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Effects of Caffeine Ingestions Concomitant to Incremental Running Test on Lipid Peroxidation and Enzymatic Antioxidant in Active Men

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Abstract

Purpose: The aim of the current research was to assess effects of caffeine (5 and 9 mg.kg $^{-1}$) ingestions concomitant to incremental running test on malondialdehyde (MDA) as lipid peroxidation marker and superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) as enzymatic antioxidants.

Material and Methods: Ten subjects were tested on a treadmill until exhaustion on three separate occasions between which there were intervals of 5 days. Every volunteer underwent three conditions containing placebo or caffeine doses that were consumed one hour before exercise (pre-test). Blood samples were collected pre-test, before caffeine or placebo consumption and immediately after exercise (post-test).

Results: Results showed that in comparison to placebo condition, caffeine doses decreased MDA levels and increased GPx activities significantly (P< 0/05). Regardless of 5 mg.kg $^{-1}$ caffeine ingestion that had no significant effect on CAT and SOD activities (P< 0/05), 9 mg.kg $^{-1}$ caffeine intake significantly increased and decreased CAT and SOD respectively (P< 0/05), when compared to placebo. For MDA levels, there was no significant difference between caffeine doses (P< 0/05), but these differences for CAT, SOD and GPx were significant (P< 0/05).

Discussion and Conclusion: In conclusion caffeine doses approximately had equal effects on lipid peroxidation decline, while these effects on enzymatic antioxidant with 9 mg.kg $^{-1}$ caffeine intake were better than 5 mg.kg^{-1} caffeine.

Keywords: Oxidative stress, Free radicals, Progressive exercise, Bruce protocol, Caffeine doses

Introduction

Though regular exercise training has numerous health benefits, it can also be seen as an intense physical activity in which, the production of Reactive Oxygen Species (ROS) increases. Exercise can cause an imbalance between ROS and antioxidant and result in oxidative stress [3]. The oxidative stress condition is one wherein the prooxidant-antioxidant balance is disturbed and there occurs an imbalance of redox status in favor of the former [19]. On the other side, to protect cells against ROS, non-enzymatic antioxidants like glutathione (GSH), uric acid, ceruloplasmin , albumin, transferring, ferritin, melatonin and vitamins A, C, and E concomitant to enzymatic antioxidants like CAT, GPx and SOD co-operate with each other [12]. Besides the pointed antioxidants, caffeine (1,3,7- trimethyloxanthine) is also an efficient biological antioxidant proven by several investigations [2,7,12,13,18,20]. Dalmazio et al (2005) reported that caffeine highly scavenges ROS like hydroxyl and excited status of oxygen and protects biological molecules against these species. Besides antioxidant activity of caffeine is similar to GSH and is significantly higher than ascorbic acid.

A group of researchers believe that the antioxidant effect of caffeine is due to caffeine metabolites. In human body, caffeine breaks down in two main metabolites; 1- methylxanthine (1- X) and 1- methyluric acid (1- U). The antioxidant effect of 1- X is equivalent to ascorbic acid and 1- U to uric acid [15]. Among sport science texts, there are a few investigations that have been studied caffeine effects on exercise-induced oxidative stress (EIOS). Olcina et al. (2006) investigated the effect of 5 mg.kg^{-1} caffeine intake

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on EIOS with ergometer test until exhaustion. The results did not point to any prooxidant or antioxidant effect. In another research, Olcina et al. (2008) evaluated the effect of 5 $mg.kg^{-1}$ caffeine ingestion on EIOS by a steady- state test on the ergometer. The results indicated an enhancement in oxidative stress by MDA level elevations. With regards to the results of recent studies, it is difficult to come to a conclusion about the antioxidant effect of caffeine. With the hypothesis that caffeine is an antioxidant supplement, this study will respond to this question " what dose(s) of caffeine has an antioxidant effect on EIOS? "

Therefore, the aim of this study was to assess the effect of caffeine dose (5 and 9 $mg.kg^{-1}$) ingestions concomitant to incremental exercise on MDA levels as lipid peroxidation marker and SOD, GPx and CAT as the enzymatic antioxidants in blood serum of active men.

