Effect of coffee on endothelial function in healthy subjects: the role of caffeine

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A B S T R A C T

Coffee is one of the most widely used pharmacologically active beverages. The present study was designed to evaluate the acute effect of coffee ingestion on endothelial function in healthy individuals, and the potential role of caffeine. We studied 17 healthy young adults (28.9 ± 3.0 years old; nine men), who were regular non-heavy coffee drinkers. The endothelial performance was estimated by endothelium-dependent FMD (flow-mediated dilatation) of the brachial artery before and 30, 60, 90 and 120 min after ingestion of a cup of caffeinated coffee (80 mg of caffeine) or the corresponding decaffeinated beverage (< 2 mg of caffeine) in two separate sessions, following a randomized single-blind cross-over design. There was no difference in baseline FMD values between the two sessions [7.78 compared with 7.07% after caffeinated and decaffeinated coffee respectively; $P = $ NS (not significant)]. Caffeinated coffee led to a decline of FMD (7.78, 2.86, 2.12, 4.44 and 4.57% at baseline, 30, 60, 90 and 120 min respectively; $P < 0.001$). This adverse effect was focused at 30 ($P = 0.004$) and 60 min ($P < 0.001$). No significant effect on FMD was found with the decaffeinated coffee session (7.07, 6.24, 5.21, 7.41 and 5.20%; $P = $ NS). The composite effect of the type of coffee consumed over time on FMD was significantly different ($P = 0.021$). In conclusion, coffee exerts an acute unfavourable effect on the endothelial function in healthy adults, lasting for at least 1 h after intake. This effect might be attributed to caffeine, given that decaffeinated coffee was not associated with any change in the endothelial performance.

INTRODUCTION

Coffee is one of the most widely used pharmacologically active beverages. Coffee contains not only caffeine, but also some additional substances such as carbohydrates, proteins, lipids, glycosides, minerals and other contaminants [1]. Although evidence concerning the impact of coffee and caffeine on cardiovascular risk is rather conflicting, recent data suggest that caffeine may have an unfavourable effect on vascular performance.

Epidemiological studies suggest that regular consumption of caffeine is associated with adverse cardiovascular outcomes [2–5], but this is not a consistent finding in all relevant studies [6,7]. On the other hand, there is extensive evidence that both acute and chronic use of caffeine at dietary doses may increase BP (blood pressure) [8–10]. Moreover, we [11] and others [12–14] have shown that caffeine may possibly have an unfavourable effect on arterial elastic properties.

Vascular endothelium is a metabolically active paracrine tissue with a pivotal role in the regulation of cardiovascular homoeostasis. Endothelial function has been identified as an independent predictor of cardiovascular morbidity and mortality [15,16].

Data regarding the effect of coffee and caffeine on endothelial function are limited [17]. In the present

Key words: caffeine, coffee, endothelial function, decaffeinated coffee, flow-mediated dilatation, nitric oxide.

Abbreviations: BP, blood pressure; FMD, flow-mediated dilatation; NID, nitrate-induced dilatation; NO, nitric oxide; NS, not significant.

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randomized placebo-controlled cross-over study, we sought to investigate whether caffeine affects endothelial function in apparently healthy individuals.

METHODS

Study population

We studied 17 healthy young adults (mean age, 28.9 years; range, 25–38; nine men), who were recruited among the personnel of our hospital. All volunteers were regular, but non-heavy, coffee consumers (they used to drink at least one, but no more than two cups, of coffee daily), non-smokers and had a body mass index $< 30\ kg/m^2$. None had a history of arterial hypertension, diabetes mellitus or hypercholesterolaemia, or was on vasoactive medications or vitamin supplementation. All female participants excluded use of birth-control pills and were examined during the follicular phase of menstrual cycle. Each study day was preceded by a 12–24 h period of abstinence from coffee, tea, cola, alcohol and flavonoid-containing beverages.

