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ORIGINAL ARTICLE

Dose-dependent effects of decaffeinated coffee on endothelial function in healthy subjects

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Background/Objectives: Coffee is known to contain antioxidant substances whose effects may be blunted because of caffeine that may unfavorably affect the cardiovascular system. This study was designed to investigate the acute dose-dependent effects of decaffeinated coffee (DC) on endothelial function measured by the brachial artery flow-mediated dilation (FMD).
Subjects/Methods: A total of 15 (8 men and 7 women) healthy nonobese subjects underwent a single-blind, crossover study. Subjects ingested one and two cups of decaffeinated Italian espresso coffee in random order at 5- to 7-day intervals.
Results: In the hour following the ingestion of two cups of DC, FMD increased (mean ± s.e.m.): 0 min, 7.4 ± 0.7%; 30 min, 8.0 ± 0.6%; 60 min, 10.8 ± 0.8%; $P<0.001$) as compared to consumption of one cup of DC (0 min, 6.9 ± 0.7%; 30 min, 8.4 ± 1.2%; 60 min, 8.5 ± 1.1%; 3 × 2 repeated-measures analysis of variance: $P=0.037$ for time × treatment effect). Blood pressure did not differ between groups, and basal heart rate was lower in the two-cup group at baseline and 60 min.
Conclusions: The present study demonstrated a significant acute favorable dose-dependent effect of decaffeinated espresso coffee on endothelial function. Further studies are needed to investigate the effects of chronic use of DC especially with respect to caffeinated coffee and in subjects with cardiovascular diseases.
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Introduction

The effect of coffee on cardiovascular metabolism and disease has been controversial (Bonita *et al*., 2007; Klatsky *et al*., 2008). Epidemiological investigations (Silletta *et al*., 2007; Larsson *et al*., 2008; van Woundenbergh *et al*., 2008) have generally indicated the protective effects of coffee on the development of cardiovascular diseases (CVD), however, short-term studies have shown otherwise (Mukamal *et al*., 2004; Zampelas *et al*., 2004). A possible explanation of these conflicting reports may be that coffee contains different compounds with opposing cardiovascular effects. Acutely, caffeine may induce unfavorable cardiovascular responses such as increased blood pressure and heart rate, but substances such as the chlorogenic acids that possess antioxidant properties may have long-term protective effects (Fujioka and Shibamoto, 2008). An impaired regulation between the balance of oxidant and antioxidant substances is characteristic of endothelial dysfunction that may be a key step in understanding the pathogenesis of atherosclerosis, hypertension and heart failure (Fuchgott and Zawadzki, 1980; Deanfield *et al*., 2007). Traditional cardiac risk factors induce endothelial dysfunction, defined by a reduced bioavailability of nitric oxide, either because of reduced production, increased inactivation or both (Fuchgott and Zawadzki, 1980; Deanfield *et al*., 2007). In this context, it is not well defined if dietary antioxidants may have a protective role on endothelial function and CVD.

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We recently investigated the acute effects of espresso coffee compared to decaffeinated coffee (DC) on endothelial function measured *in vivo* as flow-mediated dilation (FMD) of the brachial artery (submitted, *Eur J Clin Nutr*) to validate the only other study currently examining the comparative impact of caffeinated and DC on endothelial function (Papamichael *et al*., 2005). As DC ingestion led to a nonsignificant rise in brachial artery FMD, we hypothesized that antioxidant substances contained in either brand of coffee acutely improve endothelial function. In this study, we investigated the acute dose-dependent effects of DC on endothelial function following administration of decaffeinated espresso coffee in healthy subjects.

Materials and methods

Subjects
A total of 15 nonobese healthy hospital employees voluntarily participated in the study after responding to our medical center announcement. There was no incentive provided to the participants. The study period was from April 2008 to May 2008. Inclusion criteria included ages 25–50 years and body mass index (BMI; 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 more than 2 cups of coffee, weekly ingestion of more than one commercial caffeinated beverage, abstaining from chocolate or other flavonoid containing beverages the day preceding the measurements. 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 (ISRCTN41583899).