Material and Methods

Subjects

Ten male students (age: 21.4±1.6 years, height: 176±4.6 cm, weight: 72.7±8.5 kg, BMI: 23.31±2.39 kg/m^2) from the faculty of physical education of Mazandaran university, Iran voluntarily participated in this study. They did a minimum nine hours of exercise every week. The subjects were informed about the purpose and conditions of the test and gave their written consent to the authors to participate in this study. During the implementation of the test, the participants were not supposed to consume any amphetamine, codeine, acetaminophen, antioxidant nutrients, psychologic and stimulant pharmaceuticals, caffeic materials like cola, coffee, tea, etc., 24 hours before the test sessions. Besides, they should not engage in severe physical activity or exercise within 72 hours before the test. This research underwent ethics evaluation and was thereby approved by the University Ethics Committee before being conducted.

Procedures

In this study, every subject underwent three conditions (starch as placebo, 5 and 9 $mg.kg^{-1}$ caffeine doses which were all taken with water one hour before exercise test). Bruce protocol was done on a treadmill until exhaustion. The Test was taken on three separate days with 5-day intervals between the sessions. Blood samples were collected before caffeine or placebo ingestions and exercise test (pre-test) and immediately after the test (post-test).

Measurements

Blood samples were taken from the anticubital vein in sitting situations. blood serum was separated through centrifugation and was stored at -20 ^{0} C for further analysis . Serum samples were analyzed using enzymatic colorimetric intra assay method to determine the concentrations of SOD, CAT (Jaica, Shizuoka-Japan) and GPx (cayman company, MI-USA). ,MDA concentrations were assessed applying TBARS chemical colorimetric assay (cayman company, MI-USA).

Statistical analyses

dependent variables of the study were MDA, SOD, CAT and GPx, whereas independent variables were caffeine supplement and incremental exercise. Descriptive statistics was used to determine means and standard divisions) and One-Way ANOVA with post-hoc LSD test were used to compare the groups. Statistical analyses were performed using SPSS (version 16). and the significance level was set at (P<0/05).

Results

Table 1 shows Mean ± SD values of MDA, SOD, CAT and GPx, pre- and post-test. For 9 $mg.kg^{-1}$ caffeine, MDA and SOD levels decreased significantly (P< 0/05) (figures 1 and 2) whereas CAT and GPx had significant elevations (P< 0/05) (figures 3 and 4). With 5 $mg.kg^{-1}$ caffeine ingestion MDA and CAT decreased significantly (P< 0/05), but GPx was elevated significantly (P< 0/05). There were no significant changes for SOD by 5 $mg.kg^{-1}$ caffeine ingestion either (P< 0/05). Placebo amounts for MDA, SOD and GPx increased significantly (P< 0/05), but there were significant decreases in CAT levels (P< 0/05).

Table 2 also presents results of One- Way ANOVA and LSD test. For MDA amounts, there were significant differences between caffeine doses and placebo (P< 0/05), but these differences were not significant between the caffeine doses (P< 0/05). There were significant differences among placebo and caffeine doses for GPx (P< 0/05). Differences for CAT and SOD were significant between 9 $mg.kg^{-1}$ caffeine and placebo and between the caffeine doses (P<0/05), but these differences were not significant between 5 $mg.kg^{-1}$

caffeine and placebo (P<0/05).

Table 1: pre- and post-test levels of MDA, SOD, CAT and GPx (Mean ± SD).

	9 mg.kg $^{-1}$ caffeine		**5 mg.kg $^{-1}$ caffeine**		**placebo**	
	Pre-test	**Post-test**	**Pre-test**	**Post-test**	**Pre-test**	**Post-test**
MDA	3.31±0.72	2.66±0.52	3.9±0.6	3.16±0.86	3.35±0.47	3.75±0.47
SOD	105±5	93±3	113±5	115±6	103±5	110±5
CAT	7.17±1.15	9.84±2.68	12.26±2.33	9.82±1.61	16.98±2.97	13.6±2.54
GPx	116±7.72	200±8.66	110±5.12	133±6.48	118±6.17	153±6.26

