J Food Sci Nutr
Vol 16, p 278~281 (2011)
http://dx.doi.org/10.3746/jfn.2011.16.3.278

J Food Science and Nutrition

- Research Note -

Effects of Coffee on Physical Performance in Mice

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Abstract

In this study, coffee was shown to effectively inhibit L6 muscle cell death and ATP reduction induced by hydrogen peroxide damage. Additionally, two weeks of oral administration of 7 mg/kg coffee extracts to mice resulted in a 33% increase in treadmill running time relative to that seen in the distilled water administered group. Blood analysis showed decreased lactate content, which was increased by exercise. Thus, these data suggest that coffee intake may enhance exercise capacity and inhibit damage due to excessive exercise.

Key words: coffee, exercise, L6, antioxidant, ATP

INTRODUCTION

The coffee tree, a tropical evergreen shrub of the genus *Coffea* of the family Rubiaceae, is commercially cultivated around the world. The most commonly grown species are arabica, robusta, and liberica, of which arabica accounts for 75% of total global production (1-3). The coffee bean, a processed product of the fruit of the coffee tree, produces one of the world's favorite beverages, and has been shown to have preventative effects against obesity, diabetes, cancer, cardiovascular disease, and brain disease (4-6). When ingested, particularly during exercise, coffee increases superoxide dismutase (SOD), catalase, and malondialdehyde (MDA) contents and reduces high density lipoprotein (HDL)-cholesterol contents in the body (7). However, its protective effects on muscle cells have not yet been reported. The coffee bean's major constituents include caffeine, chlorogenic acid, trigonelline, 5-caffeoylquinic acid, amino acids, tannins, and saccharides. Among these, caffeine has been noted to stimulate the central nervous system and the peripheral nervous system, thus causing anxiety, excitement, and insomnia when ingested excessively; however, caffeine is also effective in alleviating fatigue and improving exercise capacity when moderately ingested (8-11).

Regular and proper exercise has been shown to help prevent and alleviate hypertension, stroke, cardiovascular disease, diabetes, hyperlipidemia, and cancer (12,13), but strenuous exercise can generate excessive reactive oxygen species and induce oxidative damage to muscle tissues (14,15). In this study, the protective effects of coffee against reactive oxygen species in muscle cells were examined by measuring coffee's effect on cell viability and adenosine triphosphate (ATP) production in L6 muscle cells after treatment of the cells with both hydrogen peroxide and different concentrations of coffee. Additionally, coffee was administered orally to mice, and its effects on treadmill running time and blood indices were measured to assess any changes in exercise capacity.

MATERIALS AND METHODS

Sample preparation

Colombian supremo green coffee beans of *Coffea arabica*, produced in 2010, were purchased from Coffee Nuri (Seoul, Korea) and roasted for 16 min at 245°C using a coffee roaster (CBR-101A, Gene cafe, Seoul, Korea), and then extracted for 5 min with a coffee machine (BCO 120T, Delonghi, Saddle Brook, NJ, USA) using distilled water at 100°C. The obtained extract was then filtered through a paper filter and freeze-dried for use as experimental coffee samples.

Cell culture

L6 muscle cells were distributed by the Korean Cell Line Bank and cultured in DMEM (Dulbecco's Modified Eagle's Medium, Gibco, Grand Island, NY, USA) with 10% FBS (Fetal bovine serum, Gibco) and 1% AA (Antibiotic Antibiotics, Gibco) at 37°C, 5% CO_2.

Cell viability

Cell viability was measured via an MTT assay. In order to evaluate the protective effects of coffee on muscle cells, L6 cells were seeded in 96-well plates at a concentration of 1×10^5 cells/mL and incubated for 24 hr, then

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treated with each concentration of coffee and 1.5 mM H_2O_2 and incubated for an additional 24 hr. Next, the cells were incubated with MTT (0.5 mg/mL in PBS) solution and the absorbance was measured at 540 nm. The protective effect of coffee on L6 cells was expressed as a percentage (%) by calculating the recovery rate of the coffee treatment against the cell death rate observed in the hydrogen peroxide treatment group.

