load	82 ± 4	59–119
Blood insulin (μU/ml)		
Basal	14.9 ± 1.4	8.0–20.0
2 h After glucose oral load	50.0 ± 8.4	22.0–86.0
Total cholesterol (mg per 100 ml)	179 ± 7	121–237
HDL-cholesterol (mg per 100 ml)	64 ± 6	22–103
Triglycerides (mg per 100 ml)	72 ± 5	38–132
Uric acid (mg per 100 ml)	4.8 ± 0.3	2.9–8.1
Blood pressure (mm Hg)		
Systolic	114 ± 3	90–130
Diastolic	73 ± 2	55–90
Heart rate (beats/min)	73 ± 3	55–92

Abbreviations: BMI, body mass index; HDL, high-density lipoprotein.

Results

The physical and clinical characteristics of subjects included in the study are reported in Table 1. The effects of CC and DC on FMD are reported in Table 2 and Figure 1. Despite no time × treatment effect being observed, both systolic and diastolic blood pressures were higher in the hour following ingestion in the CC group. In particular, the average increase in systolic blood pressure from baseline was 2.7% at both 30 min ($P<0.05$) and 60 min ($P<0.05$) after CC ingestion; similarly, diastolic blood pressure increased from baseline at an average of 5.9% at both 30 min ($P<0.05$) and 60 min ($P<0.05$) after CC. The FMD significantly decreased from baseline until reaching an average maximum of 22.1% at 60 min ($P<0.05$) after CC ingestion. Both systolic and diastolic blood pressure and FMD did not significantly

Table 2 Changes in vital signs and in brachial artery flow-mediated dilation after ingestion of caffeinated or decaffeinated espresso coffee[a]

	Coffee		P-*value*[b]	
	Caffeinated (N = *20*)	*Decaffeinated* (N = *20*)	*Time*	*Time* × *treatment*
Systolic blood pressure (mm Hg)				
Basal	113 ± 2	112 ± 2		
30 min	116 ± 2*	111 ± 2	0.75	0.22
60 min	116 ± 2*	111 ± 2		
P-value[c]	0.003	0.22		
Diastolic blood pressure (mm Hg)				
Basal	68 ± 2	66 ± 2		
30 min	72 ± 2*	68 ± 2	0.30	0.27
60 min	72 ± 2*	67 ± 2		
P-value[c]	0.001	0.07		
Heart rate (beats/min)				
Basal	68 ± 2	69 ± 2		
30 min	69 ± 2	69 ± 1	<0.01	0.63
60 min	67 ± 2	68 ± 2		
P-value[c]	0.27	0.08		
Flow-mediated dilation (%)				
Basal	7.7 ± 0.6	6.9 ± 0.6		
30 min	6.3 ± 0.7	8.1 ± 0.9	0.98	<0.005
60 min	6.0 ± 0.8*	8.5 ± 0.9		
P-value[c]	0.04	0.12		

Abbreviation: ANOVA, analysis of variance.
[a]All values are mean ± s.e.m.
[b]3 × 2 ANOVA for repeated measures.
[c]*P*-value compares within group values between basal, 30 min and 60 min for each variable within each group.
Paired *t*-test: *$P <$ 0.05 vs both decaffeinated coffee and basal value.

change after DC. Table 3 also reports the changes in metabolic variables following coffee ingestion. There were no differences in glucose concentration in either group, but reductions in insulin and C-peptide concentration and in the homeostasis model assessment of insulin resistance were observed in the CC group. DC patients had no change in any of their variables. No significant correlation was observed between changes in FMD and changes in parameters, including insulin and C-peptide concentrations, and systolic and diastolic blood pressure.

Discussion

This study shows that in non-obese healthy subjects, CC acutely induces significant endothelial dysfunction. Conversely, DC ingestion seems to improve the endothelial function, although the change in FMD approached but did not reach statistical significance. To our knowledge, this is the first study that has assessed the effects of espresso coffee on FMD in healthy subjects with a double-blind, crossover study design in an Italian cohort.

