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THE EFFECT OF CAFFEINE SUPPLEMENTATION ON TRAINED INDIVIDUALS SUBJECTED TO MAXIMAL TREADMILL TEST.

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Corresponding author E-mail: vivimana@hotmail.com**Abstract**

Background: Intense physical training increases oxidative stress and inflammation, resulting into muscle and cellular damage. The aim of this study was to analyze the effect of caffeine supplementation on trained young individuals subjected to two treadmill maximal tests.

Materials and Methods: It was a double-blind and crossover study comprising 24 active individuals within the age group 18-30 years. The comparisons were conducted: the effect of exercise (week 1 x 2) and caffeine intake (GC x GP) on thiobarbituric acid (TBARS), interleukin 6 (IL-6), interleukin 10 (IL-10) and superoxide dismutase (SOD) variables during pre-exercise time (30 min. after caffeine or placebo intake) and post-exercise (5 min after treadmill test).

Results: The comparison between weeks 1 and 2 showed increase in the first week, in the following items: TBARS, IL-6 and IL-10 in the GC and GP groups. The comparison within the same week showed that GC individuals presented lower post-exercise TBARS values in the first and second weeks; IL-6 presented higher post-exercise values in the GC group in both weeks. The paired analysis comparing pre- and post-exercise, with and without caffeine showed that IL-6 presented higher post-exercise values in the GC group.

Conclusion: Caffeine used by athletes can decrease oxidative stress. The increased IL-6 suggest that this ergogenic supplement may stimulate muscle hypertrophy, since IL-6 has myokine effect. However, the caffeine effect on IL-6 level and muscle hypertrophy increase should be better investigated in future studies.

Keyword: Caffeine; Exercise; Interleukin; Oxidative stress.

Introduction

In recent years, studies have shown the association between exercise and reactive oxygen species (ROS). Several studies have shown that individuals or animals given to training protocol have higher levels of antioxidant enzymes and of some non-enzymatic oxidants in their muscles. Thus, these individuals and animals demonstrate greater resistance to physical-exercise induced oxidative stress (Troup et al, 1998; Scheffer et al, 2012). In addition, exercise promotes induced inflammatory response through increased serum levels of pro-inflammatory cytokines (IL-1b, TNF- α , IL-6), which is followed by the release of anti-inflammatory cytokines (IL-6, IL-10, IL-4, IL-5, IL-13 and IL-1ra) (Petersen & Pedersen, 2005). Such metabolic burden imposed by exercising is associated with the type, duration and intensity of the exercise (Scheffer et al, 2012). Caffeine is a very common drug and, because of its ability to alter the performance during exercise practice, it has been used as ergogenic aid in sports competitions. Its physiological effects include coronary and cerebral vasoconstriction, smooth muscle relaxation and decreased insulin sensitivity. At high concentrations, caffeine may have antioxidant action; it "sequesters" free radicals (FR) and protects the cells from oxidative damage. The antioxidant action concern the receiving of unpaired free radical (FR) electrons;

thus, it prevents chain reaction inhibition. Accordingly, caffeine is a powerful antioxidant and peroxy hydroxyl radical (Troup et al, 1998). Studies have shown that caffeine use increases alveolar ventilation during exercise. Such increased oxygen consumption during and after exercise may enhance ROS production in different tissues. Caffeine supplementation may inhibit the deleterious effects caused by the presence of free radicals (Pinho et al, 2006; Turley & Gerst, 2006; Aguiar et al, 2008). Therefore, the aim of the present study was to analyze the effect of caffeine supplementation on the oxidative stress, inflammatory markers, performance and physiological variables of young individuals subjected to two maximum treadmill tests in a week interval.

Materials and Methods

A double blind, randomized and crossover study comprising 24 active individuals from Cuiaba City – Mato Grosso State was conducted (Fig. 1). The sample included individuals from both genders, within 18-30 year age group who have been training for at least 12 months (aerobic and resistance), presenting BMI (body mass index) from 20 to 30 kg/m².