ATP production

ATP production in L6 cells was measured via an ATP bioluminescence assay kit (HS II, Roche, Mannheim, Germany). The L6 cells were seeded on a 96-well plate at a concentration of 1×10^5 cells/mL and incubated for 24 hr, treated with a specified concentration of coffee and 1.5 mM H_2O_2 at the same time, and then incubated for an additional 24 hr. The cells were then lysed and an ATP bioluminescence assay was conducted using a luminometer (Molecular Devices, Sunnyvale, CA, USA).

Experimental animals

Male ICR mice, each weighing approximately 350 g, were obtained from Central Lab Animal Inc., Seoul, Korea, and acclimated for 1 week prior to use in the experiment. The animals were maintained at 23°C with 50% relative humidity, and feed and water were provided *ad libitum* during the experimental period. Their body weights were measured once a week.

Exercise test

An aerobic exercise load test was conducted using a treadmill. In the preliminary exercise to assess the mice's ability to adapt to exercise, the animals were made to exercise at 5 m/min for 5 minutes on the first day, 10 m/min for 10 minutes on the second day, 15 m/min for 15 minutes on the third day, and 20 m/min for 20 minutes on days 4～5. Individual animals with significantly lower exercise capacity were excluded. Animals selected in the preliminary exercise experiment were divided into 3 groups－the non-exercise group, the exercise group, and the exercise＋coffee group; each group contained 7 mice. Starting on the sixth day of the experiment, the mice were administered 0.25 mL of either distilled water or coffee dissolved in distilled water (10 mg/mL) orally, and were made to exercise at 20 m/min for 20 minutes in the afternoon for two weeks. On the final day, blood samples were drawn from the orbital vein immediately after exercise and centrifuged at $2,000\times g$ for 10 minutes to separate the plasma, and then stored at -70°C for analysis.

Blood analysis

The glutamate-oxaloacetate transaminase (GOT), glutamate-pyruvate transaminase (GPT), creatinine and lactate contents in the plasma samples were analyzed via colorimetry using a hematology analyzer (7020, HITACHI, Tokyo, Japan) at DooYeol Biotech. The GOT, GPT, and lactate analysis reagents were purchased from Wako (Osaka, Japan), while the creatinine analysis reagent was purchased from Daiichi (Tokyo, Japan).

Caffeine content analysis

The caffeine content of the prepared coffee extract was quantified via HPLC. For HPLC, an analytical liquid chromatographic apparatus (Jasco Co., Tokyo, Japan) with a SunFire™ C18 column (5 μm, 4.6×250 mm, Waters, Milford, MA, USA) was used. The mobile phase was a mixture solvent of methanol : water : acetic acid (20:79 :1, v/v) with a 1 mL/min elution rate, and sample detection was conducted at 280 nm (16). The analytical reference material, caffeine, was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Statistical analysis

The experimental results were expressed as means± SD, and the significance of differences between the experimental and control groups at the $p<0.05$ was determined using Student's *t*-test.

RESULTS AND DISCUSSION

Strenuous exercise causes excessive reactive oxygen species to develop, and thus induces oxidative damage to muscle tissues. To determine whether coffee exerts a beneficial protective effect on muscle cells, the protective effect of coffee against hydrogen peroxide-induced oxidative damage was measured in the L6 muscle cells. Initially, the L6 cells were treated with coffee at a concentration of 10 ppm and checked for the absence of cell death. Then, 0.1, 1, and 10 ppm coffee were treated for 24 hr with 1.5 mM hydrogen peroxide. The L6 cells treated by 1.5 mM hydrogen peroxide exhibited a 52.5% rate of cell death, whereas cells treated along with coffee exhibited a reduction in cell death in a dose-dependent manner (Fig. 1). The reduction rates of cell death were 4.3, 16.0, and 18.2% in the 0.1, 1, and 10 ppm coffee treatment groups, respectively. These results indicate that coffee intake might prevent intensity exercise-induced oxidative damage to muscle cells in the body. Similarity, Choi et al. (7) reported that coffee intake can promote activities of antioxidant enzyme in physically trained rats.