The study was carried out according to the Declaration of Helsinki and approved by the Local Scientific Committee. All subjects gave written informed consent before entering the study.

Study design: assessment of endothelial function

The study was carried out using a randomized single-blind (operator) placebo-controlled cross-over design. All participants were examined in the morning on two separate days in a quiet temperature-controlled room after an overnight fast. During the first study day, after a rest period of 15 min, endothelial function was estimated by the endothelium-dependent FMD (flow-mediated dilatation) of the right brachial artery. A high-resolution linear array ultrasonic transducer (128 XP; Acuson, Mountain View, CA, U.S.A.) was used.

The method to assess endothelial function by FMD of the brachial artery has been described in detail previously [18,19]. The subject was in supine position and the right brachial artery was imaged in the longitudinal plane above the antecubital fossa. In brief, two-dimensional and Doppler images of the right brachial artery for diameter and flow determination were initially recorded under resting conditions. A pulsed Doppler velocity signal was obtained from a sample volume placed in the middle part of the artery. A cuff placed on the forearm was inflated to suprasystolic levels (50 mmHg above systolic BP), and the arterial inflow was thus interrupted. After 4.5 min, the cuff was deflated leading to reactive hyperaemia. Brachial artery was continuously scanned from 30 s before to 90 s after cuff deflation. Hyperaemic time-averaged velocity was assessed by a Doppler signal obtained within the first 15 s after cuff deflation. All scans were recorded on S-VHS tape.

The volunteers were then randomized to either drink a cup of freshly brewed caffeine-containing instant coffee (80 mg of caffeine), followed by drinking 200 ml of water, or the corresponding decaffeinated beverage (< 2 mg caffeine), which was used as a control for caffeine, and 200 ml of water. Resting and hyperaemic arterial scans were obtained 30, 60, 90 and 120 min after intake as described previously [19]. At 10 min after the last cuff deflation, endothelium-independent NID (nitrate-induced dilatation) was estimated by measuring brachial artery diameter 4 min after delivering a single 0.4 mg dose of nitroglycerine spray sublingually.

After 2 days, all participants returned and FMD and NID were estimated after ingestion of the opposite kind of beverage, according to the same schedule. Recorded scans were analysed by two independent observers, who were unaware of the study design and status of coffee drinking. Brachial artery diameter was measured at end-diastole (peak of R wave of the surface ECG) by placing electronic calipers at the adventitia–media interface (M-line) of the anterior and posterior arterial walls. Hyperaemic artery diameter was measured 60 s after cuff deflation. Three cardiac cycles were analysed and measurements were averaged. Resting and hyperaemic brachial artery flow was calculated as the product of the respective time-averaged velocity with heart rate and vessel cross-sectional area. FMD was calculated according to the equation:

$$\text{FMD}\ (\%) = [(\text{hyperaemic diameter} - \text{resting diameter})/\text{resting diameter}] \times 100$$

The inter- and intra-observer variability for brachial diameter measurements in our laboratory is 0.1 ± 0.12 and 0.08 ± 0.19 mm respectively, and FMD variability measured on two different days was 1.1 ± 1 % (absolute value).

Systolic and diastolic BP were measured at the left brachial artery by using a mercury sphygmomanometer. Measurements were obtained 6–8 min before each brachial artery scan.

Statistical analysis

Data are expressed as means $\pm$ S.D. All variables were tested for homogeneity of variance and normal distribution using the Kolmogorov–Smirnov criterion before any statistical analysis was applied. Baseline parameters between the two sessions were compared using Student's *t* test for paired measures. In order to evaluate the composite effect of the type of coffee consumed over time on the parameters of interest, an overall 5×2 ANOVA for repeated measures was performed [five periods (baseline, 30, 60, 90 and 120 min) $\times$ two types of beverage (caffeinated versus decaffeinated coffee)]. ANOVA for repeated measures was also performed in order to detect significant changes in variables over time within the two sessions separately. When a significant interaction

Table 1 Cardiovascular parameters before and after drinking a cup of caffeine-containing (a) and decaffeinated (b) coffee

Values are means ± S.D.