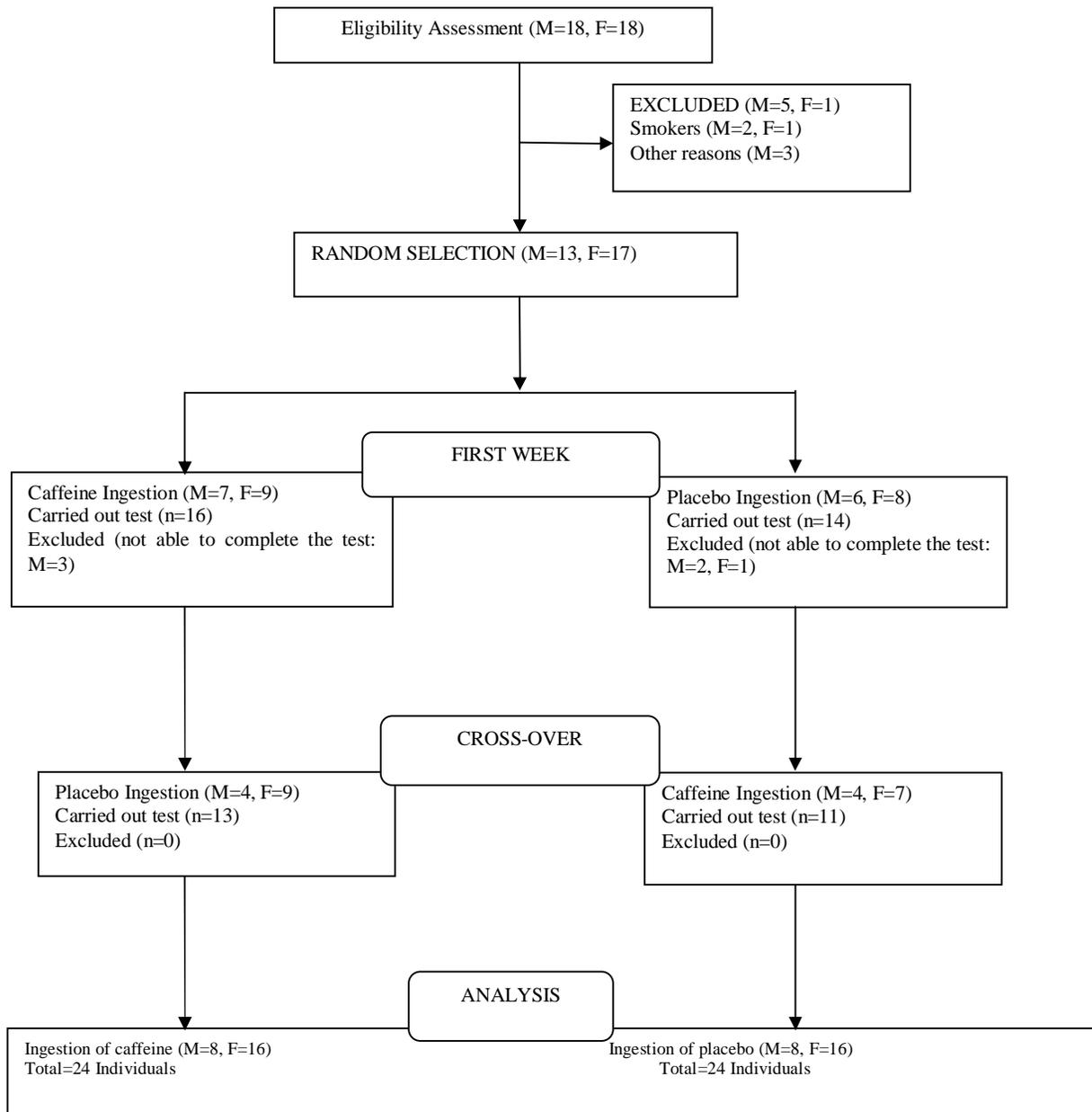


Figure 1: Flowchart of the Participants.

Individuals with chronic disease who used the medication to alter metabolism; pregnant women; individuals with pre-existing lung diseases, in treatment of malignancies, with autoimmune diseases and taking corticoids; smokers and former smokers (with less than six-month suspension) were excluded from current study. All the individuals were instructed not to take dietary supplements in the week prior to the test and during the testing weeks. All participants agreed to participate in the study and signed All participants agreed to participate in the study and signed the consent form and consent form. The present study was approved by the Ethics Committee of Júlio Muller University Hospital (CEP/ HUIJM- registry number: 171.075).