Our results parallel a study of 17 healthy subjects testing an instant CC or DC with randomized single-blind (operator) crossover design (Papamichael *et al*., 2005). Results showed a significant reduction in FMD after CC ingestion with a maximum at 60 min, but a nonsignificant reduction of FMD with DC ingestion, contrary to the trend that we observed. Although FMD was measured for 2 h, this may lead to

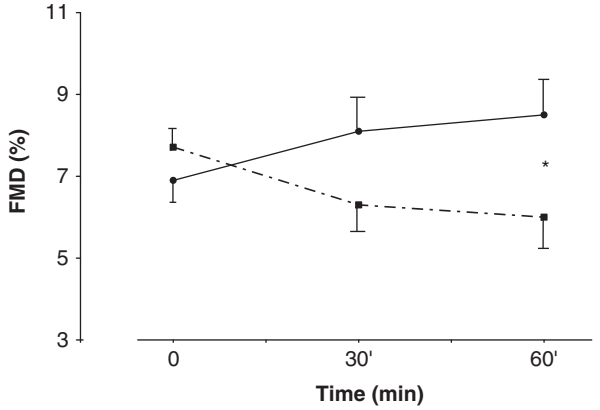

Figure 1 Brachial artery flow-mediated dilation (FMD) before (0 min) and after (30 min, 60 min) ingestion of one cup of caffeinated (dotted line) or decaffeinated (solid line) espresso coffee. Data are expressed as mean ± s.e.m. and are represented by vertical bars ($n = 20$). All data were analyzed by using a one-way ANOVA (analysis of variance) for repeated measures. The effect was significant ($P < 0.05$) for caffeinated coffee, with a significant difference observed at 60 min compared with 0 min ($P < 0.05$; Bonferroni's *t*-test). *$P < 0.05$ indicates significant difference between caffeinated coffee and decaffeinated coffee.

Table 3 Changes in metabolic variables after ingestion of espresso coffee[a]

	Coffee		P-*value*[b]
	Caffeinated	*Decaffeinated*	
Glucose (mg per 100 ml)			
Basal	85.5 ± 2.2	83.1 ± 1.3	0.30
60 min	82.2 ± 1.8	83.2 ± 1.4	0.56
P-value[c]	0.12	0.94	
Insulin (μU/ml)			
Basal	15.8 ± 0.9	14.4 ± 0.7	0.08
60 min	15.0 ± 0.8	15.2 ± 1.1	0.82
P-value[c]	0.047	0.41	
C-peptide (ng/ml)			
Basal	1.25 ± 0.09	1.21 ± 0.09	0.07
60 min	1.18 ± 0.09	1.13 ± 0.09	0.27
P-value[c]	0.007	0.12	
HOMA-IR			
Basal	3.37 ± 0.23	2.95 ± 0.16	0.47
60 min	3.06 ± 0.19	3.14 ± 0.25	0.80
P-value[c]	0.019	0.36	

Abbreviation: HOMA-IR, Homeostasis model assessment of insulin resistance.
[a]All values are mean ± s.e.m.
[b]Paired *t*-test: caffeinated vs decaffeinated.
[c]Paired *t*-test: basal vs 60 min.

excessive patient discomfort and potential harm due to persistent sphygmomanometer compression. Furthermore, the coffee preparation was not thoroughly standardized, thereby introducing bias into their results. By measuring caffeine content in a systematic manner, as we did in our own study, we feel justified in the accuracy and precision of our results.

We cannot exclude the possibility that the 22% reduction in FMD may be extended on the basis of the slope of the time course (Figure 1). Subjects were examined for a limited 1 h period to exclude confounders that may influence FMD, such as prolonged fasting, stress or artificial ischemic episodes imposed by frequent FMD measurements (Moens *et al*., 2005). As the reduction in FMD was not observed after DC ingestion, we can reasonably attribute the effects of CC on endothelial function to the presence of caffeine. In contrast with this hypothesis, two studies on tea consumption (Duffy *et al*., 2001; Alexopoulos *et al*., 2008) included a caffeine control group and showed that acutely administered caffeine had no effect on FMD. However, these studies differ slightly with our own study. In particular, Duffy *et al*. measured the FMD 2 h after caffeine ingestion, whereas we showed significant changes 1 h after coffee ingestion. The above study included participants with coronary artery disease, a group of patients expected to have lower FMD (Kitta *et al*., 2009), which may not have further been suppressed by caffeine. Finally, despite being withheld for 12–24 h beforehand, the interference of concurrent vasoactive medications cannot be excluded. In the study by Alexopoulos *et al*., FMD was measured at 30, 90 and 120 min, and not at 60 min after caffeine ingestion. However, 50% of studied subjects were smokers, and it was not specified whether they were habitual coffee consumers, a condition that, if present, may induce caffeine tolerance (Riksen *et al*., 2009). Other confounding factors may have influenced the Alexopoulos study results, as the presented basal average value of FMD before caffeine ingestion (4.35%) is generally considered low for healthy non-obese subjects (Kitta *et al*., 2009).

