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Basic nutritional investigation

Freeze-dried instant coffee can promote the activities of antioxidant enzymes and induce weight loss but also aggravate the plasma cholesterol profile in rats

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ABSTRACT

Objective: This study investigated the effect of instant coffee on antioxidant enzyme activity and plasma cholesterol profile during exercise in rats.
Methods: Forty eight rats were fed a control diet with water (C) or a control diet with a coffee solution (CF). At the end of week 4, animals in each dietary group were subdivided into three exercise groups: before exercise (BE), during exercise (DE), and after exercise (AE). DE groups were exercised on a treadmill for 1 h immediately before being sacrificed. Animals in the AE groups were allowed to take a rest for 1 h after exercise. Antioxidant enzyme activities of the C and CF groups were evaluated with activities of catalase in plasma and superoxide dismutase, the ratio of reduced glutathione to oxidized glutathione, and the level of malondialdehyde in the liver. Plasma concentrations of triacylglycerol, total cholesterol, and high-density lipoprotein cholesterol were also compared.
Results: Final body weights and food intakes of the CF group were significantly lower than those of the C group. Catalase activities of the CF group were higher than those of the C group BE and AE. Reduced glutathione/oxidized glutathione of the CF group was significantly higher than that of the C group BE and DE. Superoxide dismutase activities of the CF group were higher than those of the C group regardless of exercise. Compared with the C group, there was an increase of total cholesterol and a decrease of high-density lipoprotein cholesterol levels in the CF group. Malondialdehyde levels in the CF group were higher than those in the C group BE and AE.
Conclusion: It is suggested that freeze-dried instant coffee can promote activities of antioxidant enzymes and induce weight loss but also aggravate the plasma cholesterol profile in rats.

Introduction

Despite the controversy for human health [1,2], coffee is one of the most widely consumed beverages in the world. Habitual coffee drinking has been reported to decrease the risk of mortality and chronic diseases including cancer, and to be an effective ergogenic and weight-loss aid [3,4]. These favorable health effects are supported by several plausible mechanisms because of the presence of different biological compounds such as caffeine, diterpenes, caffeic acid, polyphenols, and volatile aroma and heterocyclic substances [5]. However, many studies have also reported that coffee may increase blood cholesterol [6], serum homocysteine [7], and blood pressure [8], which can be important factors of increasing coronary heart disease risk. Some meta-analyses have observed statistically significant associations between coffee consumption and coronary heart disease for the highest-intake group (more than five cups/day), whereas other meta-analyses have concluded that moderate to high coffee consumption (three to six cups/day) is not significantly associated with an increased risk of coronary death or heart attack [9,10]. However, most of these results on the potential risk and benefit of coffee on human health have been from in vitro studies and there is little direct evidence that coffee intake modulates the body's antioxidant system or affects the blood lipid profile in vivo. These antioxidant systems during and after exercise are especially important for recuperation from exercise because strenuous exercise has been reported to induce an imbalance between free radical production and the body's antioxidant defense systems [11], and the contribution of free radical damage to the development of atherosclerosis has been established [12]. Therefore, the purpose of this study was to

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investigate the effect of coffee intake on antioxidative status and lipid profile under exercise-induced oxidative stress in vivo.

Materials and methods

Experimental diets

Forty eight male Sprague-Dawley rats (Daehanbiolink Co., Seoul, Korea) weighing 95 to 105 g were fed a control diet with water (C) or a control diet with a coffee solution (CF). The C diet was a vitamin-free casein-based semisynthetic diet that met AIN-93 recommendations. Coffee was fed as a solution of freeze-dried coffee (Daehanbiolink Co.) and the average daily amount of coffee intake per rat was 0.12 g of freeze-dried instant coffee/100 g of body weight. This concentration was based on the rationale that a 60-kg maximum human intake of coffee as a usual drink is 10 cups/d, with 7.2 g of freeze-dried instant coffee/cup, and does not exceed an estimated upper limit of caffeine (895 mg/d) based on reports that converted this limit to 0.12 g of freeze-dried instant coffee/100 g of body weight per day [13–16].

