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Effect of caffeine contained in a cup of coffee on microvascular function in healthy subjects

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ABSTRACT

Recent epidemiological studies have demonstrated that coffee drinking is associated with reduced mortality of cardiovascular disease. However, its precise mechanisms remain to be clarified. In this study, we examined whether single ingestion of caffeine contained in a cup of coffee improves microvascular function in healthy subjects.

A double-blind, placebo-controlled, crossover study was performed in 27 healthy volunteers. A cup of either caffeinated or decaffeinated coffee was drunk by the subjects, and reactive hyperemia of finger blood flow was assessed by laser Doppler flowmetry. In an interval of more than 2 days, the same experimental protocol was repeated with another coffee in a crossover manner. Caffeinated coffee intake slightly but significantly elevated blood pressure and decreased finger blood flow as compared with decaffeinated coffee intake. There was no significant difference in heart rate between caffeinated and decaffeinated coffee intake. Importantly, caffeinated coffee intake significantly enhanced post-occlusive reactive hyperemia of finger blood flow, an index of microvascular endothelial function, compared with decaffeinated coffee intake.

These results provide the first evidence that caffeine contained in a cup of coffee enhances microvascular function in healthy individuals.

1. Introduction

Coffee is the most widely consumed beverage in the world (1). Coffee contains a variety of pharmacologically active ingredients, and it has long been argued whether coffee drinking is beneficial or harmful for cardiovascular disease (2–4). Recently, a large cohort study, in which more than 400,000 participants were prospectively followed up for 13 years, has demonstrated that coffee consumption is associated with reduced mortality of cardiovascular disease (5). Moreover, a meta-analysis of 23 prospective studies has provided quantitative evidence that coffee intake is inversely related to cardiovascular disease mortality (6). These findings suggest the beneficial cardiovascular actions of coffee. However, its precise mechanisms remain to be elucidated.

The vascular endothelium synthesizes and releases several vasodilating substances, such as prostacyclin, nitric oxide, and endothelium-derived hyperpolarizing factors (EDHF). Evaluation of endothelial function has been shown to provide important prognostic information in patients with cardiovascular disease, as evidenced by the facts that the severity of endothelial dysfunction can predict future cardiovascular events (7, 8) and that improvement of endothelial function by pharmacological interventions reduces the risk of cardiovascular disease. Acute effects of caffeine, a major

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pharmacologically active ingredient of coffee, on human endothelial function of large conduit arteries have been examined in several previous studies by using ultrasound-based measurement of brachial artery diameter during post-occlusive reactive hyperemia. However, the results of those studies are quite inconsistent (9–13). It is generally accepted that flow-dependent dilation of conduit arteries is mediated primarily by nitric oxide (14), while in the microcirculation EDHF rather than nitric oxide have been suggested to play a major role in the reactive hyperemic response (15). Microvessels, but not large arteries, regulate tissue blood blow and systemic blood pressure, and thereby play a key role in the circulatory system. However, no study has ever addressed the effect of caffeine on microvascular function.

Based on the above background, we examined in this study the effect of single ingestion of a cup of caffeinated and decaffeinated coffee on finger microvascular function in healthy subjects by laser Doppler flowmetry.

2. Methods

2.1. Subjects

We recruited twenty-seven healthy subjects (13 men and 14 women; 22–30 years old [mean age, 23.7 ± 2.2]; mean body weight, 58.4 ± 15.1 kg; mean height, 162.9 ± 9.6 cm) in our university, and the subjects who wanted to take part in the study voluntarily were investigated. Subjects taking any medication or smokers were excluded from the study, and the experiments were performed when the subjects were well conditioned. All volunteers were asked to abstain from caffeine-contained beverages at least 12 h before the study. All subjects gave written informed consent, and invasive experiments including blood sampling were approved by the Clinical Trial Ethics Committee of the University of the Ryukyus, according to the declaration of Helsinki and the ethical standard.