Antioxidants contained in coffee may also be responsible for the beneficial effects of DC ingestion on FMD. In fact, our group showed a dose-dependent favorable effect of DC on FMD (Buscemi *et al*., 2009a). Caffeic acid and chlorogenic acid are present in coffee, both of which have antioxidant properties (Daglia *et al*., 2000), which thereby may mitigate the detrimental effects of caffeine contained in CC on FMD. We cannot therefore exclude the fact that the same amount of caffeine might produce additional negative effects on endothelial function when ingested with other commercial beverage preparations different from coffee that do not contain antioxidants (Frary *et al*., 2005). Both animal (Suzuki *et al*., 2006) and human (Esposito *et al*., 2003) studies have shown that antioxidants improve endothelial function. In fact, antioxidants are also present in chocolate, which can be rich in flavanols (polyphenols) known to increase FMD and affect endothelial dysfunction (Grassi *et al*., 2008). However, in this study, no measurement was performed of serum concentrations of oxidative stress markers that would have drawn more specific conclusions concerning the role of oxidative stress in coffee-induced changes in endothelial function.

Our results support earlier evidence of the association between increased blood pressure and coffee (Whitsett *et al*., 1984; Casiglia *et al*., 1991) or caffeine intake (Casiglia *et al*., 1991; Nurminen *et al*., 1999), suggesting that CC may have acute unfavorable cardiovascular effects in healthy subjects. However, in a study by Corti *et al*. (2002), espresso DC increased systolic, but not diastolic, blood pressure in four subjects, 30 and 60 min after coffee ingestion, suggesting that components of coffee other than caffeine may be responsible for the pressor effect. The results of our study, and similarly those of the report on DC (Buscemi *et al*., 2009a), do not support the conclusions by Corti *et al*., which may be because of the small number of subjects studied or other methodological bias. For instance, they investigated the acute pressor effects of DC after ingestion of three cups of coffee. As these authors do not report the concentrations of caffeine in each cup of DC, we cannot exclude that a significant amount of this substance was consumed initially by the subjects studied. We cannot exclude the fact that ingestion of three nonsweetened cups of DC may activate, in nonhabitual coffee drinkers, the sympathetic nervous system independently from their content.

An interesting potential hypothesis is that the effects that we observed on FMD and blood pressure may be even more relevant in subjects who are genetically 'slow caffeine metabolizers,' as recently defined by Cornelis *et al*. (2006). In their cohort, the carrier frequency of this gene was 54%, and it may therefore act as a confounder that can explain the inconclusive results of epidemiological studies examining coffee consumption and cardiovascular risk (Klatsky *et al*., 2008). Further studies are needed to assess this hypothesis.

Our results suggest that coffee might influence glucose homeostasis. Although speculative, plasma glucose may remain unchanged in fasting conditions, despite decreases in insulin production, if peripheral insulin sensitivity increases and/or hepatic glucose output decreases. A previous study showed in 10 patients that CC impairs postprandial glucose and insulin sensitivity compared with DC (Moisey *et al*., 2008). On the contrary, CC impairs insulin secretion in the fasting state. These data imply that CC unfavorably affects glucose homeostasis, particularly in diabetic patients (Lane *et al*., 2008), subjecting patients with comorbidities to higher cardiovascular risk.

Despite the nature of our study design, we acknowledge the limitations of this pilot study. The decaffeinated group serves as a placebo group in this study, but it cannot entirely substitute for a caffeine group; therefore, our results can only be generalized to caffeine-containing coffee consumers. This volunteer cohort of hospital employees may be healthier than others. It is unclear whether the washout period of 5–7 days would be adequate in accurately measuring FMD.

However, owing to the crossover design, we would expect the differences to be similar in both groups. We acknowledge that we were grossly underpowered, but our results are meant to provide directions for future studies in examining the effect of caffeinated products on endothelial function. Whether or not acute effects indeed have an impact on cardiovascular events in the short- or long-term is unknown; however, our results can provide some preliminary data on the understanding of such events. This was a homogeneous population without known risk factors. In reality, patients with chronic diseases would be on medications, some of which have antiinflammatory effects (Buus *et al*., 2007; Mäki-Petäjä *et al*., 2007). Further studies need to be performed on such subjects to observe any possible coffee–disease interaction on endothelial function. Finally, the study results are limited to the effects of espresso coffee and not of other preparations, thereby limiting its external validity to other coffee or populations.

We believe that coffee may show unfavorable acute cardiovascular and metabolic effects with regard to endothelial function. The 'coffee paradox,' though, remains unresolved. Further studies need to investigate whether antioxidants contained in coffee can overcome caffeine's harmful effects to further our understanding of the conflicting results of the protective effects of coffee consumption on diabetes and cardiovascular disease.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This study was supported in part by the Italian Ministry of Education (ex 60% funds, 2007) and by the Associazione Onlus Nutrizione e Salute, Italy. The authors are gratefully indebted to the voluntary subjects who participated in the study and to Giovanna Seddio and Giovanni De Canzio for their invaluable technical support in the laboratory work.

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