(a)

		Time after drinking				
	Baseline	30 min	60 min	90 min	120 min	*P* value
Heart rate (beats/min)	70 ± 11	64 ± 9	65 ± 10	62 ± 8	64 ± 7	0.003
Systolic BP (mmHg)	107 ± 12	111 ± 12	110 ± 11	108 ± 11	108 ± 11	NS
Diastolic BP (mmHg)	72 ± 9	75 ± 11	76 ± 10	76 ± 9	75 ± 9	0.032
Resting vessel diameter (mm)	3.42 ± 0.17	3.51 ± 0.13	3.43 ± 0.19	3.40 ± 0.12	3.44 ± 0.17	NS
Flow at rest (ml/min)	74 ± 10	66 ± 11	82 ± 15	64 ± 12	88 ± 16	NS
Hyperaemia (%)	192 ± 31	253 ± 61	173 ± 44	153 ± 22	131 ± 52	NS

(b)

		Time after drinking				
	Baseline	30 min	60 min	90 min	120 min	*P* value
Heart rate (beats/min)	69 ± 12	69 ± 8	64 ± 6	62 ± 7	62 ± 9	0.001
Systolic BP (mmHg)	108 ± 11	108 ± 12	106 ± 16	106 ± 12	107 ± 11	NS
Diastolic BP (mmHg)	71 ± 9	71 ± 10	71 ± 11	71 ± 10	72 ± 9	NS
Resting vessel diameter (mm)	3.43 ± 0.73	3.43 ± 0.61	3.56 ± 0.72	3.44 ± 0.72	3.46 ± 0.64	NS
Flow at rest (ml/min)	91 ± 14	69 ± 12	98 ± 19	67 ± 9	85 ± 13	NS
Hyperaemia (%)	158 ± 30	218 ± 36	160 ± 33	128 ± 29	128 ± 44	NS

occurred, the location of pairwise differences was determined by using the Bonferroni post-hoc test. A P value < 0.05 was considered statistically significant.

RESULTS

Baseline characteristics

There were no differences in baseline systolic and diastolic BP, heart rate, resting brachial artery diameter, flow and the degree of reactive hyperaemia between the two sessions [P = NS (not significant)]. Likewise, baseline FMD values were similar (7.78 ± 5.08 compared with 7.07 ± 4.62 % for caffeinated and decaffeinated coffee sessions respectively; P = NS).

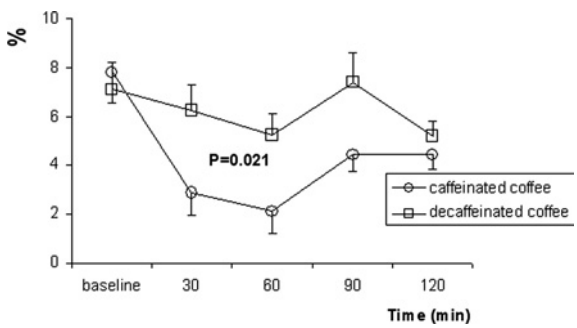

Figure 1 FMD of the brachial artery after intake of caffeinated or decaffeinated coffee

Error bars represent S.E.M.

Changes after regular or decaffeinated coffee

Table 1 shows the values of cardiovascular parameters and their interaction with time during the caffeinated and the decaffeinated coffee sessions.

Heart rate was significantly decreased with both caffeinated (by 7.5 beats/min at 90 min; Table 1a) and decaffeinated coffee (by 6.7 beats/min at 120 min; Table 1b), but the type of coffee × time interaction on heart rate was not significant. Systolic BP did not change throughout the study, whereas diastolic BP increased with caffeinated coffee (maximum at 90 min, by 3.9 mmHg; $P = 0.032$; Table 1). The effect of the two types of coffee on diastolic BP was significantly different ($P = 0.006$).