2.2. Study design

A double-blind, placebo-controlled, crossover study was performed. All participants were examined on two separate days in a quiet temperature-controlled room. Instant coffee of 2 g with or without caffeine (Taster's Choice™, Nestlé, Vevey, Switzerland) was prepared with 150 ml hot water. Neither sugar nor milk was added. A cup of the caffeinated or decaffeinated coffee was ingested in each subject. Hemodynamic variables and reactive hyperemic response were measured before and every 15 min after coffee intake. In a pilot study, we were not able to continue this experiment more than 75 min because some subjects complained of strong pain due to repeated cuff-compression or a fixed position of the test arm. Thus, we set the experiment time for 75 min. In an interval of more than 2 days, the same experimental protocol was repeated with another coffee in a crossover manner. Blood pressures were measured at the brachial artery using a sphygmomanometer (BP-103i, Nihon Colin, Komaki, Japan). A manchette was placed around the right upper arm, and a mean value of three measurements was used for the statistical analyses. Heart rate was obtained from the sphygmomanometer. The subjects were in a sitting position throughout the experiments.

2.3. Assessment of microvascular function

Finger blood flow was measured by a laser Doppler flowmeter (ALF21, Advance, Tokyo, Japan). A flow-probe (type C) was placed at the tip of the left index finger or thumb. Blood flow was calculated by measuring Doppler shifts derived from moving erythrocytes per photon and the mean photon frequency. As the number of Doppler shifts is proportional to the erythrocyte volume and velocity, blood flow is the product of linearized volume and velocity (16). Post-occlusive reactive hyperemia of finger blood flow was assessed as an index of microvascular endothelial function. A cuff was placed on the left upper arm, and reactive hyperemia of finger blood flow was induced by inflating a cuff for 1 min in order to interrupt arterial blood flow and then deflating it. Peak hyperemic flow was defined as the highest blood flow immediately after cuff deflation. Reactive hyperemia was calculated according to the following equation:

Reactive hyperemia (%) = [(peak hyperemic flow − resting flow)/resting flow] × 100

2.4. Measurement of caffeine and catecholamine levels

Venous blood samples were collected before and 30 min after coffee ingestion in five volunteers. The plasma caffeine levels and caffeine contents in decaffeinated and caffeinated coffee were analyzed by high performance liquid chromatography (HPLC; LC-10AD, Shimadzu, Kyoto, Japan) (17). Plasma catecholamine levels were measured by SRL Inc. (Tokyo, Japan) using the HPLC method.

2.5. Statistical analysis

Statistical analyses were performed by a two-way ANOVA followed by a Bonferoni/Dunn post hoc test. When paired or unpaired data were compared, a paired or unpaired Student's *t*-test, respectively, was applied. The computer software StatView-J 5.0 (SAS Institute Japan Ltd, Tokyo, Japan) was used for the statistical analyses. A value of $P < 0.05$ was considered to be statistically significant. Results are expressed as mean ± SD.

Reproducibility of laser Doppler flowmetry was expressed as within-subject coefficients of variability. In our laboratory, the intra-day variability for finger blood flow was 6.3% (range: 0–27.1%) and that for reactive hyperemia assessed by laser Doppler flowmetry was 21.6% (0–54.2%), and the day-to-day variability for finger blood flow was 26.2% (0–76.1%) and that for reactive hyperemia was 33.7% (0–102%). According to the previous studies, the coefficient of variance < 35% can be deemed acceptable (18).

3. Results

3.1. Caffeine content in decaffeinated and caffeinated coffee and plasma caffeine levels before and after coffee intake

Caffeine content in decaffeinated vs. caffeinated coffee was markedly different (1.37 ± 0.09 vs. 54.5 ± 3.4 mg, respectively) (Fig. 1A). Before coffee intake, plasma caffeine levels were identical between subjects with decaffeinated and caffeinated coffee intake. However, 30 min after coffee intake, plasma caffeine levels were markedly increased in the subjects with caffeinated coffee intake (from 0.75 ± 0.85 to 1.57 ± 1.30 μg/ml, $P < 0.05$), but not in those with decaffeinated coffee intake (from 0.76 ± 0.57 to 0.77 ± 0.60 μg/ml) (Fig. 1B).