A person not directly involved in the study was responsible for splitting the groups supplemented with caffeine (CG, 6 mg/kg) (Simmonds et al, 2010) or placebo/starch (PG). Caffeine and starch were handled in a pharmacy in capsules containing 50 milligrams. Subjects were randomly selected to receive the caffeine or starch capsules. All subjects were identified through numbered sheets and distributed weekly in two groups (CG or PG). Subsequently, the sheets were sealed in an envelope opened at the end of the study. The sheets were shown to the researchers and to the participants who had ingested caffeine and placebo at the end of the experiment. A structured questionnaire was initially applied to collect socio-demographic data and history of symptoms, previous respiratory diseases, smoking, as well as to collect information about physical activity practices. After the questionnaires were applied, the participants' lung function was assessed through spirometric testing in order to check the integrity of their respiratory system. The test was conducted in the One Flow- Clement Clark spirometer, which has seven-liter capacity and generates direct graphic records, according to the recommendation by the American Thoracic Society-ATS (Redlich et al, 2014).

Basal venous blood samples were collected (before the caffeine or placebo) from all individuals after the spirometric test in order to assess the oxidative stress. The formation of substances reactive to Thiobarbituric acid (TBARS) and the superoxide dismutase (SOD) activities were checked. The inflammatory markers were assessed through the analysis of Interleukin-6 (IL-6) and interleukin-10 (IL-10). The blood sample collection was conducted 30 minutes after the caffeine or placebo intake. Next, the maximum treadmill test was initiated in indoor environment at room temperature. The heart rate (HR), systolic blood pressure (SBP) and diastolic blood pressure (DBP) were assessed two minutes after exercise interruption. Five minutes after test interruption the participants were once more subjected to blood collection. These procedures were repeated after one week in crossover.

The blood collection was performed in the Laboratory of the Research Center for Physical Fitness, Computers, Metabolism, Sport and Health of Federal University of Mato Grosso (NAFIMES-UFMT). It was conducted under the responsibility of an accredited pharmacist who followed all hygienic and aseptic standards. Five milliliters of blood were directly transferred to appropriate tubes and centrifuged for 15 minutes at 3000xg in order to separate the plasma from the blood cells. The plasma was stored at -80°C in "Eppendorf" tubes (Zoppi et al, 2003) for further oxidative stress and inflammatory parameter analyses.

The incremental treadmill test was applied to all participants in the first and second weeks. The individuals kept on walking on the treadmill at 3 km/h, without slope, for one minute in order to adapt to the ergometer. Next, the treadmill slope was increased to 1% and kept at such rate throughout the whole test period. The speed was increased to 6 km/h in the second minute, consecutive 1 km/h increases were performed each following minute until reaching the full speed (15 km/h).

The statistical analysis was conducted in the Minitab (version 16.0) and in the SPSS PASW (version 18.0) software. The Kolmogorov-Smirnov test was applied as curve distribution analysis and it showed normal and abnormal distributions between data. The t-test was adopted for the unpaired data with normal distribution and the Mann Whitney test was applied to the abnormal distribution in order to compare the groups. The paired-T test was used in the pairwise comparison of individuals with normal distribution, and the Wilcoxon test was adopted for those with abnormal distribution. The herein adopted statistical significance was $p < 0.05$.

Results

Eighteen (18) male (M=18) and 18 female subjects (F=18) were included in the experiment and 12 were excluded. Of the 12 excluded ones, three were smokers (M=2 and F=1), two were taking anabolic substances (M=2), one had a respiratory disease (M=1) and six did not finish the test in the first week (M=5 and F=1).

In the first week of testing, the caffeine group randomly comprised 16 people (M=7 and F=9); and the placebo group, 14 (M=6 and F=8) as it was previously described. Six participants did not complete the test in the first week. The cross-over design was put in place in the second week of testing through the inversion of caffeine vs. placebo use (13 individuals in the placebo group (M=4 and F=9); and 11 in the caffeine group (M=4 and F=7)). All subjects have completed the test. At the end of the tests, 24 individuals who had caffeine and 24 who had not (M=8 and F=16) were analyzed.

When the CG and PG groups were separately analyzed by taking into account the comparison between the two weeks in order to test the different responses of the variables, it was found that the TBARS, IL-6 and IL-10 levels had increased in both groups in the first week (Table 1).