Exercise and sample collection

At the end of week 4, animals in each group were subdivided into three exercise groups: before exercise (BE), during exercise (DE), and after exercise (AE). The BE groups were sacrificed without exercise at the end of week 4. The DE groups were exercised on a treadmill (15° incline, 0.5–0.8 km/h) for 1 h. Animals in the AE groups were allowed to rest for 1 h AE. The exercise used in this study was anaerobic exercise to induce the maximal oxidative stress in vivo. This intensity and duration of exercise were based on the rationale that the maximal lactate steady-state workload, an important marker of endurance exercise capacity, of treadmill running has been reported to be 15 to 20 m/min for 21 to 25 min and correspond to 60% of maximal speed achieved during incremental exercise testing in mice and rats, and exhaustive exercise was defined as 25% higher than the maximal lactate steady-state workload [17,18]. Thus, the animals of this study were exercised on a treadmill (15° incline, 0.5–0.8 km/h) for 1 h to develop an anaerobic state. At the respective time points, animals were sacrificed by decapitation under light ether anesthesia. Immediately after decapitation, liver was rapidly removed and stored at −70°C until analyzed. Blood was collected in a heparinized tube and immediately centrifuged (3000 rpm, 20 min, 4°C) for the separation of plasma and erythrocytes.

Biochemical analysis

The activity of plasma catalase was determined with a commercial kit (Catalase-520, Bioxytech, Foster City, CA, USA). The activity of superoxide dismutase (SOD), the ratio of reduced glutathione to oxidized glutathione (GSH/GSSG), and levels of malondialdehyde (MDA) were determined in liver cytosol. Because tissues such as the liver, heart, and brain constituently express more antioxidant enzymes with a higher oxygen consumption rate, antioxidant supplementation produces a better oxidative status in these organs [19,20], and plasma SOD and MDA levels were not stable in the sample preparation methods adopted in this study, liver cytosol was prepared for SOD and MDA measurements.

Liver was homogenized in cold Tris-KCl buffer (0.1 M). The homogenized solution was centrifuged (8000 × *g*, 4°C, 30 min). The supernatant was then centrifuged (10 000 × *g*, 4°C, 30 min). This supernatant was ultracentrifuged (105 000 × *g*, 4°C, 90 min) and separated the cytosol. SOD activity was determined with a commercial kit (SOD-525, Bioxytech). The GSH/GSSG was determined with a commercial kit (GSH/GSSG-412, Bioxytech). Levels of MDA were determined with a commercial kit (MDA-586, Bioxytech). Plasma triacylglycerol (TG) and total cholesterol (TC) were analyzed with a commercial kit (Youngdong Phamaceutical Co., Seoul, Korea). High-density lipoprotein cholesterol (HDL-C) was analyzed with a commercial kit based on the same analytical method as TC after the precipitation of very low-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and chylomicron with polyethylene glycol.

Statistical analysis

All data were subjected to analysis of variance and tested for significant differences by Duncan's multiple-range test (SAS Institute, Cary, NC, USA) and $P < 0.05$ was considered statistically significant. The significance of differences between the C and CF groups was analyzed using a *t* test at $P < 0.05$.

Results

Table 1 lists the effects of coffee intake on body weight and food efficiency ratio. Final body weights and food intake of the CF group were significantly lower than of the C group, but the food efficiency ratio was significantly higher than that of the C group.

Table 1
Effect of coffee intake on BW and FER in rats*

	Group		*t* Test
	C	CF	
Initial BW (g)	70.00 ± 3.24	69.94 ± 3.24	NS†
Final BW (g)	310.23 ± 26.23	274.54 ± 24.60	
Food intake (g/d)	24.46 ± 2.72	19.45 ± 2.82	
FER	0.41 ± 0.04	0.44 ± 0.04	

BW, body weight; C, control diet; CF, coffee intake; FER, feed efficiency ratio
* Values presented as mean ± SD ($n = 8$).
† No significant difference between C group and CF group by *t* test ($P < 0.05$).

