

Effects of the Consumption of Caffeinated and Decaffeinated Instant Coffee Beverages on Oxidative Stress Induced by Strenuous Exercise in Rats

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Abstract Many authors attribute the antioxidant activity of brewed coffee to its caffeine content. In addition, caffeine intake has been associated with increased performance during physical exercise. This study analyzed the *in vivo* effects of drinking caffeinated and decaffeinated instant coffee (8%, w/v) on oxidative stress and antioxidant enzyme activity in the anterior tibialis muscles of rats subjected to intense exercise. It was observed that exercise induced lipid peroxidation (estimated using malondialdehyde) and protein oxidation (evaluated by determining the formation of carbonyl groups) in the muscle ($P < 0.05$). Decaffeinated instant coffee and caffeine solution did not exhibit antioxidant activity *in vivo*. Caffeinated instant coffee beverage intake did not induce changes in superoxide dismutase and glutathione peroxidase activities but was able to diminish lipid and protein oxidation in the anterior tibialis muscles of rats after exercise ($P < 0.05$), contributing to a reduction in the oxidative stress triggered by exercise.

Keywords Coffee · Oxidative stress · Caffeinated · Decaffeinated · Exercise · Muscle

Abbreviations

ANOVA Analysis of variance
CC Rats treated with caffeinated instant coffee and not subjected to intense exercise

CD Rats treated with decaffeinated instant coffee and not subjected to intense exercise
CF Rats treated with caffeine solution and not subjected to intense exercise
CK Serum creatine kinase
CNPq Conselho Nacional de Desenvolvimento Científico e Tecnológico
Exer Rats subjected to intense exercise and treated with the vehicle (water)
Exer.CC Rats treated with caffeinated instant coffee and subjected to intense exercise
Exer.CD Rats treated with decaffeinated instant coffee and subjected to intense exercise
Exer.CF Rats treated with caffeine solution and subjected to intense exercise
Fapemig Fundação de Amparo à Pesquisa do Estado de Minas Gerais
FES Functional electrical stimulations
GPx Glutathione peroxidase
SOD Superoxide dismutase
TBARS Thiobarbituric acid-reactive substances

Introduction

Although physical training can benefit the health of organisms, exceeding the recommended loads through strenuous and heavy sports or exercises can cause muscular microtrauma [1]. Tissue damage resulting from acute or chronic exercise ranges from considerable fiber disruption to subcellular damage [2, 3]. Data in the literature have suggested that oxidative stress might be involved not only in the process of muscle damage induced by intense muscle contractions, but also in reducing the maximum force of

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contraction observed after high-intensity exercise [2, 3]. Thus, the activation of the antioxidant systems during and after exercise is especially important for recuperation from exercise [2, 3].

Coffee is a beverage widely consumed by people in all social classes, including athletes [4]. Coffee is known as a stimulant, a property mainly attributed to caffeine, but among the components in coffee, many may be considered bioactive, such as diterpenes, caffeic acid and polyphenols. Therefore, there is growing interest in the effects of this beverage on health [5].

Some studies have reported that roasted coffee possesses mutagenic and pro-oxidant activities *in vitro* [6]. However, other authors noted that roasted coffee can act as a potent antioxidant *in vitro* and *in vivo* [7–10]. Mursu et al. [11] reported that the consumption of filtered coffee does not have any detectable effects on lipid peroxidation in healthy nonsmoking men, whereas Natella et al. [12] demonstrated an increase in the resistance to oxidative modification of low-density lipoprotein isolated from the plasma of healthy volunteers after ingestion of 200 ml of coffee beverage. In animals treated with the coffee, the reduction in the concentration of malondialdehyde has been observed in different organs [13–15].

Some authors attribute the antioxidant activity of the drink to the caffeine content [16]. However, other compounds present in coffee beverage, such as chlorogenic acid and Maillard reaction compounds, can also act as antioxidants [8, 10].

Despite studies showing the antioxidant properties of coffee, most studies have examined the effects of coffee consumption on the formation of lipid peroxidation products. To date, few studies have investigated the effects of drinking coffee on the modulation of the antioxidant defense system, especially after exercise performance.

The aim of this study was to analyze the possible *in vivo* effects of drinking caffeinated and decaffeinated instant coffee on oxidative stress and the enzyme activities of the components of the antioxidant defense system in rats subjected to intense exercise.

Materials and Methods

Coffee Sample and Preparation of the Beverage Samples of caffeinated and decaffeinated instant coffee were obtained from a local trader in Alfenas. Coffee drinks were prepared according to the instructions on the package.