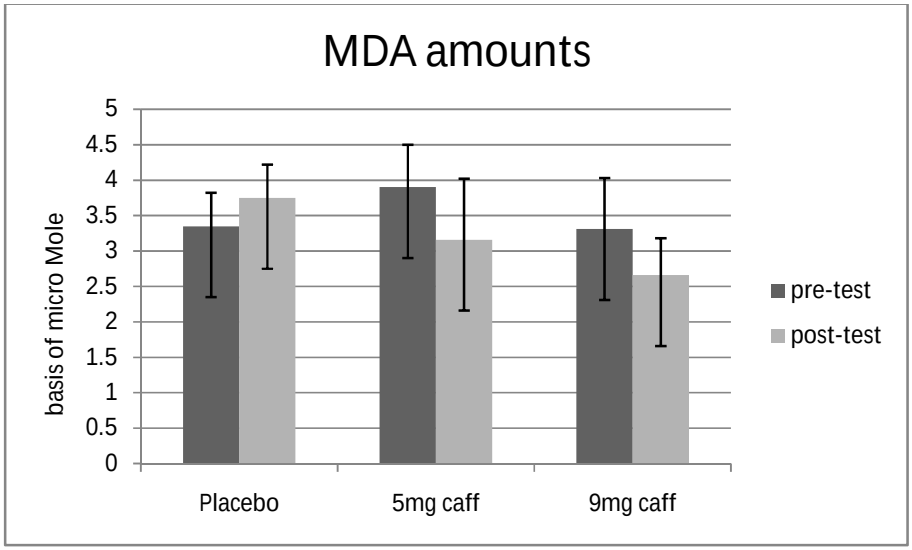

Figure 1: pre- and post-test MDA amounts for caffeine and placebo.

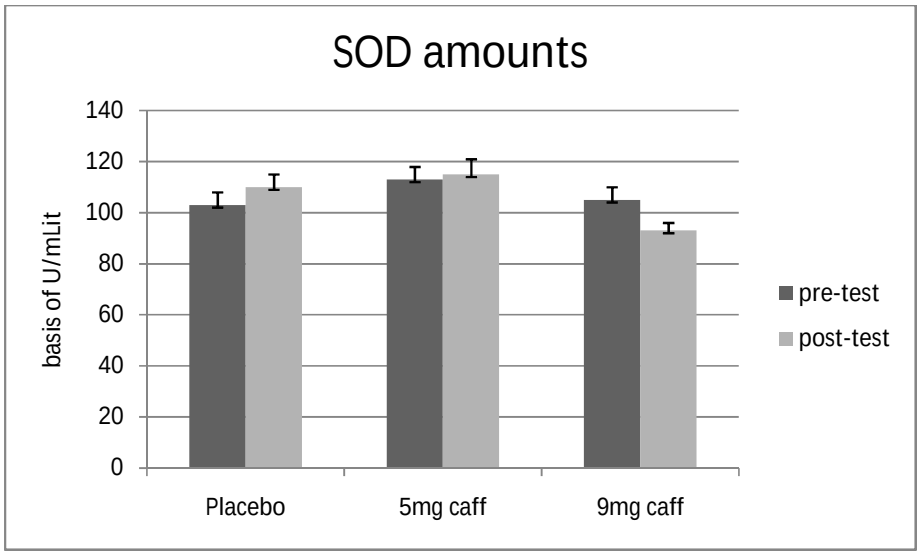

Figure 2: pre- and post-test SOD amounts for caffeine and placebo.

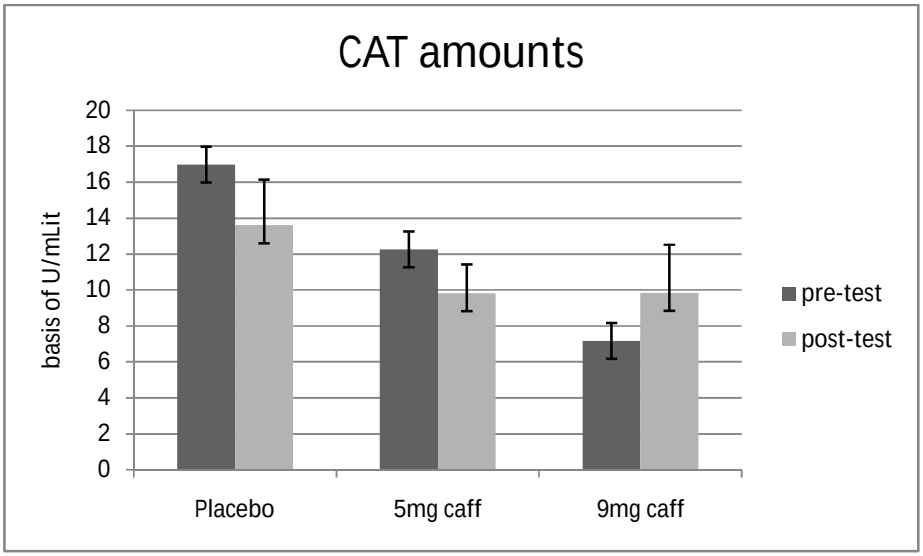

Figure 3: pre-and post-tes CAT amounts for caffeine and placebo.