3.2. Effects of caffeinated coffee intake on blood pressure and finger blood flow

Before coffee intake, there were no significant differences in baseline hemodynamic variables (i.e., systolic, diastolic, and mean blood pressures, finger blood flow, vascular resistance, or heart rate) in the subjects with decaffeinated and caffeinated coffee intake (Table 1). However, caffeinated coffee intake, but not

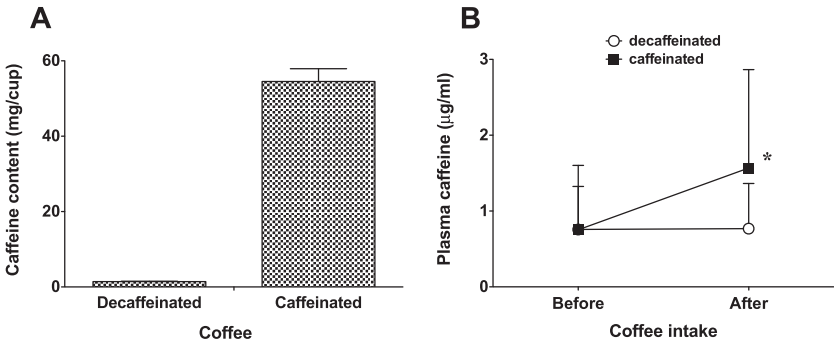

Fig. 1. Caffeine contents in a cup of decaffeinated and caffeinated coffee (A) and plasma caffeine levels before and 30 min after single intake of caffeinated and decaffeinated coffee (B). Data are expressed as mean ± SD. *$P < 0.05$ between before and after coffee intake by a paired *t*-test.

decaffeinated coffee intake, caused slight but significant elevations of systolic, diastolic and mean blood pressures by maximally 2.7, 3.2 and 2.8 mmHg, respectively (each $P < 0.01$, Fig. 2). Furthermore, caffeinated coffee intake significantly reduced finger blood flow (as assessed by laser-Doppler flowmetry, $P < 0.01$, Fig. 3A) and significantly increased vascular resistance of the finger vascular bed when compared with decaffeinated coffee intake ($P < 0.01$, Fig. 3B). On the other hand, there was no significant difference in heart rate in the subjects with decaffeinated and caffeinate coffee intake (Fig. 3C).

3.3. Effects of caffeinated coffee intake on reactive hyperemia of finger blood flow

Before coffee intake, post-occlusive reactive hyperemia of finger blood flow, an index of microvascular endothelial function, were comparable between the subjects with decaffeinated and caffeinated coffee (8.7 ± 4.3 and 10.0 ± 3.4 ml/min/100 g, respectively). However, caffeinated coffee intake significantly enhanced post-occlusive reactive hyperemia of finger blood as compared with decaffeinated coffee intake ($P < 0.01$, Fig. 4).

3.4. Plasma catecholamine levels

Plasma norepinephrine levels did not significantly differ between the subjects with decaffeinated and caffeinated coffee intake at baseline (336 ± 132 vs. 317 ± 165 pg/ml) and at 30 min after the intake (271 ± 95 vs. 272 ± 125 pg/ml). Plasma epinephrine levels also did not significantly alter between the subjects with decaffeinated and caffeinated coffee intake at baseline (35.8 ± 12.5 vs. 33.3 ± 18.5 pg/ml) and at 30 min after the intake (32.0 ± 11.2 vs. 25.8 ± 13.5 pg/ml). The respective plasma catecholamine levels did not significantly change before and after coffee intake.

Table 1
Baseline characteristics in subjects with decaffeinated and caffeinated coffee intake.