Experimental Groups The study was conducted in accordance with the ethical principles for animal experimentation adopted by the Brazilian College of Animal Experimenta-

tion and was approved by the ethics committee on animal research at the Federal University of Alfenas. Sixty-four adult male Wistar rats (*Rattus norvegicus*) weighing 270 ± 20 g, obtained from the Unifal-MG vivarium, were used. The rats were housed in a temperature-controlled room at 12 h light/dark schedule with food and water available *ad libitum*. The animals were allocated to eight groups of eight animals: animals not subjected to intense exercise and treated with water (control group); animals subjected to intense exercise and treated with water (Exer); animals treated with caffeinated instant coffee and not subjected to intense exercise (CC); animals treated with decaffeinated instant coffee and not subjected to intense exercise (CD); animals treated with caffeine solution and not subjected to intense exercise (CF); animals treated with caffeinated instant coffee and subjected to intense exercise (Exer.CC); animals treated with decaffeinated instant coffee and subjected to intense exercise (Exer.CD) and animals treated with caffeine solution and subjected to intense exercise (Exer.CF).

Coffee Drink and Caffeine Administration Freshly prepared coffee drinks (8%, w/v) were administered to animals by gavage for 21 days, once per day at 280 mg/kg/day (3.8 ml/kg/day), equivalent a daily human consumption of four 50-ml cups of coffee. Caffeine powder was used to prepare the caffeine solution (1.67%, w/v) in water at 90 °C. The caffeine solution and the water were administered to animals by gavage for 21 days, once per day at 6 mg/kg/day and 3.8 ml/kg/day, respectively. This amount was based on the caffeine content of samples of Brazilian instant coffee and the optimum interval suggested to improve physical performance, which is 3 to 6 mg/kg of pure caffeine [17, 18]. The animals were provided with commercial NuvilabCr-1® (Nuvital Nutriente S/A, Colombo, PR, Brazil) and water *ad libitum*, throughout the treatment period.

Intense Exercise Protocol At the end of 19 days, animals in the Exer, Exer.CC, Exer.CD and Exer.CF groups were exercised using functional electrical stimulation to produce standardized repetitive activation of the fast anterior tibialis muscle, according to the method of Paula et al. [19]. The functional electrical stimulations (FES) were performed on the anterior tibialis muscle at 100 Hz and On-off cycle of 1:1 (10-s contraction and 10 s rest) for 15 min using an Electro Kinesis Clinical apparatus (KW Industry National Electronic Technology Ltd.).

Obtaining Homogenate Anterior Tibialis Muscle At the end of 21 days of treatment with coffee or drinks with caffeine, all animals were anesthetized, and blood samples were obtained by cardiac puncture. The blood was centrifuged at 2,500 g for 10 min to obtain serum for creatine

kinase (CK) activity determination. Then, the animals were euthanized, and the anterior tibialis muscle was removed and divided into two portions. One part was homogenized in 0.1 M phosphate buffered saline and centrifuged at 3,000g at 4 °C for evaluation of lipid peroxidation. The second portion was homogenized in Tris–HCl pH 7.4 buffer and centrifuged at 10,000 g at 4 °C. The supernatant, containing cytosolic elements (fraction 1), and the precipitate, containing mitochondria and lysosomes (fraction 2), were separated and used to determine the levels of protein oxidation and the activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) [20].

Determination of Protein Oxidation and Lipid Peroxidation Levels The level of protein oxidation was evaluated by assessing the formation of carbonyl groups using 2,4-dinitrophenylhydrazine according to the method of Levine et al. [21]. The lipid peroxidation level was evaluated using the thiobarbituric acid-reactive substances (TBARS) test [19].

Determination of the Protein Concentration The protein concentration was determined using the Bradford method [22].

Determination of the Enzyme Activity The SOD activity was determined following the method of Oyanagui [23], assuming that 1 unit of enzyme is capable of producing 50% inhibition in the reaction. The GPx activity was determined by the method of Sinet et al. [24], using the molar extinction coefficient of reduced nicotinamide adenine dinucleotide phosphate at 340 nm. The CK activity was determined in the serum of animals by the kinetic method, at 340 nm. The procedure was based on the conversion of creatine phosphate and adenosine diphosphate to creatine and adenosine triphosphate, respectively. The results were expressed in U/L, where 1 U is equivalent to the amount of CK that resulted in the phosphorylation of 1 mmol of creatine per min at 37 °C [25].

Statistical Analysis The results were expressed as the means±SE. The obtained data were subjected to one-way analysis of variance (ANOVA) and were compared by Tukey's test, with $P<0.05$ indicating significance, using the Instat®.