Additionally, ATP production, which is an energy source required for exercise and is essential for cell survival, was measured. The results showed that coffee treatment induced an increase in the ATP level in a dose-dependent manner, when the ATP levels had been

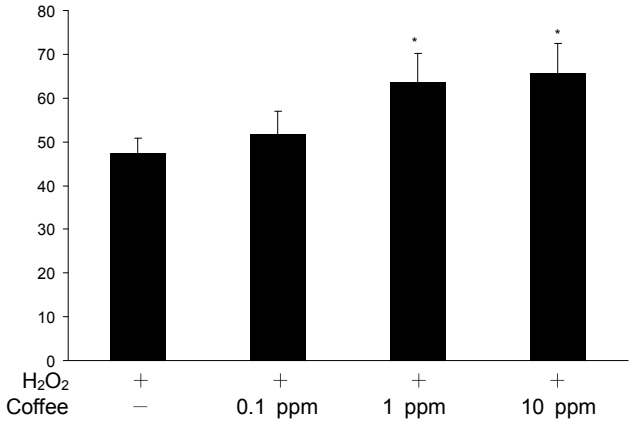

Fig. 1. Protective effects of coffee against H_2O_2-induced oxidative damage in L6 muscle cells. L6 cells were treated for 24 hr with 1.5 mM H_2O_2 and different concentrations of coffee. The data represent the means±SE of the three experiments. $^{*}p<0.05$.

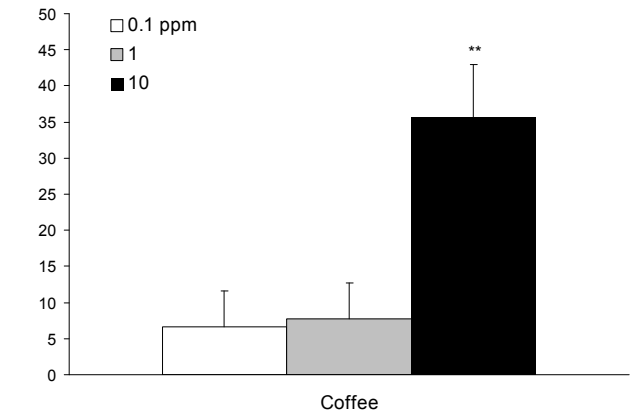

Fig. 2. Effects of coffee on ATP production in L6 muscle cells. L6 cells were treated for 24 hr with 1.5 mM H_2O_2 and different concentrations of coffee. The data are expressed as the means±SE of the three experiments. $^{**}p<0.01$.

reduced by hydrogen peroxide in the L6 cells (Fig. 2).

Effects on exercise capacity and blood indices in mice were measured using a treadmill. The changes in bodyweight and treadmill running time (time taken to be off the treadmill for more than 3 sec) observed after two weeks of coffee treatment are shown in Fig. 3 and 4. The body weights of the mice increase by 2.3 g in the non-exercise group, which was the greatest increase, a 1.1 g increase in the exercise group, and a 1.4 g increase in the exercise+coffee group, with no significant differences between the exercise and exercise+coffee groups. Treadmill running time was increased by 33%, although statistically insignificant, in the exercise+coffee group relative to the distilled water group. Other clinical studies also found exercise performance-enhancing effects of coffee (17,18).

During strenuous exercise, the levels of the metabolic byproducts of lactate and creatinine are usually increased.