Study design
The study followed a randomized, crossover, single-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 carried out in all subjects before participating in the study by MRT, SB, RR, AR, and GP who were blinded to study participant randomization. Subjects were tested in the morning after an overnight fast. FMD of the brachial artery was performed by the same investigator (SB) at baseline, 30 and 60 min after drinking one or two cups of Italian espresso DC. Ultrasound images were video-recorded and analyzed by a trained reader who was blinded to the participant's amount of coffee tested (SV). Subjects had a continuous electrocardiogram, systolic and diastolic blood pressures (10 min intervals) recorded for the duration of each test. Pulse pressure was calculated as the difference between peripheral systolic and diastolic blood pressure.

Biochemical measurements
Venous plasma glucose concentrations were measured before and at 60 min after DC ingestion using the glucose oxidase method (Instrumentation Laboratory, Milano, Italy).

Basal lipid measurements and uric acid were ascertained using common clinical chemistry methods (IL test CHOL; IL test HDL-CHOL; IL test triglycerides; IL test uric acid; Instrumentation Laboratory). Low-density lipoprotein (LDL) cholesterol concentration was calculated according to the Friedewald formula (Friedewald, 1972).

Coffee testing
Fresh DC was prepared using a commercial automatic machine (Easy Serving Espresso, Palermo, Italy) by a study nurse. 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 DC contained a mixture of 65% Robusta (variety Canephora) and 35% Arabica (A Morettino SpA, Palermo, Italy). The average caffeine content in 25 ml of DC measured by chromatography and spectrophotometry (Chemical Laboratory, Camera di Commercio Industria Artigianato e Agricoltura, Trieste, Italy) was 5 mg. No addition of sugar or milk was permitted.

Measurements
Body circumference was obtained at the umbilicus (waist circumference) and at the most prominent buttock level (hip circumference). 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 approximately 220–250 mm Hg, 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 analogue 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 samples per second. 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 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*., 2009). The intra-observer coefficient of variation for FMD was 2.9% in our laboratory.

Statistical analysis

All data are presented as means ± standard error of means. Basal pair-wise comparisons between the two treatments (one cup vs two cups of DC) were tested for statistical significance using the paired Student's *t*-test. An overall 3×2 repeated-measures analysis of variance (ANOVA) was performed to evaluate the composite effect of the amount of DC ingested (one cup vs two cups of DC) over time (three periods: baseline, 30 and 60 min) on the parameters of interest. Repeated-measures ANOVA was also carried out to detect significant changes in variables over time within the two sessions separately; Bonferroni's *t*-test was performed for individual differences between two time points (paired) when appropriate. A two-tailed $P<0.05$ was considered statistically significant. An expected difference in FMD within each experiment group due to the amount of DC ingested was estimated to be 2.5% with a standard deviation of 2.0. The power analysis showed that with an α of 0.05 and a power of 0.80, 12 subjects were needed to be investigated. All analyses were performed using Systat (Windows, version 11.0; Systat Software Inc., San Jose, CA, USA).

Results

The physical and clinical characteristics of study participants are reported in Table 1. The effects of ingested DC on FMD and other measured variables are shown in Table 2 and Figure 1. Basal blood pressure (systolic and diastolic) did not differ between groups, systolic blood pressure significantly decreased after two cups of DC ($P<0.001$) whereas diastolic blood pressure increased after one cup of DC ($P<0.001$). Pulse pressure decreased significantly ($P=0.028$) after one cup of DC and not significantly after two cups of DC ($P=0.17$). Basal heart rate was lower in the two-cup group at baseline. No significant treatment effect was observed on systolic and diastolic blood pressure, pulse pressure and heart rate. Fasting plasma glucose did not change 1 h after ingestion of either one cup (basal: 83 ± 1 vs 60 min: 84 ± 2 mg/100 ml; $P=0.79$) or two cups (basal: 85 ± 2 vs 60 min: 83 ± 2 mg/100 ml; $P=0.21$) of DC.

Flow-mediated dilation was no higher following ingestion of one cup of DC at follow-up ($P=0.11$) but increased significantly in the two-cup group ($P<0.001$). FMD was significantly higher in the two-cup group than in the one-cup group at 60 min ($P=0.04$). In the two-cup group, the FMD at 60 min was significantly higher than at baseline ($P<0.000$) and at 30 min ($P<0.004$) (Figure 1). A significant treatment effect was observed on FMD (Table 2).