Variables	Decaffeinated	Caffeinated	*P* value
Systolic BP (mmHg)	104.9 ± 12.4	106.2 ± 11.2	0.346
Diastolic BP (mmHg)	58.0 ± 8.3	59.1 ± 6.6	0.297
Mean BP (mmHg)	73.6 ± 8.8	74.8 ± 7.6	0.264
Finger blood flow (ml/min/100 g)	23.6 ± 7.7	23.3 ± 7.9	0.916
Vascular resistance (unit)	3.43 ± 1.15	3.67 ± 1.63	0.543
Reactive hyperemia (%)	40.8 ± 25.4	50.3 ± 27.1	0.125
Heart rate (bpm)	74.6 ± 9.4	74.3 ± 8.6	0.815

BP = blood pressure, Vascular resistance = vascular resistance of the finger vascular bed (finger blood flow/mean BP), Reactive hyperemia (%) = 100 × (post-occlusive increase in finger blood flow)/(baseline finger blood flow).

4. Discussion

To the best of our knowledge, this is the first study examining the acute effect of caffeine on endothelial function in the human finger cutaneous microcirculation. The present study demonstrates that an intake of caffeine contained in a cup of coffee may cause a favorable effect on microvascular endothelial function assessed by a noninvasive laser Doppler flowmetry method in Japanese young healthy subjects.

4.1. Pressor effect of caffeine

In the present study, the plasma caffeine concentration after caffeinated coffee intake attained 1.6 μg/ml. This concentration of caffeine has been shown to act as an antagonist of adenosine A_1/A_{2A} receptors (19, 20). As adenosine causes vasodilation in most vascular beds (21), caffeine would induce an increase in vascular resistance. Thus, slight but significant rises in blood pressure observed after caffeinated coffee intake in the present study may, in part, be caused by an increase in basal vascular tone derived from the adenosine antagonism of caffeine, as found by an early study (22). In addition, a direct stimulatory effect of caffeine on myocardial contractility (23) might be involved in a significant increase in blood pressure seen after caffeinated coffee intake.

4.2. Effect of caffeine on microvascular function

The present finding that caffeine ingestion, even at a small dose (54.5 mg = less than 1 mg/kg), improves microvascular endothelial function is consistent with a previous study (24) using venous occlusion plethysmography demonstrating that the acute administration of caffeine at an extremely large dose (300 mg) augments vasodilator responses of forearm vessels to intra-arterial infusion of the endothelium-dependent agonist acetylcholine.

In contrast to our study, however, two previous reports using ultrasound-based measurement of brachial artery diameter during post-occlusive reactive hyperemia demonstrated that caffeinated coffee ingestion impaired endothelial function in healthy volunteers (9, 12). In addition, two other studies showed that acutely administered caffeine had no effect on endothelial function assessed by the brachial artery vasoreactivity measurement (10, 11). Although the reason for conflicting with our data cannot be fully explained at present, it seems plausible that the difference in the type of vessels used for assessing vascular function was mainly involved. Laser Doppler flowmetry employed in the present study measures microvascular function in cutaneous arterioles and capillaries, whereas the ultrasound-based measurement of brachial artery diameter reflects 'macrovascular' function in large conduit