Higher TBARS values were found in the first week in the pre- and post-exercise in both groups through the categorization of the analysis and by taking into account the test moment (pre-exercise and post-exercise), and by comparing CG (pre-exerc. sem1 x pre-exerc. sem2; post-exerc. sem1 x post-exerc. sem 2) and PG (idem CG). The IL-10 presented higher values in the first week in the pre- and post-exercises in the CG group, only. The IL-6 showed increase trend in the post-exercise moment in the first week in the PG group only (Table 2).

Table 1: Distribution of oxidative stress and inflammation makers after maximum treadmill test with and without caffeine supplementation. Cuiabá-MT, 2013.

Variables Mean (SD)	With Caffeine (CG)			Without Caffeine (PG)		
	Week 1	Week 2	p-value	Week 1	Week 2	p-value
TBARS (μ M)*	21.0(15.7)	5.2(3.7)	<0.0001	25.3(20.3)	9.5(7.6)	<0.0001
IL-6 (pg/mL) ⁺	2.0(1.2)	0.9(2.0)	<0.0001	1.5(1.3)	0.9(1.6)	0.06
IL-10 (pg/mL)*	3.7(3.5)	1.14(1.2)	<0.0001	3.2(3.5)	1.7(1.9)	0.03

Caption: CG: caffeine group (6mg/kg, 30 min prior to testing); PG: placebo group (starch capsule); TBARS (thiobarbituric acid); IL-6 (interleukin-6); IL-10 (interleukin 10). * unpaired T Test; + Mann Whitney test. Statistical significance ($p < 0.05$).

Table 2: Pre and post-exercise analysis of the CG and PG groups in the first and second weeks, in maximal test on treadmill. Cuiabá-MT, 2013.

Variables Mean (SD)	With Caffeine (CG)				Without Caffeine (PG)			
	Week 1		Week 2		Week 1		Week 2	
	pre-ex	post-ex	pre-ex	post-ex	pre-ex	post-ex	pre-ex	post-ex
TBARS(μ M)	25.2(19.6) ^a	16.7(9.5) ^A	5.4(3.8) ^b	5.0(3.8) ^B	22.6(20.2) ^a	34.5(24,2) ^A	6.3(5,4) ^b	15.0(8.0) ^B
IL-6 (pg/mL)	2.1(1.22) ^a	2.3(1.2) ^A	1.2(1.98) ^a	1.9(3.0) ^A	1.4(1,2) ^a	1.2(1.4) ^A	1.5(2.1) ^a	0.14(0.33) ^B
IL-10(pg/mL)	3.6(4.8) ^a	3.6(2.3) ^A	0.71(0.88) ^b	1.53(1.3) ^B	3.2(2.9) ^a	3.8(5.2) ^A	2.1(2.7) ^a	2.1(1.9) ^A

Caption: CG: caffeine group (6mg/kg, 30 min prior to testing); PG: placebo group (starch capsule); pre-ex: pre-exercise (before the test); post-exercise (5 minutes after test interruption); TBARS (thiobarbituric acid); IL-6 (interleukin-6); IL-10 (interleukin 10). unpaired T test; lowercase letters compare pre-exercise moments within the same group (CG or PG); uppercase letters compare post-exercise moments within the same group (CG or PG). Statistical significance ($p < 0.05$).

The HR and SBP were higher in the first week than in the second in the pre-exercise moment, in both groups (CG, PG) ($p < 0.05$). There was no difference in the enzyme superoxide dismutase (SOD) levels. The total test time (performance) presented the highest mean in the second week, but it had no statistical difference.

When each particular week was compared in order to analyze the behavior of variables with and without caffeine intake before and after-exercise, it was found that the individuals who had caffeine (CG) showed lower TBARS values in the post-exercise moment in the first and second weeks. On the other hand, the IL-6 levels showed higher values in the post-exercise moment with caffeine intake (CG) in the first and the second weeks. However, the IL-10 levels showed no difference (Table 3). The HR and SBP values were higher in the post-exercise moment in both groups when they were compared to the pre-exercise moment.

Table 3: Analysis of pre- and post-exercise moments in the first and second weeks in the CG and PG groups in the maximal treadmill test. Cuiabá, 2013.