Table 1 Characteristics of study participants

	Mean ± s.e.m.	*Range*
Sex (male/female)	8/7	
Age (years)	29 ± 3	26–42
Body weight (kg)	70.0 ± 3.6	55.4–91.8
BMI (kg/m^2)	24.3 ± 0.9	20.4–27.8
Waist circumference (cm)	85 ± 3	67–102
Hip circumference (cm)	93 ± 2	82–103
Blood glucose (mg/100 ml)		
Basal	84 ± 3	71–101
2 h post-glucose oral load	85 ± 5	78–127
Total cholesterol (mg/100 ml)	175 ± 9	134–216
HDL cholesterol (mg/100 ml)	57 ± 5	32–86
LDL cholesterol (mg/100 ml)	103 ± 6	65–142
Triglycerides (mg/100 ml)	75 ± 8	54–141
Uric acid (mg/100 ml)	4.4 ± 0.5	3.1–6.4
Blood pressure (mm Hg)		
Systolic	111 ± 4	95–125
Diastolic	72 ± 3	60–85
Heart rate (beats/min)	71 ± 3	54–96

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

Discussion

The present study demonstrated a significant favorable dose-dependent effect of consuming decaffeinated espresso coffee on endothelial function. Substances contained in coffee, presumably the chlorogenic acids that have antioxidant effects, exert a positive action on the endothelium, which is likely favorable in further enhancing our understanding of the underlying abnormalities of endothelial dysregulation in cardiovascular and metabolic diseases. Our results suggest that DC may acutely improve endothelial function in all likelihood due to such antioxidants.

Brachial artery FMD is a reference method for *in vivo* measurements of endothelial function in humans and has been shown to predict cardiovascular events (Gokce *et al*., 2002; Widlansky *et al*., 2003; Yeboah *et al*., 2007; Rossi *et al*., 2008). Endothelial function following ingestion of two cups of DC demonstrated a progressive increase of 45% above baseline values. Interestingly, there was a nonsignificant, yet profound 23% mean increase in FMD after the ingestion of only one cup of DC at 1 h. Whether this effect reached a plateau is unknown but potentially this relationship may have more amplitude or be ascertained if measured continuously or past the 1 h mark. Following the ingestion of two cups of DC the FMD reached the peak value at 1 h being not significantly changed at 30 min of FMD. Although frequent measurements of FMD may lead to excessive patient discomfort, future studies that aim to

Table 2 Changes in vital signs and in brachial artery FMD following ingestion of different amounts of decaffeinated espresso coffee

	Decaffeinated coffee			
	One cup (25 ml)	*Two cups (50 ml)*	P-*value*[a]	
	N = *15*	N = *15*	*Time*	*Time* × *Treatment*
Systolic blood pressure (mm Hg)			0.10	0.68
Basal	112 ± 2	112 ± 2		
30 min	111 ± 2	110 ± 2		
60 min	111 ± 2	111 ± 2		
Diastolic blood pressure (mm Hg)			0.18	0.15
Basal	66 ± 2	68 ± 2		
30 min	67 ± 2	68 ± 2		
60 min	67 ± 2	67 ± 2		
Pulse pressure (mm Hg)			0.005	0.76
Basal	45 ± 3	44 ± 2		
30 min	43 ± 2	42 ± 2		
60 min	44 ± 2	43 ± 2		
Heart rate (beats/min)			0.009	0.31
Basal	69 ± 2	66 ± 2*		
30 min	69 ± 1	68 ± 2		
60 min	68 ± 2	65 ± 2		
Flow-mediated dilation (%)			0.000	0.037
Basal	6.9 ± 0.7	7.4 ± 0.7		
30 min	8.4 ± 1.2	8.0 ± 0.6		
60 min	8.5 ± 1.1	10.8 ± 0.8		

All values are mean ± s.e.m.
*Student's paired *t*-test: $P<0.05$ vs one-cup treatment.
[a]3 × 2 repeated-measures ANOVA.