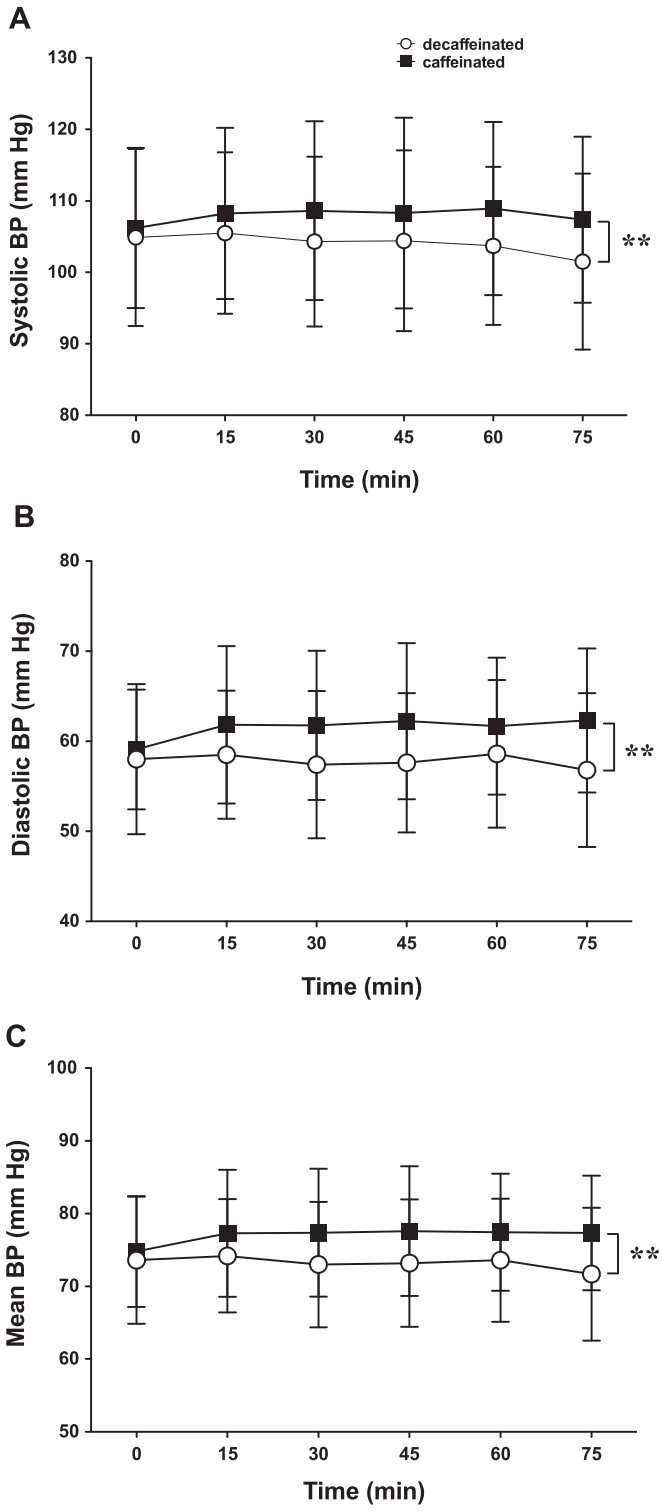

Fig. 2. Effects of caffeinated and decaffeinated coffee intake on systolic (A), diastolic (B) and mean (C) blood pressures (BP). Data are expressed as mean ± SD. $^{**}P < 0.01$ between caffeine (−) and caffeine (+) by ANOVA.

