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Full paper

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