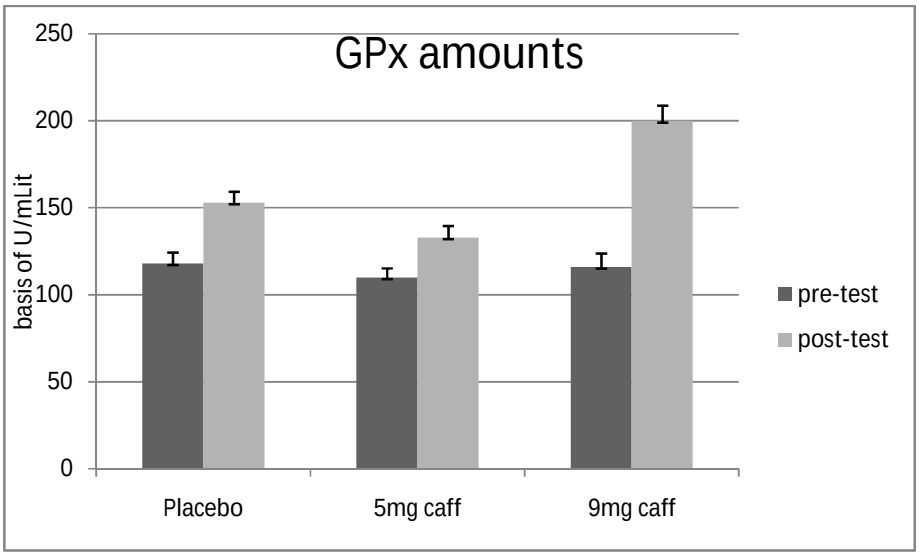

Figure 4:pre- and post-test GPx amounts for caffeine and placebo.

Table 2: one- way ANOVA and LSD-post hoc test results for caffeine and placebo.

Awerage of differences for	Betweem placebo and 9mg.kg^{-1} caffeine	Betweem placebo and 5mg.kg^{-1} caffeine	Betweem 9mg.kg^{-1} and 5mg.kg^{-1} caffeine
MDA	0.938 *	1.139 *	0.277
SOD	26 *	8.9	17.1 *
CAT	6.13 *	1.01	5.11 *
GPx	48.8 *	11.7 *	60.5 *

Discussion and Conclusion

In this work, the effects of two caffeine doses (5 $mg.kg^{-1}$ and 9 $mg.kg^{-1}$) on exercise- induced oxidative stress (EIOS) were assessed by MDA measurement as a lipid peroxidation marker and SOD, GPx and CAT assessments as non-enzymatic antioxidants.

Results of MDA values indicated that there were significant differences between placebo and caffeine doses (P<0/05) which were inconsistent with the findings of Olcina et al (2006, 2008). Olcina et al. (2006) assessed effects of 5 $mg.kg^{-1}$ caffeine ingestion on oxidative stress in inactive male subjects. Exercise was done on the ergometer until exhaustion and the Results did not show any prooxidant or antioxidant effects for 5 $mg.kg^{-1}$ caffeine consumption. In another work, Olcina et al. (2008) investigated the effect of 5 $mg.kg^{-1}$ caffeine in exercising at a steady state with 75 percent of Vo_{2max} on the ergometer. Subjects practiced at a constant intensity for 30 minutes and then continued the activity until exhaustion. Results demonstrated that 5 $mg.kg^{-1}$ caffeine ingestion one hour before exercise promoted oxidative stress through enhancing MDA. The differences among the findings of the present research and those of the mentioned study may be due to differences in the intervals between the test sessions, , fitness levels of the participants, supplement dosages, type and duration of exercise protocols and the number of the participants. Zimmermann (2003) in an investigative report pointed that strenuous exercise particularly in unconditioned individuals can produce oxidative damage and aerobic exercise training strengthens these antioxidant defenses. Also Bloomer et al (2007) have argued that discrepancies in oxidative stress studies may be due to the type, dosage and timing of administration of the antioxidants. Moreover Cooper et al (2002) have noticed that factors including training status, different exercise protocols, age and gender could all play a role which might influence the study differences.

Some non-sport scientific researches, which investigated caffeine effect on lipid peroxidation in animals, indicated caffeine decreased MDA levels [10,18] but other studies have shown an increase in MDA levels [14]. The findings of the current study regarding MDA levels showed no differences between caffeine doses. In other words, the applied caffeine doses in the present study did not have any advantages over each other in attenuating lipid peroxidation.