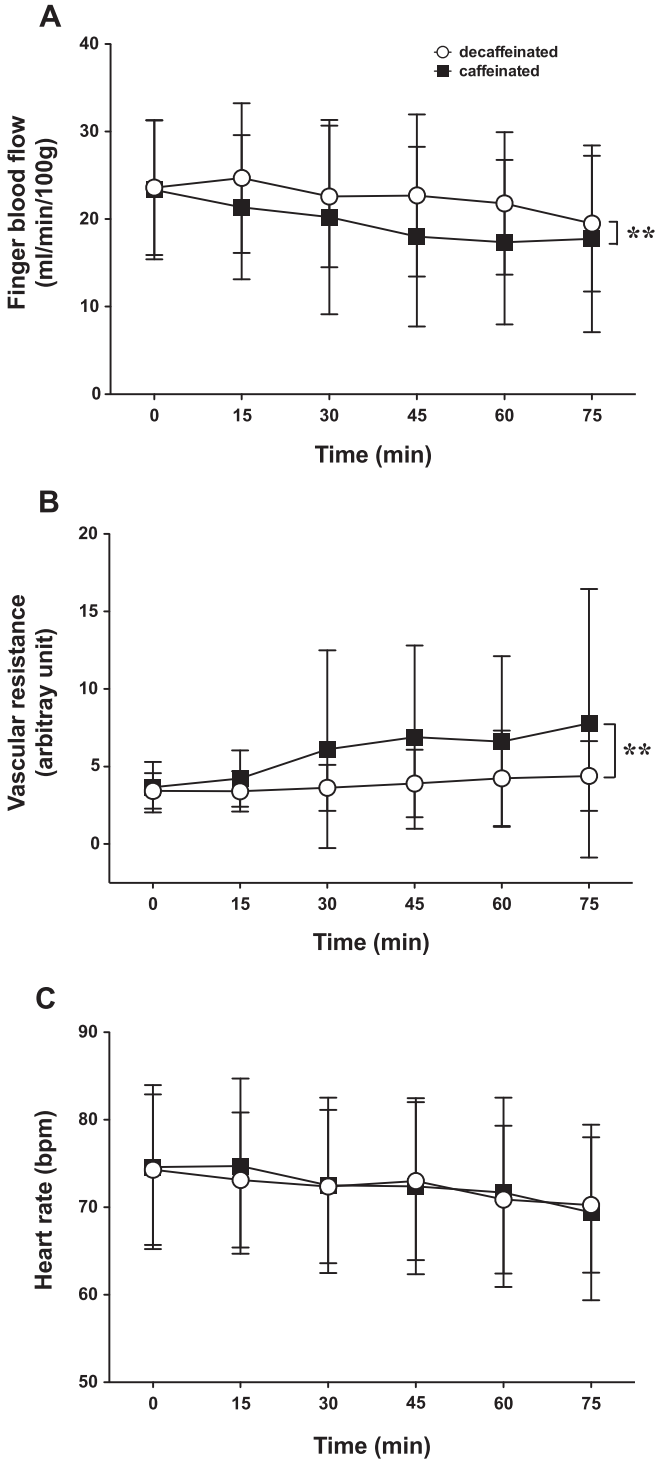

Fig. 3. Effects of caffeinated and decaffeinated coffee intake on finger blood flow (A), vascular resistance of the finger vascular bed (B), and heart rate (C). Data are expressed as mean ± SD. $^{**}P < 0.01$ between caffeine (−) and caffeine (+) by ANOVA.

arteries. Indeed, some previous studies have described that brachial artery responses to reactive hyperemia do not correlate with microvascular function as measured by agonist infusion studies or laser Doppler flowmetry (25, 26). It is generally considered that flow-dependent dilation of conduit arteries is mediated primarily by nitric oxide (14). By contrast, contribution of nitric oxide to post-occlusive reactive hyperemia in microvessels appears minimal (27, 28). Instead, EDHF may have a major role in the reactive hyperemic response in the microcirculation (15). Although the nature and mechanisms of EDHF remain uncertain, EDHF response has been proposed to be divided into two broad categories as follows: the first (classical) EDHF pathway is associated with endothelial cell hyperpolarization due to the opening of endothelial calcium-

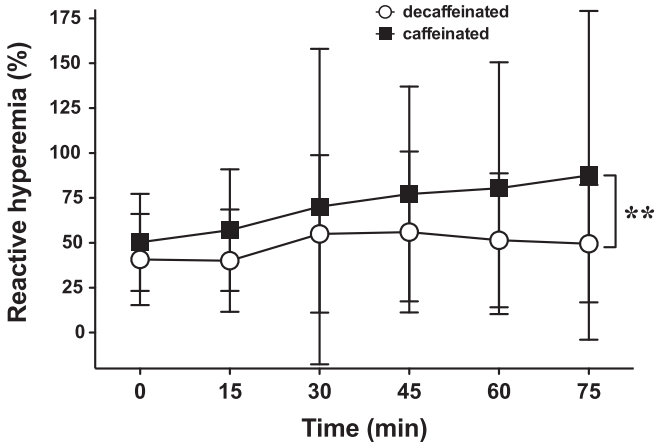

Fig. 4. Effect of caffeinated and decaffeinated coffee intake on post-occlusive reactive hyperemia of finger blood flow. Reactive hyperemia was calculated according to the following equation: reactive hyperemia (%) = [(peak hyperemic flow − resting flow)/resting flow] × 100. Data are expressed as mean ± SD. $^{**}P < 0.01$ between caffeine (−) and caffeine (+) by ANOVA.