Oxidative status in the rats was evaluated using the activities of catalase and SOD and GSH/GSSG as direct measurements and the level of MDA as an indirect long-term measurement. Figures 1–4 show the effect of coffee intake on SOD activity. Coffee intake induced a significant increase in SOD activities. SOD activities in the CF groups were significantly higher than those of the C groups BE, DE, and AE. In the C and CF groups, SOD activities DE and AE were significantly higher than those BE. In the CF groups, exercise induced more significant increases of SOD activity and this increased activity was maintained AE. Catalase activities of the CF groups were significantly higher than those of the C groups BE and AE, but there were no significant differences between the C and CF groups DE. In the C groups, exercise induced a significant increase in catalase activity, and this increased activity returned to the BE level AE. However, in the CF groups, this increased activity was maintained AE. The GSH/GSSG of the CF groups was significantly higher than those of the C groups BE and DE, but there was no significant difference in GSH/GSSG between the C and CF groups AE. In the C and CF groups, no significant differences were observed in GSH/GSSG regardless of exercise. The MDA levels of the CF groups were significantly higher than those of the C groups BE and AE. However, DE there was no significant difference in MDA levels between the C and CF groups. In the C groups, there were no differences in MDA levels regardless of exercise, but in the CF groups, MDA levels DE were significantly lower than those BE and AE.

The differences in plasma TG and cholesterol profiles are presented in Table 2. There were no significant differences

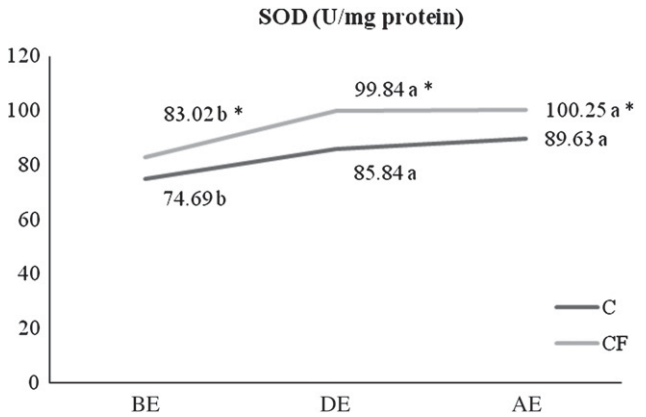

Fig. 1. The effect of coffee intake and exercise on SOD activity levels in rats determined by one-way analysis of variance and *t* test. Data are means. Letters in the same lines and asterisks in the same *x*-axis are significantly different at $P < 0.05$. AE, after exercise; BE, before exercise; C, control diet; CF, control diet plus coffee intake; DE, during exercise; SOD, superoxide dismutase.

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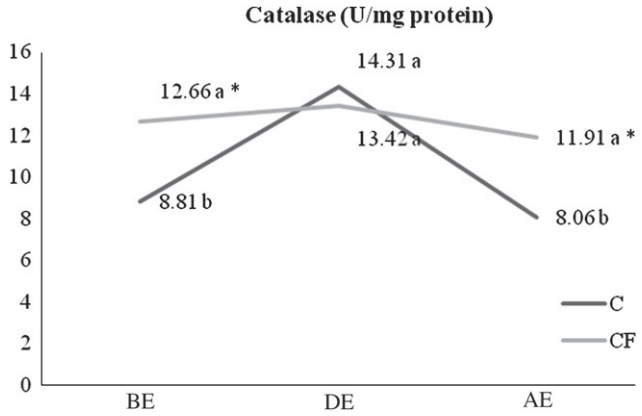

Fig. 2. The effect of coffee intake and exercise on CAT activity levels in rats determined by one-way analysis of variance and *t* test. Data are means. Letters in the same lines and asterisks in the same *x*-axis are significantly different at $P < 0.05$. AE, after exercise; BE, before exercise; C, control diet; CAT, catalase; CF, control diet plus coffee intake; DE, during exercise.