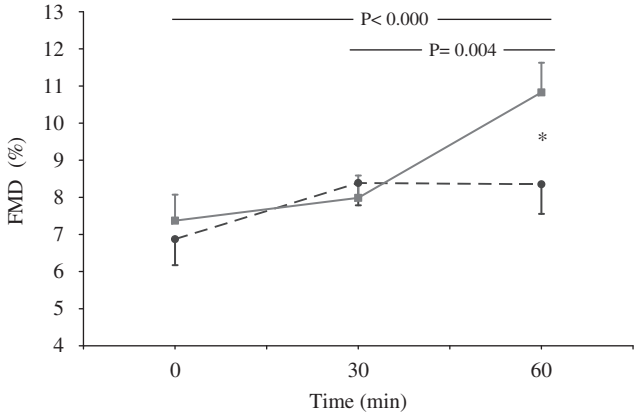

Figure 1 Brachial artery flow-mediated dilation (FMD) before (0 min) and after (30 and 60 min) ingestion of one (dotted line) or two (solid line) cups of decaffeinated espresso coffee. Data are expressed as mean ± s.e.m. represented by vertical bars ($n=15$). The effect was significant ($P< 0.001$, one-way ANOVA) for two cups of decaffeinated coffee with a significant difference observed at 60 min compared with 0 min ($P<0.000$) and 30 min ($P=0.004$; Bonferroni's *t*-test). *$P<0.05$ significant difference between one and two cups of decaffeinated coffee.

investigate the time duration of increased FMD may extend measurements at 2 and 3 h avoiding those at 30 min. Papamichael *et al*. (2005) were the first to examine the effect of both caffeinated and decaffeinated instant coffees, not expresso as in ours, on endothelial function using similar techniques but in fact measured FMD up to 120 min. However, they did not demonstrate any changes in FMD in the decaffeinated group, nor did they examine the dose-dependent effects of multiple cups of DC. In addition, their study population may have been impacted by certain confounding variables as participants were abstaining from caffeinated products for 12–24 h before participation, which may question the validity of their results. A small number of other studies have examined similar proposing hypotheses but their study design and outcomes differed than ours (Karatzis *et al*., 2005; Umemura *et al*., 2006). Study participants in one study abstained from nicotine for only 24 h (Umemura *et al*., 2006), a compound known to affect endothelial function despite smoking cessation. Both Karatzis and Vlachopoulos *et al*. (2005) examined the impact of caffeine on arterial wave forms but not specifically on endothelial function. Karatzis did not observe any effect on their augmentation index or waveforms in the decaffeinated groups. Finally, Mahmud and Feely (2001) demonstrate in only seven subjects the impact of both caffeinated and DC on arterial stiffness and not FMD. Such differences in study design and outcomes make comparisons to our own study rather difficult to make and none of these studies demonstrate dose-dependent effects of DC on FMD.

As participants in this study were healthy volunteers that were not habitual consumers of DC, we cannot therefore

exclude that tolerance mechanisms may mitigate the effects of DC on prolonged FMD. However, other foods and beverages that contain natural antioxidants seem to exclude the possibility that they have a reduced effect on FMD with chronic use. Kim *et al*. (2006) demonstrated that the consumption of 8 g per day (about eight cups) of green tea for 2 weeks improved FMD by 29%. Similarly, FMD was increased after flavanol-rich dark chocolate consumption (100 g per day) for 15 days (Grassi *et al*., 2008).

In general, only slight changes in blood pressure and heart rate were observed after DC ingestion, but this study does not show univocal conclusions. The ingestion of two cups of DC induced a significant reduction of systolic blood pressure and heart rate, especially at 1 h in the latter case. However, diastolic blood pressure increased after the ingestion of one cup of DC and this remains a contradictory result without a clear interpretation. When pulse pressure variation was considered, we observed a slight reduction after DC ingestion with a significant time effect without any significant treatment effect. Because pulse pressure reflects arterial stiffening, the latter seems not to be influenced by the DC dose. The pulse pressure reduction might rather have been a consequence of a progressive reduction of adrenergic tone due to relaxation, as the significant reducing time effect on heart rate might suggest. Owing to both the small effect we observed on blood pressure and also due to our small sample size, we were insufficiently powered to demonstrate any statistically significant changes. Further studies on larger samples of subjects with hypertension may give a conclusive answer about the effects of DC on blood pressure and heart rate.