Variables Mean (SD)	Week 1				Week 2			
	With Caffeine (CG)		Without Caffeine (PG)		With Caffeine (CG)		Without Caffeine (PG)	
	pre-ex	post-ex	pre-ex	post-ex	pre-ex	post-ex	pre-ex	post-ex
TBARS(μ M)	25.2(19.5) ^a	16.7(9.5) ^A	22.6(20.1) ^a	34.5(24.1) ^B	5.3(3.8) ^a	5.0(3.8) ^A	6.3(5.3) ^a	14.9(8.0) ^B
IL-6(pg/mL)	2.1(1.2) ^a	2.3(1.2) ^A	1.4(1.2) ^a	1.2(1.3) ^B	1.2(1.9) ^a	1.9(2.8) ^A	1.5(2.1) ^a	0.19(0.36) ^B
IL-10 (pg/mL)	3.6(4.8) ^a	3.6(2.3) ^A	3.2(2.9) ^a	3.8(5.2) ^A	0.71(0.8) ^a	1.5(1.3) ^A	2.1(2.7) ^a	2.1(1.2) ^A

Caption: CG: caffeine group (6mg/kg, 30 min prior to testing); PG: placebo group (starch capsule); pre-ex: pre-exercise (before the test); post-exercise (5 minutes after test interruption); TBARS (thiobarbituric acid); IL-6 (interleukin-6); IL-10 (interleukin 10). unpaired T test; lowercase letters compare the pre-exercise moments within the same week; capital letters compare post-exercise moments within the same week. Statistical significance ($p < 0.05$).

Differences in the TBARS and IL-6 values were observed in the analysis of paired individuals through the comparison of test moments (pre and post-exercise) with and without caffeine intake. Nevertheless, IL-6 showed higher values in post-exercise with caffeine intake and it suggests that caffeine influences the increase of this cytokine (Table 4). The total test time (performance) presented the highest mean in the caffeine group, but it had no statistical difference.

Table 4: Paired analysis results of oxidative and inflammatory stress in the pre- and post-exercise with and without caffeine supplementation. Cuiabá-MT, 2013.

Variables Mean (SD)	Pre-exercise			Post-exercise		
	With Caffeine (CG)	Without Caffeine (PG)	p- value	With Caffeine (CG)	Without Caffeine (PG)	p- value
TBARS (μ M)*	16.1(17.5)	13.7(16.1)	0.68	11.4(9.4)	23.9(19.7)	0.001
IL-6 (pg/mL)*	1.7(1.6)	1.5(1.7)	0.60	2.1(2.2)	1.4(2.3)	0.002
IL-10 (pg/mL) ⁺	2.3(3.8)	2.6(2.8)	0.75	2.6(2.1)	2.9(3.6)	0.79

Caption: CG: caffeine group (6mg/kg, 30 min prior to testing); PG: placebo group (starch capsule); TBARS (thiobarbituric acid); IL-6 (interleukin-6); IL-10 (interleukin 10). * Paired t test; + Wilcoxon test. Statistical significance ($p < 0.05$).

Discussion

The present study found increased TBARS, IL-10, heart rate and arterial blood pressure in the first week of testing in both groups (CG and PG). Anxiety and stress in the pre-test moment could have influenced the increase of the herein assessed variables (Weinberger, 2001; Brudey et al, 2015). However, stress and / or anxiety was not analyzed in the present study. It suggests that further studies with human subjects must be conducted in order to assess oxidative stress and inflammatory markers under anxiety condition during physical tests and competitions.

There was increased IL-6 and reduced TBARS levels with caffeine supplementation. According to such findings, intense exercises have increased IL-6 expression, but with no significant effect on the anti-inflammatory cytokines, IL-4, IL-10 and IL-13 levels (Della-Gatta et al, 2014; Skarpańska-Stejnborn et al, 2015; Souglis et al, 2015). The IL-6 has shown reduction due to aerobic training in patients with bronchial hyperresponsiveness and moderate or severe asthma (França-Pinto et al, 2015), and in obese youngsters subjected to resistance training (Shultz et al, 2015).

According to Petersen & Pedersen (2005), besides IL-8 and IL-15 cytokines, IL-6 has been called exercise factor or myokine. The regulatory activity of IL-6 in the inflammatory process has been considered a primary regulator of the acute-phase response in exercise practicing. This cytokine is produced at high concentrations in the skeletal muscle tissue by the leukocytes and endothelial cells through the signaling of pro-inflammatory cytokines and ROS. Its secretion is related to the intensity and duration of, as well as to the amount of muscle mass involved in the exercise. Serrano et al. (2008) reported that IL-6 also regulates satellite cells migration, in order to promote muscle tissue hypertrophy. Thus, the present results suggest that caffeine may stimulate muscle growth through IL-6 increase.