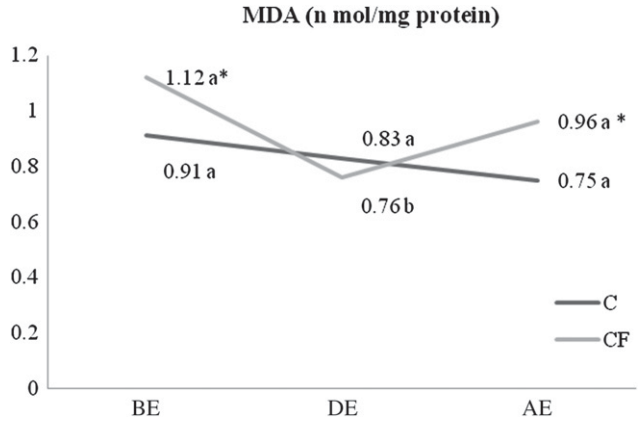

Fig. 4. The effect of coffee intake and exercise on MDA levels in rats determined by one-way analysis of variance and *t* test. Data are means. Letters in the same lines and asterisks in the same *x*-axis are significantly different at $P < 0.05$. AE, after exercise; BE, before exercise; C, control diet; CF, control diet plus coffee intake; DE, during exercise; MDA, malondialdehyde.

between the C and CF groups in plasma TG levels BE and AE, but plasma TG level in the CF groups was significantly lower than in the C groups DE. In the C groups and CF groups, there were no significant differences among BE, DE, and AE in plasma TG levels. The CF groups showed a significant increase in TC and a significant decrease in HDL-C BE, but TC levels in the CF groups were decreased significantly and showed no difference from those in the C groups DE and were significantly lower than those of the C groups AE. HDL-C levels of the CF groups were significantly lower than those of the C groups regardless of exercise. In the C groups and CF groups, HDL-C levels were increased significantly AE.

Discussion

Because the final body weights and food intakes of the CF groups were significantly lower than those of the C groups, whereas the food efficiency ratio was higher than that of the C groups, the decreased final body weight of the CF group might be the result of decreased food intake due to a loss of appetite in the CF group. These results are supported by a previous finding that consumption of caffeine, one of the major biologically active components of coffee, reduces energy intake by 22% [21]. Moreover, a positive relation between satiety and daily caffeine intake has been shown in men and women [22]. From their cohort study, Lopez-Garcia et al. [23] reported that subjects who increased their caffeine consumption over 12 years gained less weight than those who decreased their caffeine consumption.

This study provides evidence that coffee intake strengthens the effect of exercise on the antioxidant system under exercise-induced oxidative stress. The hypothesis that coffee intake can increase the antioxidant system was verified by results from two different studies. First, the activities of SOD and catalase and GSH/GSSG were higher in the CF groups than in the C groups BE. The increased SOD and catalase activities in the CF group in this study might be due to the action of chlorogenic acid of coffee because coffee is one of the richest sources of chlorogenic acid, which is a strong antioxidant in vitro [24]. This increase of catalase and SOD activities in the rat liver has been observed in another study [25].

Second, in the C and CF groups, exercise induced a significant increase in SOD activity; these increased levels remained high even after 1 h of recuperation. Although exercise induced significant increases in catalase activity and these levels returned

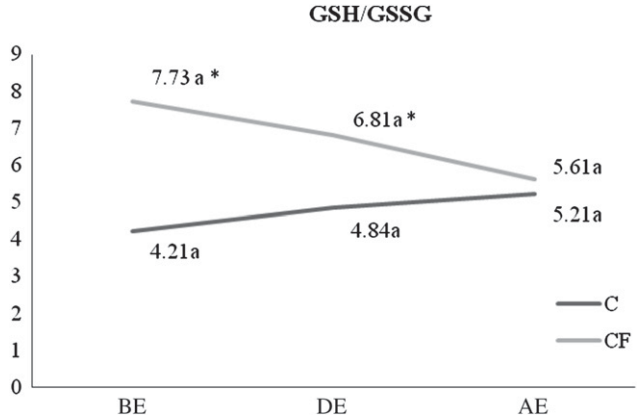

Fig. 3. The effect of coffee intake and exercise on the GSH/GSSG in rats determined by one-way analysis of variance and *t* test. Data are means. Letters in the same lines and asterisks in the same *x*-axis are significantly different at $P < 0.05$. AE, after exercise; BE, before exercise; C, control diet; CF, control diet plus coffee intake; DE, during exercise; GSH/GSSG, ratio of reduced glutathione to oxidized glutathione.