Results and Discussion

Several studies have shown an increase in CK activity and lipid peroxidation in different tissues after intense muscle contractions [19, 26, 27]. However, differences in lifestyle, such as diet and level of physical capacity, can influence the

results. Moreover, it is important to remember that several reactive oxygen species can react at the spot where they are generated. For these reasons, we used rats as an experimental model, because this model allows us to obtain a more rigorous standardization of the experimental conditions than is possible when studying humans. The FES protocol is based on the production of contractions through electrical stimulation, which depolarizes the motor nerve, producing a synchronous response in all motor units of the muscle [28]. This synchronization promotes an efficient contraction, and stimulation with frequencies between 50 and 100 Hz induces tetanic contractions [28]. In the present study, it was noted that 48 h after completing FES at 100 Hz, the activity of CK was significantly higher ($P=0.0006$) in the exercise group (589.17 ± 61.073 U/L) than in the control group (285.14 ± 46.785 U/L). In addition, FES induced a significant increase in lipid peroxidation in the anterior tibialis muscles of rats in the exercise group relative to that in the muscles of the control group (Table 1).

These results show that the FES protocol was effective in inducing muscle contractions in the same way as observed during intense physical exercise. This is corroborated in the literature; previous publications suggest that there is a correlation between high-intensity exercise and oxidative stress [19, 26, 27, 29, 30].

Because mitochondria is an important source of reactive oxygen species (ROS) and because antioxidant enzymes are distributed among cell compartments, the homogenized anterior tibialis muscle was divided into two fractions, and analyses of the protein oxidation and the activities of SOD and GPx were performed using both fractions (fractions 1 and 2) [19]. The adopted exercise protocol induced an increase in the oxidation of proteins in both analyzed fractions (Table 1), but no significant change was observed in the activity of these enzymes after completing the exercise protocol (Table 2).

The intracellular activity of these enzymes protects cells against superoxide anions, hydrogen peroxide and lipid hydroperoxides [3]. However, there are conflicting reports in the literature regarding the activities of enzymes such as SOD and GPx after physical exercise. These conflicting data could be attributed to the different exercise protocols adopted in these studies; the body's response to exercise is highly dependent on factors such as the type, duration and intensity of the performed exercise [1, 30]. In addition, most of these studies did not compare the enzyme activities between different cellular compartments [1, 30].

Despite the existence of other antioxidants that were not analyzed in this work, SOD and GPx are the first line of defense against oxidative stress [3]. The results obtained in this study suggest that the activity of these antioxidant enzymes was not activated in a manner proportional to the need induced by the pro-oxidant events. In these situations,

Table 1 The effect of caffeinated coffee, decaffeinated coffee and caffeine on the protein oxidation and lipid peroxidation levels in the homogenate of the anterior tibialis muscle of rats

Groups	Protein oxidation (μg carbonyl/mg prot.)		Lipid peroxidation (μmol MDA/mg prot.) Total homogenate
	Fraction 1	Fraction 2	
Control	0.339 \pm 0.036	1.862 \pm 0.267	49.85 \pm 5.52
Exer	*0.52 \pm 0.048	*3.39 \pm 0.495	*114.41 \pm 4.9
CC	0.326 \pm 0.021	1.565 \pm 0.166	61.13 \pm 3.42
CD	0.322 \pm 0.021	1.764 \pm 0.268	68.06 \pm 5.38
CF	0.459 \pm 0.049	2.856 \pm 0.582	73.79 \pm 7.66
Exer.CC	#0.315 \pm 0.02	#1.64 \pm 0.298	#76.27 \pm 1.69
Exer.CD	0.420 \pm 0.035	2.158 \pm 0.358	91.48 \pm 1.40
Exer.CF	0.406 \pm 0.039	1.755 \pm 0.367	117.18 \pm 9.5

These results are the mean \pm standard error of five determinations per treatment. * P <0.05 relative to the control, caffeinated coffee (CC) and decaffeinated coffee (CD) groups. # P <0.05 when compared with the exercise group (Exer). CF=caffeine, Exer.CC=exercise and caffeinated coffee, Exer.CD=exercise and decaffeinated coffee, Exer.CF=exercise and caffeine

therapeutic intervention to prevent the denaturing of biologically active molecules becomes important [31, 32].

The data collected in this study show that drinking caffeinated instant coffee was effective in protecting muscle tissue against the lipid peroxidation and protein oxidation induced by highly intense exercise. Thus, these results corroborate published data showing that drinking caffeinated instant coffee has an antioxidant effect *in vivo* both in animals and in humans [12–15, 33, 34].

In contrast, it was observed that drinking decaffeinated instant coffee or caffeine solution alone had no significant effect on the biomarkers of oxidative stress in the anterior tibialis muscle of the animals that we studied.

These results allow us to suggest that despite the boost to physical performance engendered by caffeine consumption, as reported by some authors [18, 35], caffeine alone is not able to protect the muscle against the oxidative stress induced by

intense exercise when administered in doses similar to those found in caffeinated coffee drinks.