activated K^+-channels, and the second EDHF pathway does not require endothelial hyperpolarization but involves the endothelial release of factors that hyperpolarize vascular smooth muscle cells by opening various myocyte K^+-channels such as calcium-activated K^+-channels (29). Experimental studies with animal and human vessels have demonstrated that the activation of vascular smooth muscle Ca^{2+}-activated K^+ channels probably contributes to the EDHF component of reactive hyperemia in microvessels (30, 31). Thus, microvascular endothelial function assessed by laser Doppler flowmetry may reflect the bioavailability of endothelium-dependent hyperpolarization via the activation of Ca^{2+}-activated K^+ channels in the endothelium and/or vascular smooth muscles.

4.3. Possible mechanisms involved in the beneficial effect of caffeine on microvascular function

In addition to the action on adenosine receptors, caffeine has been known to have a variety of pharmacological properties, including inhibition of phosphodiesterase (32), and calcium release from intracellular calcium stores via ryanodine-sensitive calcium channels (33). Interestingly, several electrophysiological experiments have displayed that caffeine at concentrations ranging from 10^{-6} to 10^{-3} M evokes calcium-dependent hyperpolarization in endothelial cells and vascular smooth muscle cells as a result of increased outward K^+ current (34–36). These data suggest that caffeine-induced release of calcium from intracellular calcium stores elicits the activation of calcium-activated K^+-channels in these cells. Considering that EDHF, unlike nitric oxide, has a major role in microvascular reactive hyperemia, it is possible that caffeine has the potential to augment the reactive hyperemic response of microvessels through amplifying hyperpolarization caused by EDHF. This may explain a favorable effect of caffeine on microvascular endothelial function in the present study, because the plasma concentration of caffeine was estimated to be nearly 10^{-5} M (Fig. 1B). It is intriguing that previous experiments in rats have shown that treatment with blockers of calcium-activated K^+-channels dose not affect baseline blood pressure or vascular conductance but attenuates vasodilator responses of resistance vessels produced by endothelium-dependent vasodilators such as acetylcholine (37, 38). These findings indicate that calcium-activated K^+-channels contribute little to the regulation of basal blood pressure but participate in responses to endothelial stimulation, and may be related to the present results that caffeine intake produced enhancement of microvascular endothelial function in spite of the occurrence of a slight increase in baseline blood pressure.

Several clinical studies (13, 39–41) have shown that caffeine exerts acute beneficial metabolic effects such as increased concentrations of adiponectin, a marker of anti-inflammatory and insulin-sensitizing effects (42). In addition, a cross-sectional study has reported that coffee consumption is inversely associated with a plasma marker of inflammation (C-reactive protein) and that of endothelial dysfunction (E-selectin) (43). Thus, these preferable properties of caffeine, besides the effect on endothelial function, may partly account for the beneficial cardiovascular effect of long-term coffee consumption.

4.4. Study limitations

Our study has some potential limitations to be considered. First, the number of subjects examined in this study may have been so small as to provide conclusive proof, although statistically significant effects were found. Second, the long-term effects of caffeine ingestion on endothelial function remain unknown. Third, we did not ask female subjects about the menstrual cycle, and it is thus unknown to what extent its phases affected the finger blood flow response. Finally, assessment of microvascular function was performed solely in Japanese healthy young volunteers. We have not yet elucidated whether or not caffeinated coffee intake ameliorates microvascular endothelial function not only in healthy subjects but also in patients with cardiovascular disease. These issues remain to be examined in future studies.

5. Conclusion

Our double-blind, placebo-controlled, crossover study has demonstrated, for the first time, that caffeine at the amount contained in a cup of coffee may cause improvement of microvascular endothelial function in healthy subjects.

Conflict of interest

None.

Acknowledgments

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