Table 2
Effect of coffee intake on levels of TG, TC, and HDL-C in rats*

	Group		*t* Test
	C	CF	
TG (mg/dL)			
BE	155.13 ± 39.84[a]	127.38 ± 25.26[a]	NS†
DE	133.13 ± 35.43[a]	105.13 ± 30.80[a]	
AE	142.13 ± 74.20[a]	132.50 ± 49.72[a]	NS
TC (mg/dL)			
BE	83.13 ± 16.47[a]	95.50 ± 24.14[a]	
DE	73.88 ± 13.58[a]	74.25 ± 10.10[b]	NS
AE	89.00 ± 19.28[a]	82.13 ± 19.72[ab]	
HDL-C (mg/dL)			
BE	51.04 ± 19.54[ab]	46.83 ± 10.85[ab]	
DE	45.62 ± 12.74[b]	37.11 ± 10.50[b]	
AE	67.36 ± 19.07[a]	51.07 ± 12.53[a]	

AE, after exercise; BE, before exercise; C, control diet; CF, coffee intake; DE, during exercise; HDL-C, high-density lipoprotein cholesterol; TC, total cholesterol; TG, triacylglycerol

* Values presented as mean ± SD ($n = 8$). Values with different superscript letters within a column are significantly different from each other at $P < 0.05$.

† No significant difference between C and CF groups by *t* test ($P < 0.05$).

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to basal values after 1 h of recuperation in the C group, the increased catalase activity of the CF group remained high even after 1 h of recuperation. The GSH/GSSG in the CF group was significantly higher than in the C group BE and DE. Previous research using enzyme measurements showed that the activity of glutathione peroxidase was only marginally increased after coffee consumption, although a significant increase of SOD activity was detected [26]. Thus, it is suggested that coffee intake strengthens the effect of exercise on the antioxidative system under exercise-induced oxidative stress because there is considerable evidence that the antioxidant defense system increases with exercise [27–29]. Because coffee has been reported effective in inhibiting lipid peroxidation in the 1,1-diphenyl-2-picrylhydrazyl assay and decreasing diene conjugates and caffeine has a significant ability to scavenge highly reactive free radicals such as lipid peroxyl radical ($LOO^{\bullet}$) when rat liver microsomes are used, protecting crucial biological molecules against these species in vitro [30], MDA levels of the CF groups were expected to be lower than those of the C groups in this study. However, MDA levels of the CF groups were significantly higher than those of the C groups BE and AE, although there were no significant difference between the C and CF groups DE. Thus, contrary to the hypothesis, it is suggested that coffee intake does not inhibit lipid peroxidation in vivo. Also, because MDA levels DE were significantly lower than those BE and AE in the CF groups and there were no differences in MDA levels regardless of exercise in the C groups, it is suggested that exercise alleviated the result of an accelerated peroxidation in the CF groups.

Although the cholesterol-raising effect of coffee has long been controversial, this study confirmed that coffee intake induced a significant increase in TC, decrease in HDL-C, and no effect on plasma TG levels BE. The diterpenes cafestol and kahweol in coffee may be responsible for this cholesterol-raising effect [31–33]. However, TC levels in the CF groups were decreased significantly and showed no difference from that in the C group DE and significantly lower than those in the C group AE and HDL-C levels were increased significantly AE. Thus, it is suggested that exercise can alleviate the aggravated plasma cholesterol profile caused by coffee intake.

Conclusion

It is suggested that an intake of 0.12 g of freeze-dried instant coffee per 100 g of body weight per day may increase the antioxidant system under exercise-induced stress in vivo but may also induce a significant increase in TC and a significant decrease in HDL-C.

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