Despite the favorable acute effects of DC on FMD, and possibly to a limited extent on systolic blood pressure and heart rate, we cannot extrapolate our results to chronic DC consumption on patient health. Furthermore, we did not measure antioxidant concentrations during the study, nor were we able to obtain them from the manufacturer. As such, we can only hypothesize that this was the reason why FMD improved in a dose-dependent effect acutely. Whether certain antioxidants are responsible for such effects is unknown. In addition, whether this can be further generalized to all other antioxidant supplementations, who themselves may have possible safety and tolerability problems (Vivekananthan *et al*., 2003; Bjelakovic *et al*., 2004; Miller *et al*., 2004) that can have an impact on cardiovascular metabolism, is unclear. Clearly, interaction with other foods is highly possible. For instance, studies have demonstrated the antioxidant capacity of dark chocolate and green and black tea, all are also strongly reduced by milk (Serafini *et al*., 1996, 2003; Langley-Evans, 2000). By omitting the addition of milk, we possibly may have had a homogenous sample that could accurately lead to proper function. However, although the above statements are likely physiologically plausible, they are undoubtedly speculative and should not be considered causative.

Our study had two major limitations. We did not include a corresponding control or placebo group, to which we could compare the effects of DC. Ideally, this would allow us to have a prespecified end point or effect. Although we are unaware of any studies that indeed contrast these entities, we partially deal this with the reproducibility of FMD in each group. Alternatively, such a study was a natural follow-up to a previous study evaluating the differences on FMD between caffeinated coffee and DC (Buscemi *et al*., submitted, *Eur J Clin Nutr*).

This study is to the best of our knowledge the first to compare the acute dose-dependent effects of DC on FMD with a randomized, crossover, single-blind design. Owing to the different volumes of DC tested, it was not possible to design a double-blind study, however we feel that the knowledge of the amount of DC ingested by the study participants could not elicit or influence different FMD responses *per se*. Our study was meant to be an exploratory one and our results should be interpreted with caution. Larger sample sizes would be needed to ascertain fully this relationship on FMD.

Caffeinated and decaffeinated espresso coffee appear to have opposing effects on FMD. This may have possible health implications considering the number of persons consuming coffee worldwide. In particular, also DC might be included among those foods or beverages that are known to have antioxidant effect or a favorable influence on endothelial function. In patients with CVD, DC may in fact be a safer alternative than caffeinated coffee, although specific studies examining these relationships in this population would be needed. Further studies are needed to investigate the effects of chronic use of DC especially with respect to caffeinated coffee and in subjects with CVD.

Conflict of interest

The authors declare no conflict of interest.

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References

Barac A, Campia U, Panza JA (2007). Methods for evaluating endothelial function in humans. *Hypertension* **49**, 748–760.

Bjelakovic G, Nikolova D, Simonetti RG, Gluud C (2004). Antioxidant supplements for prevention of gastrointestinal cancers: a systematic review and meta-analysis. *Lancet* **364**, 1219–1228.

Bonita JS, Mandarano M, Shuta D, Vinson J (2007). Coffee and cardiovascular disease: *in vitro*, cellular, animal, and human studies. *Pharmacol Res* **55**, 187–198.

Buscemi S, Verga S, Tranchina MR, Cottone S, Cerasola G (2009). Effects of hypocaloric very-low-carbohydrate diet vs Mediterranean diet on endothelial function in obese women. *Eur J Clin Invest* **39**, 339–347.

Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA *et al*. (2002). Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol* **39**, 257–265.

Deanfield JE, Halcox JP, Rabelink TJ (2007). Endothelial function and dysfunction. Testing and clinical relevance. *Circulation* **115**, 1285–1295.

Friedewald WT (1972). Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* **18**, 499–502.

Fuchgott RF, Zawadzki JV (1980). The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* **288**, 373–376.

Fujioka K, Shibamoto T (2008). Chlorogenic acid and caffeine contents in various commercial brewed coffees. *Food Chem* **106**, 217–221.

Gokce N, Keaney JF, Hunter LM, Watkins MT, Menzoian JO, Vita JA (2002). Risk stratification for postoperative cardiovascular events via noninvasive assessment of endothelial function. *Circulation* **105**, 1567–1572.

Grassi D, Desideri G, Necozione S, Lippi C, Casale R, Properzi G *et al.* (2008). Blood pressure is reduced and insulin sensitivity increased in glucose-intolerant, hypertensive subjects after 15 days of consuming high-polyphenol dark chocolate. *J Nutr* **138**, 1671–1676.

Karatzis E, Papaioannou TG, Aznaouridis K, Karatzi K, Stamatelopoulos K, Zampelas K *et al*. (2005). Acute effects of caffeine on blood pressure and wave reflections in healthy subjects: should we consider monitoring central blood pressure? *Int J Cardiol* **98**, 425–430.

