

## SHORT COMMUNICATION

# Coffee and endothelial function: a battle between caffeine and antioxidants?

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Although coffee is largely consumed by adults in Western countries, controversy exists about its impact on the cardiovascular system. We recently demonstrated that caffeinated and decaffeinated espresso coffee have different acute effects on endothelial function in healthy subjects, measured using flow-mediated dilation (FMD) of the brachial artery. In this study, we measured the anti-oxidant capacity of two coffee substances in terms of free stable radical 2,2-diphenyl-1-picryl-hydrazyl 50% inhibition ( $I_{50}$  DPPH). The caffeinated coffee had a slightly higher anti-oxidant capacity than decaffeinated espresso coffee ( $I_{50}$  DPPH:  $1.13 \pm 0.02$  vs  $1.30 \pm 0.03 \mu\text{l}$ ;  $P < 0.001$ ). We suggest that the unfavourable effects observed after caffeinated coffee ingestion are due to caffeine and that the antioxidant activity is responsible for the increased FMD observed after decaffeinated coffee ingestion. Further clinical and epidemiological studies are needed to understand the chronic effects of coffee consumption on health.

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### Introduction

Although coffee and caffeine are largely consumed by adults in Western countries, controversy exists about its effects on overall and cardiovascular health (Klatsky *et al.*, 2008). We recently demonstrated (Buscemi *et al.*, 2010) that endothelial function in healthy subjects, measured using flow-mediated dilation (FMD) of the brachial artery, is significantly lower in the hour following the ingestion of 25 ml of espresso coffee. Both systolic and diastolic blood pressure increased and, based on C-peptide serum concentrations, insulin secretion was likely reduced. On the contrary, there was a dose-dependent significant increase in FMD when one (25 ml) or two (50 ml) cups of decaffeinated coffee were ingested (Buscemi *et al.*, 2009) (Figure 1). The two mixtures of coffee used in these studies were similar, with the exception that one underwent a decaffeination procedure. We attributed these differences to the caffeine content that, acutely,

exceeded the favourable effects of other substances contained in coffee, as compared with our observations in the decaffeinated experiment. In order to further understand these processes, we subsequently measured the anti-oxidant capacity of the two coffee substances.

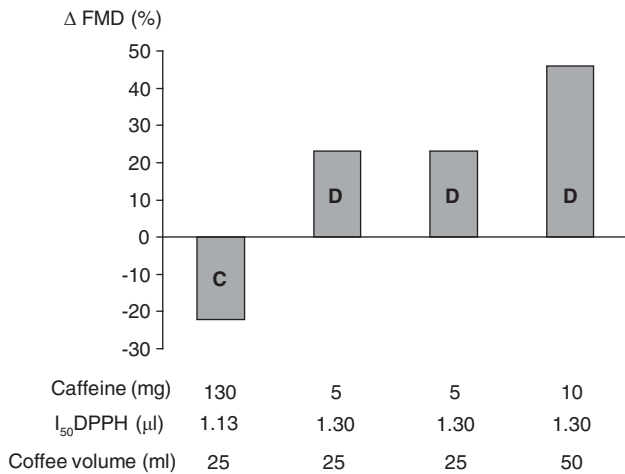
### Materials and methods

The free stable radical 2,2-diphenyl-1-picryl-hydrazyl (DPPH) was used to ascertain the radical scavenging activity of coffee samples. The method proposed by Brand Williams *et al.* (1995) was followed with minor modifications. Briefly, a 50- $\mu\text{l}$  sample diluted with ethyl alcohol was added to 3 ml of 0.004% DPPH ethanolic solution. After 150 min of incubation at 25 °C with continuous shaking in the dark, the absorbance was measured at 517 nm using a Beckman DU-640 spectrophotometer (Beckman Coulter, Milan, Italy). Free radical scavenging activity was measured as free radical DPPH inhibition percentage:  $I\% = (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 100$ , where  $A_{\text{control}}$  was the absorbance of the control reaction (50  $\mu\text{l}$  ethanol + 3 ml DPPH solution). The absorbance of the sample was read on the blank (50  $\mu\text{l}$  sample + 3 ml ethanol).

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**Figure 1** Mean percent change of brachial artery  $\Delta$ FMD in healthy subjects following the ingestion of different volumes of caffeinated (C) or decaffeinated (D) espresso coffee in two different experiments (Buscemi *et al.*, 2010, first two bars;  $P < 0.005$ ; Buscemi *et al.*, 2009, last two bars;  $P < 0.05$ ), relative caffeine content and anti-oxidant properties expressed in terms of free stable radical  $I_{50}$  DPPH.

Four sample dilutions were tested to obtain the inhibition percentage in the range 30–70%. The determinations were carried out in triplicate. Dose–response curves (sample amount vs  $I\%$ ) were created to calculate the original sample amount ( $\mu$ l) corresponding to 50% of inhibition ( $I_{50}$ ).

## Results

The  $I_{50}$  DPPH was shown to be  $1.13 \pm 0.02 \mu$ l for caffeinated coffee and  $1.30 \pm 0.03 \mu$ l for decaffeinated coffee (Student's unpaired  $t$ -test:  $P < 0.001$ ). Figure 1 summarizes our previously reported results regarding the effects of caffeinated (Buscemi *et al.*, 2010) and decaffeinated (Buscemi *et al.*, 2009) coffee on the brachial artery FMD with respect to the characteristics of the two tested espresso coffees.

## Discussion

This study indicates that both caffeinated and decaffeinated espresso coffee, which acutely influence FMD (Buscemi *et al.*, 2009, 2010), have significant anti-oxidant properties. This activity was approximately 15% higher in caffeinated coffee, probably because of a slight loss of antioxidant substances with decaffeination procedures (Parras *et al.*, 2007). We suggest that the unfavourable effects observed after caffeinated coffee ingestion are due to caffeine and that the antioxidant activity is responsible for the increased FMD

observed after decaffeinated coffee ingestion. This hypothesis is in agreement with a recent study (Namdar *et al.*, 2009) that demonstrated impaired exercise-induced myocardial blood flow after the ingestion of 200 mg of caffeine even in healthy control subjects. As we previously hypothesized, the detrimental effects of caffeine cannot be acutely blunted by the concomitant ingestion of other substances with antioxidant effects as those contained in the caffeinated coffee. However, even more unfavourable effects may be expected upon ingesting caffeine without antioxidant substances, as may be the case with some commercial drinks.

Interestingly, it is well accepted that red wine contains a source of antioxidants, and *in vitro*  $I_{50}$  DPPH values range approximately 3–15  $\mu$ l (Giovanelli, 2005; Cimino *et al.*, 2007). Espresso coffee appears to have even more than threefold higher anti-oxidant capacity, resulting in equivalent *in vitro* levels of 3 cups a day (75 ml) to at least 250  $\text{cm}^3$  of red wine. Therefore, even a moderate daily consumption of coffee may provide a significant supply of antioxidants. However, further clinical and epidemiological studies are needed to understand the chronic effects of coffee consumption on health.

## Conflict of interest

The authors declare no conflict of interest.

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