Caffeinated coffee intake led to a significant ($P < 0.001$) decline in FMD, and this adverse effect was focused at 30 and 60 min after ingestion ($P = 0.004$ and $P < 0.001$ respectively; Figure 1). On the other hand, FMD did not change after drinking decaffeinated coffee (Figure 1; P = NS). The overall 5 × 2 ANOVA for repeated measures yielded a significant time × type of coffee interaction on FMD ($P = 0.021$; Figure 1).

Moreover, all the other cardiovascular parameters tested over time within and between the two sessions, i.e. the resting diameter and blood flow of the brachial artery

and the percentage degree of reactive hyperaemia, did not exhibit any significant change. The NID values were also similar (19.88 ± 6.8 compared with 20.87 ± 7.4 % for caffeinated and decaffeinated coffee respectively; $P =$ NS).

DISCUSSION

To our knowledge, this is the first study to demonstrate that, in healthy subjects, caffeine-containing coffee has an acute unfavourable effect on endothelial function. On the other hand, decaffeinated coffee did not influence endothelial performance. These results highlight the role of caffeine as a mediator of this adverse effect of caffeinated coffee on endothelial function.

Coffee and endothelium: previous studies

Considerable data concerning the direct impact of coffee and caffeine on endothelial function are lacking. Interestingly, one study [17] reports that FMD does not change 2 h after a single 200 mg oral dose of caffeine. This finding does not disagree with our results, since we observed a decline in FMD which began within the first 30 min, peaked at 60 min and lasted no more than 90 min after coffee ingestion. Moreover, given the structural similarities between the endothelial and bronchial epithelial NO (nitric oxide) pathway, it is interesting that another study [20] showed a significant decrease in exhaled NO levels 1 h after intake of caffeine (taken either as coffee with an average caffeine content of 100 mg, or as a 200 mg caffeine capsule). Finally, it is interesting that some studies [11–13] have shown that caffeine may possibly have an acute unfavourable effect on arterial elastic properties in healthy subjects, given the evidence that increased arterial stiffness is associated with endothelial dysfunction [21].

Possible mechanisms for the effects of caffeine

Although the design of our present study does not allow us to suggest any definite mechanisms for the observed changes in endothelial function, it would be interesting to review the existing evidence concerning some effects of caffeine on vascular function.

The novel finding in our present study is that caffeine induces endothelial impairment acutely, which begins during the first 30 min, peaks at 60 min and lasts no more than 90 min after caffeine intake. This immediate effect of coffee could be the result of the inhibition of sGS (soluble guanylate cyclase) by caffeine [20,22], with subsequent suppression of the conversion of GTP into cGMP. cGMP serves as the second messenger of the L-arginine/NO system and, consequently, a decrease in cGMP levels could account for the impairment of NO-mediated effects.

Moreover, acute administration of caffeine may also decrease insulin sensitivity in healthy subjects. This latter action contributes to derangement of NO production and oxidative stress, possibly through uncoupling of the endothelial NO synthase [23,24].

Finally, there are some data suggesting a possible role of caffeine in augmenting vascular oxidant stress, because caffeine increases the production of angiotensin II by inhibiting the suppressing effect of adenosine A_1 receptors on the production of renin [25]. However, even though the inhibitory effect of caffeine is more potent at adenosine A_{2A} receptors (which exert direct vasodilatory effects mainly in ischaemic conditions and mediate the process of reactive hyperaemia) than A_1 receptors [26], we did not observe any change in the degree of reactive hyperaemia in our present study. Therefore we speculate that inhibition of adenosine receptors by caffeine seems to be irrelevant to our results, at least in the time frame of this present study and with the amount of coffee used.