Kim W, Jeong MH, Cho SH, Yun JH, Chae HJ, Ahn YK *et al*. (2006). Effect of green tea consumption on endothelial function and circulating endothelial progenitor cells in chronic smokers. *Circ J* **70**, 1052–1057.

Klatsky AL, Koplik S, Kipp H, Friedman GD (2008). The confounded relation of coffee drinking to coronary artery disease. *Am J Cardiol* **101**, 825–827.

Langley-Evans SC (2000). Consumption of black tea elicits an increase in plasma antioxidant potential in humans. *Int J Food Sci Nutr* **51**, 309–315.

Larsson SC, Männistö S, Virtanen MJ, Kontto J, Albanes D, Virtamo J (2008). Coffee and tea consumption and risk of stroke subtypes in male smokers. *Stroke* **39**, 1681–1687.

Mahmud A, Feely J (2001). Acute effects of caffeine on arterial stiffness and aortic pressure waveform. *Hypertension* **38**, 227–231.

Miller ER, Pastor-Barriuso R, Dalal D, Riemersma RA, Appel L, Guallar E (2004). Meta-analysis: high-dosage vitamin E supplementation may increase all-cause mortality. *Ann Intern Med* **142**, 37–46.

Mukamal KJ, Maclure M, Muller JE, Sherwood JB, Mittleman MA (2004). Caffeinated coffee consumption and mortality after acute myocardial infarction. *Am Heart J* **147**, 999–1004.

Papamichael CM, Aznaouridis KA, Karatzis EN, Karatzi KN, Stamatelopoulos KS, Vamvakou G *et al*. (2005). Effect of coffee on endothelial function in healthy subjects: the role of caffeine. *Clin Sci* **109**, 55–60.

Rossi R, Nuzzo A, Origliani G, Modena MG (2008). Prognostic role of flow-mediated and cardiac risk factors in post-menopausal women. *J Am Coll Cardiol* **51**, 997–1002.

Serafini M, Bugianesi R, Maiani M, Valtuena S, De Santis S, Crozier A (2003). Plasma antioxidants from chocolate. *Nature* **424**, 1013.

Serafini M, Ghiselli A, Ferro-Luzzi A (1996). *In vivo* antioxidant effect of green and black tea in man. *Eur J Clin Nutr* **50**, 28–32.

Silletta MG, Marfisi RM, Levantesi G, Boccanelli A, Chieffo C, Franzosi M *et al*. (2007). Coffee consumption and risk of cardiovascular events after acute myocardial infarction. *Circulation* **116**, 2944–2951.

Umemura T, Ueda K, Nishioka K, Hidaka T, Takemoto H, Nakamura S *et al*. (2006). Effects of acute administration of caffeine on vascular function. *Am J Cardiol* **98**, 1538–1541.

van Woundenbergh GJ, Vliegenthart R, van Rooij FJ, Hofman A, Oudkerk M, Witteman JCM *et al*. (2008). Coffee consumption and coronary calcification: the Rotterdam Coronary Calcification Study. *Arterioscler Thromb Vasc Biol* **28**, 1018–1023.

Vivekananthan DP, Penn MS, Sapp SK, Hsu A, Topol EJ (2003). Use of antioxidant vitamins for the prevention of cardiovascular disease: meta-analysis of randomized trials. *Lancet* **361**, 2017–2023.

Vlachopoulos C, Panagiotakos D, Ioakeimidis N, Dima I, Stefanadis C (2005). Chronic coffee consumption has a detrimental effects on aortic stiffness and wave reflections. *Am J Clin Nutr* **81**, 1307–1312.

Widlansky ME, Gokce N, Keaney JF, Vita JF (2003). The clinical implications of endothelial function. *J Am Coll Cardiol* **42**, 1149–1160.

Yeboah J, Crouse JR, Hsu FC, Burke GL, Herrington DM (2007). Brachial flow-mediated dilation predicts incident cardiovascular events in older adults: the Cardiovascular Health Study. *Circulation* **115**, 2390–2397.

Zampelas A, Panagiotakos DB, Pitsavos C, Chrysohoou C, Stefanadis C (2004). Associations between coffee consumption and inflammatory markers in healthy persons: the ATTICA study. *Am J Clin Nutr* **80**, 862–867.