There have been several studies that introduced caffeine as an antioxidant [2,7,13,20]. So, it was not unexpected that caffeine intakes promote antioxidant system. SOD, GPx, and CAT were assessed as the enzymatic antioxidants. SOD is a substantial antioxidant enzyme in defending cells against ROS. There are three types of SOD which differ in their place of reactions and the metals that make up their active sites [12]. GPx and CAT are other enzymatic antioxidants that catalyze breakdown of H_2O_2 to H_2O and O_2 [19]. For SOD and CAT, results of the present research showed that there were significant differences between placebo and 9 $mg.kg^{-1}$ caffeine ingestion, whereas these differences were not significant between placebo and 5 $mg.kg^{-1}$ caffeine (P<0/05). In the other words, 5 $mg.kg^{-1}$ caffeine intake concomitant exercise did not change SOD activity, whereas 9 $mg.kg^{-1}$ caffeine dosage decreased SOD and increased CAT activities respectively (P<0/05). There were also significant differences in CAT and SOD levels, between 5 $mg.kg^{-1}$ and 9 mg. kg^{-1} caffeine doses concomitant exercise(P<0/05) indicating that caffeine doses had a reversal effect on SOD and CAT activities.

For GPx values, findings of this study showed that there were significant differences between placebo and caffeine doses (P<0/05). There were also significant differences between the two caffeine doses (P<0/05). The increase in the GPx levels was higher applying 9 $mg.kg^{-1}$ caffeine as compared to 5 mg. kg^{-1}.

Olcina et al (2006, 2008) did not report any increase or decrease in non-enzymatic antioxidants with 5 mg. kg^{-1} caffeine doses after exhaustive exercise. Some researches have assessed coffee and caffeine effects on oxidative stress while others only studied caffeine effects in animals in vitro studies. There are also other researches that assessed caffeine effects on antioxidant variables. However, it must be noted that unlike the current study, those researches were conducted in non-sport fields, on animals, and caffeine administration was used for a supplemental period. Demirtas et al. (2012) assessed the effects of 14 days of 30 and 100 $mg.kg^{-1}.bw^{-1}$ caffeine administration on rat's liver The results indicated a reduction in liver MDA levels and an increase in SOD, CAT and GPx levels. Birkner et al. (2006) administered NaF with

caffeine (3 $mg.kg^{-1}$.day) in rats for 50 days. Rats received NaF and caffeine with water. Results showed there were no changes in SOD levels, but CAT and GPx activities decreased and increased, respectively. Rossowska and Nakamoto (1994) demonstrated that there were no significant differences in liver and heart tissue total SOD, GPx and CAT activities between three groups of rats (control, 20 and 22 $mg.kg^{-1}$ caffeine intakes for 30 days). In a publication, Abreu et al (2011) investigated chronic coffee and caffeine ingestion effects on the cognitive function and antioxidant system of rat brains. Results showed chronic coffee and caffeine ingestion (approximately 20-40 $mg.kg^{-1}$ per day) decreased lipid peroxidation and increased GSH levels and SOD activity, whereas no changes were induced in GPx activity. The researchers concluded that chronic coffee consumption regulated the endogenous antioxidant system in the brain. They further added that these effects of coffee on the named antioxidants were especially due to caffeine intake. In another study, Choi et al (2010) assessed coffee consumption and exercise training on plasmatic antioxidant activities and plasmatic cholesterol profile of trained rats. 48 rats were divided into two groups of 1- control diet (C) and 2- control diet with coffee (CF) both of which ran on a treadmill, 30 min per day, for four weeks. . At the end of the fourth weeks the animals were divided into three groups: before exercise (BE), during exercise (DE) and after exercise (AE). Animals in BE group were sacrificed without exercise at the end of fourth week but rats in the DE group, were sacrificed after exercising for 1 hour. . The AE group was allowed to rest for an hour after exercise. In the CF group, four weeks of exercise training and coffee intake could increase the activities of CAT, but SOD in BE and AE groups did not affect DE. In conclusion, they suggested that coffee intake not only could promote activities of pointed antioxidant enzymes, but also increased MDA levels in trained rats.

In summary, results of the present study demonstrated that both caffeine doses (5 and 9 $mg.kg^{-1}$) decreased lipid peroxidation and that 9 $mg.kg^{-1}$ caffeine intake did not have any advantages over the 5 $mg.kg^{-1}$ dose in this regard. For enzymatic antioxidants, both caffeine doses enhanced GPx, but for the remaining factors (SOD and CAT), they had reverse effects, that is, 9 $mg.kg^{-1}$ caffeine ingestion out performed the 5 $mg.kg^{-1}$ dose in reducing exercise- induced oxidative stress.

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