There is contrasting evidence regarding the contribution of caffeine to the antioxidant capacity of coffee [8, 36]. Although some studies found a high antioxidant activity of phenolic compounds but not caffeine, others indicate that caffeine make a substantial contribution to the antioxidant properties of coffee [8, 36]. Brezová et al. [8] showed that caffeine was inert in the presence of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt and 1,1-diphenyl-2-picrylhydrazyl oxidants. The ingestion of caffeine 1 h before performing exercise increased the lipid peroxidation in plasma of untrained men, but did not alter the levels of malondialdehyde in the serum of trained men and women after the completion of the exercise [36]. In contrast, Inkielewicz-Stepniak et al. [37] demonstrated that caffeine administered at a dose of 4.7 mg/kg acted as

Table 2 The effect of caffeinated and decaffeinated coffee on the SOD and GPx activities in the homogenate of the anterior tibialis muscle of rats

Groups	Superoxide dismutase (U/mg protein)		Glutathione peroxidase (nmol NADPH/mg protein)	
	Fraction 1	Fraction 2	Fraction 1	Fraction 2
Control	3.45 \pm 0.4801	2.348 \pm 0.436	37.482 \pm 1.5	0.2492 \pm 0.0508
Exer	3.606 \pm 0.2164	2.735 \pm 0.5233	46.238 \pm 2.955	0.2296 \pm 0.03957
CC	3.607 \pm 0.4516	2.342 \pm 0.3168	30.048 \pm 4.334	0.179 \pm 0.01236
CD	3.329 \pm 0.3958	1.978 \pm 0.2207	36.28 \pm 2.833	0.159 \pm 0.03557
CF	3.956 \pm 0.3388	3.1 \pm 0.5949	42.633 \pm 4.149	0.2045 \pm 0.04457
Exer.CC	3.725 \pm 0.181	2.584 \pm 0.833	38.112 \pm 3.018	0.1976 \pm 0.07767
Exer.CD	3.521 \pm 0.3448	1.416 \pm 0.3662	35.452 \pm 2.475	0.2202 \pm 0.05779
Exer.CF	3.806 \pm 0.211	2.25 \pm 0.265	42.633 \pm 3.886	0.2105 \pm 0.074

These results are the mean \pm standard error of five determinations per treatment. The significance was determined by ANOVA; P <0.05. Control=control group, CC=caffeinated coffee, DC=decaffeinated coffee, CF=caffeine, Exer=exercise, Exer.CC=exercise and caffeinated coffee, Exer.CD=exercise and decaffeinated coffee, Exer.CF=exercise and caffeine

an antioxidant and was able to alleviate the adverse effects of fluoride on the nitric oxide level in brain and on lipid peroxidation in the brain, liver and kidney of rats. In addition, Abreu et al. [34] showed that chronic caffeinated coffee and caffeine ingestion reduced lipid peroxidation and increased the concentration of reduced glutathione of brain membranes of rats.

It is important to highlight that in addition to caffeine and other methylxanthines, coffee beverages contain hundreds of other dissolved antioxidant compounds, such as chlorogenic acid, which represents about 6–10% of the dry weight of the bean. Besides, the different methods used for processing, roasting and preparing the bean of the analyzed beverages may influence the chemical composition of coffee, especially the concentration of phenolic compounds present in the drink [7, 9]. It is known that the decaffeination process induces the loss of the 5-caffeoylquinic acid in brewed coffee, due to the formation of a complex between this compound and caffeine [38]. Duarte et al. [13] evaluated the effects of coffee decaffeination with dichloromethane on the *in vitro* antioxidant activity of this matrix. This study showed that decaffeination by the dichloromethane method reduces the *in vitro* antioxidant potential of coffee. These data could explain the observed divergence between the actions of caffeinated and decaffeinated instant coffees in oxidative stress induced by strenuous exercise in the anterior tibialis muscles of rats.

The exact mechanism by which caffeinated instant coffee acts as an antioxidant is not fully understood, but based on published data and our results, we suggest that this action is associated with the chemical composition and the concentration of phenolic compounds and other antioxidant substances formed during roasting [13, 38]. These compounds are probably formed by the Maillard and melanoidin reactions, in which the decaffeination process can interfere, thereby reducing the antioxidant properties of decaffeinated coffee [38].

Conclusion

From the obtained results, we conclude that the adopted exercise protocol induced lipid peroxidation and protein oxidation in the anterior tibialis muscles of the animals that we studied. Neither decaffeinated instant coffee drink nor caffeine solution showed antioxidant activity *in vivo*. Caffeinated instant coffee intake did not induce changes in antioxidant enzyme activity, but it was able to reduce the lipid peroxidation and protein oxidation levels induced by intense exercise in the anterior tibialis muscles of rats. Moderate intake (equivalent to a daily human consumption of four 50-ml cups of coffee) of caffeinated instant coffee may have beneficial effects on health, contributing to a reduction in the oxidative stress triggered by high-intensity exercise.

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