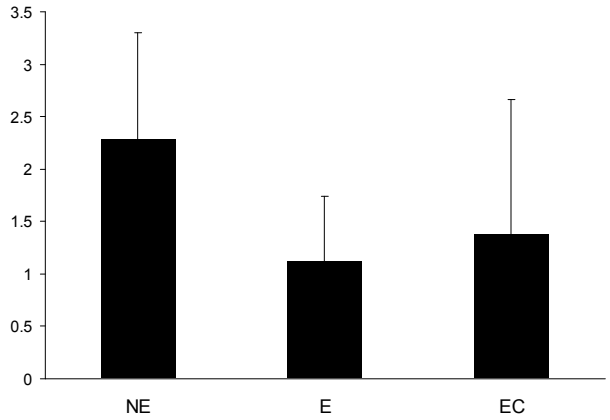

Fig. 3. Effects on body weight gain in mice (after 2 weeks). The data are expressed as the means±SE (n=7). NE, no exercise group; E, exercise group; EC, exercise and coffee-fed group.

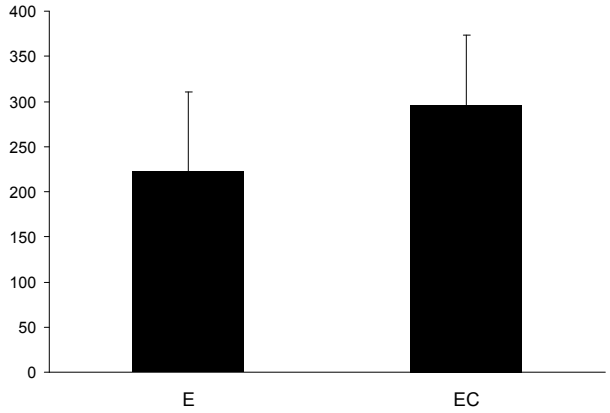

Fig. 4. Effects on running time of mice (after 2 weeks). The data are expressed as the means±SE (n=7). E, exercise group; EC, exercise and coffee-fed group.

Blood analysis results showed that in the exercise group the concentrations of GOT, GPT, creatinine, and lactate were increased as compared with the non-exercise group, and that in the exercise+coffee group the amount of lactate decreased significantly. Additionally, relatively insignificant reductions in GOT and creatinine, of 19 U/L and 0.5 mg/dL, respectively, were also observed (Table 1). Lactate accumulation has been considered one of the major causes of post-exercise muscle soreness as well as fatigue during intense exercise. Therefore, coffee intake may inhibit this fatigue and soreness.

The content of caffeine, the representative constituent of coffee, was analyzed herein via HPLC. The caffeine contained in the coffee bean was quantified as 15.3 mg/g (data not shown). This result was similar to the results obtained in the study conducted by Kim et al., in which the caffeine content of the Colombian supremo coffee bean (*Coffea arabica*) was found to be 14.06～14.31 mg/g after roasting (8). Goldstein et al. (19) reported that caffeine is effective at enhancing sport performance

Table 1. Changes in blood biochemical elements levels

	GOT (U/L)	GPT (U/L)	Creatinine (mg/dL)	Lactate (mmol/L)
NE	104±25	61±23	5.0±0.06	6.7±1.2
E	136±49	72±15	5.4±0.05	8.6±2.0
EC	117±34	102±52	4.9±0.03	6.6±0.8*

NE, no exercise group; E, exercise group; EC, exercise and coffee-fed group; n=7. *$p<0.05$ vs exercise group.

in trained athletes when consumed in low-to-moderate dosages (~3~6 mg/kg) and overall does not result in further enhancement in performance when consumed in higher dosages (>/= 9 mg/kg).

In summary, coffee effectively inhibited L6 muscle cell death and reduced ATP production caused by the addition of hydrogen peroxide. These data suggest that coffee may effectively protect against muscle cell damage induced by a rapid increase in active oxygen species due to excessive exercise. Additionally, the oral administration of coffee for two weeks in mice improved the exercise capacity and blood profiles of lactate in the mice, suggesting that coffee intake can improve exercise capacity.

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(Received August 2, 2011; Accepted September 9, 2011)