Moreover, according to Moldoveanu et al. (2001), the pro-inflammatory cytokines IL-1b and TNF- α have receptors in the liver, whose binding is a signal of the synthesis of some acute phase proteins. The further signals of these cytokines increase the IL-6 production by the monocytes, macrophages, endothelial cells, epithelial cells, fibroblasts and Skeletal muscle cells may also signal when they are produced in high amounts proteolysis of skeletal muscle tissue and inhibition of anabolic pathways.

Accordingly, IL-6 has shown an effect that may be associated with the recovery process of skeletal muscle contraction in some studies about myokine (Moldoveanu et al, 2001; Serrano et al, 2008). As caffeine has increased this cytokine's concentrations in the current study, it reinforces the assumption that caffeine may be ergogenic and indirectly help skeletal muscle hypertrophy. Thus, caffeine appears to have further effect on the release of IL-6 due to exercise practicing. These results are consistent with the study by Tauler et al. (2013). Such IL-6 level increase induced by caffeine supplementation may be explained by the increased adrenaline release in individuals who have taken caffeine (Stadheim et al, 2014; Li et al, 2015).

The use of caffeine as antioxidant resource has been described in traditional medicine (Abdel-Hady et al, 2015; Tiwari et al, 2014) and used in the various modality of sports to improve exercise performance, being described that the participants in endurance sports present higher levels of caffeine in urine when compared with other modality (Del Coso et al, 2011). The caffeine stands out as the most ergogenic substance to be taken before practicing anaerobic exercises (high intensity and short duration), because it staves off fatigue and, thus, improves performance (Buck et al, 2015). However, the present study did not find physical performance improvement in CG in comparison to PG differently from other studies (Chen et al, 2015; Del Coso et al, 2016).

Oishi et al. (2015) found that caffeine intake can actually increase muscle mass, even with low-intensity physical training due to the activation of satellite cells and of anabolic signals, fact that corroborates the results in the present study. In addition, another study found that IL-6 myokine may also control the oxidative stress condition in the skeletal muscle tissue and in immune cells (Febbraio et al, 2002). Such related effects may have, in turn, helped reducing the TBARS expression observed in CG. Cechella et al. (2014) observed that swimming and caffeine supplementation in middle-aged rats led to reduced glutathione in the gastrocnemius muscles and in the liver of the animals. It reduced the oxidative stress in these tissues, similar to what was observed in the current study. However, it is important noticing that the exercise in water causes less overhead in the musculoskeletal system, and that rats' responses may be different from humans' responses.

Studies with rats (Barcelos et al, 2014 a,b) analyzing the effects of chronic caffeine ingestion and physical training have shown that caffeine intake, alone, and caffeine intake combined with exercise, have decreased the oxidative activity in the plasma of these animals. On the other hand, other research involving athletes found no oxidative activity reduction with caffeine supplementation (Ullah et al, 2015) and found increase in oxidative stress markers after caffeine intake (Li et al, 2015).

The current study paid attention to the health of individuals who make use of caffeine to improve physical performance during training because, despite its ergogenic effect, it may help to protect against oxidative stress, favor IL-6 level increase and, consequently, increase muscle mass. However, further studies should be conducted in order to investigate the effect of IL-6 and caffeine on muscle hypertrophy.

There were some limitations in the present research such as the small number of individuals, the exclusion of participants and the loss of samples, which have made it impossible to analyze other inflammatory markers and oxidative stress such as IL-2 and TNF-alpha, respectively. Such analysis could have contributed to the greater accuracy of the present results.

Conclusions

The results of this study demonstrate that caffeine use by athletes can decrease oxidative stress. However, these findings should be viewed with caution, since it used only the TBARS and SOD markers to analyze oxidative stress levels. Some studies have shown conflicting results about caffeine and oxidative stress (Varma et al, 2010; Zeraatpishe et al, 2015).

The increased IL-6 suggested that this ergogenic supplement may stimulate muscle hypertrophy, since IL-6 has myokine effect (Petersen & Pedersen, 2005). However, the caffeine effect on IL-6 level and muscle hypertrophy increase should be investigated in future studies.

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