Therefore we hypothesize that the decline in FMD probably represents a net endothelial impairment, given that the main cardiovascular parameters which could interfere with the evaluation of FMD, such as baseline brachial artery diameter and degree of peak reactive hyperaemia, remained unaltered throughout the whole study. However, we cannot rule out an interfering effect on our present results by central neurogenic mechanisms activated by caffeine. Moreover, NID did not change after caffeinated coffee consumption, but nitroglycerine testing was performed 130 min after coffee ingestion when the effects of caffeine seemed to have mostly vanished. Consequently, we cannot exclude an effect of caffeine on smooth muscle cell function.

Clinical implications

FMD comprises a well-established method to estimate endothelial performance. It requires an endothelium-dependent response which is mediated largely by NO [27] and is closely related to coronary endothelial function [28]. Endothelial dysfunction as quantified by FMD has been regarded as an important early event in atherogenesis in subjects with risk factors for atherosclerosis, which precedes the arterial plaque formation [18]. Moreover, impaired endothelial function is associated with increased cardiovascular risk [15,16].

As already mentioned, caffeine is one of the most widely consumed drugs worldwide, with a mean consumption per capita of approx. 200 mg/day in the form of coffee, tea or soft drinks [26]. Several studies have shown that coffee, mainly because of its caffeine content, may have adverse effects on cardiovascular function, such as increase in BP, arterial stiffness and wave reflections [8–14]. Nevertheless, existing evidence concerning the impact of caffeine intake on cardiovascular outcomes

remains conflicting [2–7]. Interestingly, consumption of coffee in quantities that decrease the risk of Type II diabetes [29,30] may have an unfavourable impact on cardiovascular outcomes [4,5]. Besides the complex and not yet fully understood mechanisms of the actions of caffeine, the effect of additives (sugar and milk) and the different ways of coffee preparation may possibly also account for these discrepancies. The vascular actions of caffeine observed in the present study could comprise a possible mechanism for the association of caffeine and coffee with impaired cardiovascular performance observed in some studies.

Specific comments

The present study refers to healthy young adults and further research is needed to investigate the endothelial effects of caffeine and coffee in other populations. These volunteers were studied in the morning, after a 12–24 h abstinence from caffeine, and consumed only one cup of coffee, mimicking the typical consumption pattern. In this context, studying the effects of purified caffeine could possibly provide more information about the contribution of caffeine to the observed effects of coffee.

Our results showed that heart rate may decrease after drinking either caffeinated or decaffeinated coffee, without any significant interaction between the two types of coffee, which was also observed in another study [14]. However, our present study cannot differentiate between a possible caffeine-independent effect of coffee, due to other ingredients, or an insufficient relaxation before measurements, although all studies were performed after our subjects had rested for at least 15 min in ideal room conditions. Finally, we observed an acute increase in diastolic BP after caffeinated coffee consumption, which is in line with other studies [31]. Even though there is some evidence that acute increases in BP may impair endothelial function [32], in our present study the observed increase in diastolic BP was small, albeit significant, and, moreover, the maximum increase in diastolic BP was observed at 90 min when the adverse effect of coffee on endothelial function had almost vanished. Inversely, the unfavourable impact of caffeinated coffee on endothelial performance might contribute, at least in part, to the pressor effect of coffee found in the present and several other studies.

Given that our participants used to drink one or two cups of caffeinated coffee a day, the present study cannot clarify whether the endothelial dysfunction that we observed is superimposed on an already malfunctioning endothelium because of chronic caffeine consumption, as we did not study any caffeine-naïve subjects. Neither could we discount that the continual periodic impairment of endothelial function by caffeine in chronic drinkers might lead to a more permanent detrimental effect upon the endothelial performance. Long-term studies are required to investigate the effect of chronic caffeine intake on the endothelium, as well as its impact on future cardiovascular risk.

In conclusion, our present study shows, for the first time, that coffee exerts an acute unfavourable effect on endothelial function of healthy individuals, mainly due to its caffeine content. The clinical implications and underlying mechanisms, as well as the long-term impact of this finding, need further elucidation. Studies that involve estimation of endothelial function should control for caffeine intake.

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