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Effects of Caffeine Supplementation on Oxidative Stress, Exercise-Induced Muscle Damage and Leukocytosis

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ABSTRACT

Background: Athletes use variety of ergogenic aids such as caffeine to improve their performance. Many factors such as oxidative stress, muscular-damage and decreased immune function may have negative effect on athletic performance. However effects of caffeine on mentioned factors in female athletes after supramaximal exercise are rare and obscure. ***Methods:*** In this double-blind, cross-over study, 26 female basketball players supplemented with 5mg.kg^{-1} caffeine or dextrose as a placebo followed by Wingate test. Blood samples were obtained before and 5 minutes post-exercise for determining the serum malondialdehyde (MDA), total antioxidant capacity (TAC), creatine kinase (CK) activity and blood's white blood cells (WBC). Kolmogrov-smirnov statistic test and paired t-test were used to analyze data. ***Results:*** After the Wingate test, WBC, lymphocyte, granulocyte count and serum MDA levels, were increased significantly in both groups($P<0.001$). No significant differences were observed in increased levels between caffeine supplemented and placebo groups($P>0.05$). Furthermore the changes in CK activity and TAC levels were not statistically significant in any of the groups ($P>0.05$). ***Conclusion:*** The findings indicated that 5mg.kg^{-1} caffeine supplementation did not have significant adverse effect on oxidative stress, exercise-induced muscle damage and leukocytosis after Wingate test.

Introduction

While regular exercise training is associated with numerous health benefits,[1] an acute exercise increases oxidative stress and muscular damage, especially when the exercise intensity is high.[1,2] Oxidative stress may result in oxidative damage and consequently decrease immune function, increase fatigue[3] and decrease athletic performance.[4]

The possible pathways of oxidative stress in anaerobic exercise includes transient and acute muscular deoxygenation, which resembles the ischemia-reperfusion, xanthine oxidase activation, catecholamine autoxidation, and NADPH oxidase activation, prostanoid metabolism, phagocyte respiratory burst activity, disruption of iron containing proteins, and altered calcium homeostasis.[5] Mechanical stress is also another hypothesis used to explain the increment of free radicals.[6] The muscle tissue damage caused by high levels of force, initiates the inflammation process that eventually produces oxygen free radicals and lipid peroxidation. In addition, the strong muscle contractions during exercise may cause micro-tears both in muscle and in the vascular endothelium, which also affect the migration of white cells.[7] This damage leads to a temporary loss of the exercising capacity of muscle for force production and has implications for increase in muscle post exercise soreness.[8] Tidball reported that muscle micro-damage is an inflammatory factor during and after exercise.[9]

Many approaches are used to improve athletic performance such as ergogenic supplements. Since caffeine has been removed from the list of prohibited substances by the World Anti-Doping agency in 2004, it has recently been increasingly used as ergogenic supplement by many athletes.[10] The ergogenic properties of caffeine have been studied extensively and previous results have indicated that low-to-moderate (~3-6 mg/kg) dosages of caffeine are ergogenic for many kinds of sports.[10,11]

The effects of caffeine on oxidative stress are equivocal, with some studies indicating an antioxidant effect,[12-14] and others demonstrating proxidant effect[15,16] or no effect.[17] The ergogenic effect of caffeine may be due to its hypoalgesic properties.[18,19] The hypoalgesic effect with effort perception altered

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could be the cause of a higher exercise induced micro-injuries[20] and risk of muscle damage in athletes.[21]
It has been reported that caffeine ingestion prior to exercise enhances the activation of both the hypothalamic-pituarity-adrenal axis and the autonomic nervous system, which in turn, may affect the immune response to exercise.[22-24] Some researchers have demonstrated that the leucocytes and lymphocytes have been increased due to stress of exercise,[21,25] and caffeine administration can lead to an aggravation of the muscle injury after exercise.[25]
Although the benefits of caffeine on different types of exercise performance, including aerobic and anaerobic have been studied, its effect on human exercise-induced oxidative stress, muscle damage and immune system after supramaximal exercise are still obscure and controversial,[21] and since some studies showed different metabolism of caffeine between males and females due to factors such as lower CYP1A2 (Cytochrome P450 1A2) activity among females[26] and the effect of estrogen,[27,28] we conducted this study to evaluate the effect of caffeine solely on female athletes.

Materials and Methods

Female basketball players (n=26) participated in this double blind, placebo-controlled, crossover design study and each person participated on 3 separate occasions at the same time of the day. VO_2max (maximal oxygen consumption) and anthropometric measurements were carried out during the first visit. The following 2 sessions, the subjects consumed gelatin capsules containing 5 mg/kg caffeine or dextrose as a placebo, and after 70 minutes a 30-second Wingate test was conducted. The participants (aged 18-30 years) were recruited by advertisement from sports centers and 26 potential volunteers were selected. Athletes who had cardiovascular, inflammatory diseases, musculoskeletal disorders and history of medical or surgical events that might influence the study outcome, took any types of supplements (such as vitamins, antioxidants, protein, creatine, etc.) or had usual intake of caffeine containing OTC (over the counter) drugs and NSAIDs (nonsteroidal anti-inflammatory drugs), 6 weeks prior to the test and during the study were excluded from the study. Habitual caffeine intake from coffee, tea, soft drinks, chocolate, sport drinks was assessed via a questionnaire. As daily caffeine use of our subjects was 116.88 mg.day^{-1} they were not caffeine dependat. The participants were instructed to drink plenty of fluids over the 24-hour period preceding the test, get 6-8 hours of sleep at night before the test and also were asked to continue their usual physical activity and dietary intake during the days of the study and also abstain from vigorous physical activity 24 hours prior to the test. All testing subjects were verbally explained in detail, about the procedure, its risks and benefits, and a written informed consent was obtained. Approval to this trial was granted by the ethical committee of Tabriz University of Medical Sciences.
The baseline demographic characteristics of athletes were presented in table 1.

Table 1. Subjects characteristics (n=26)

Variable	Mean ± SD
Age (year)	24.22 ± 2.65
Weight (Kg)	57.33 ± 6.97
Height (m)	1.643 ± 5.45
BMI (Kg.(m^2)$^{-1}$)	21.62 ± 1.98
Caffeine intake (mg.day^{-1})	116.88±26.73
Body fat (%)	21.94 ± 2.86
Vo_{2max} (ml/kg/min)	40.84 ± 4.07

BMI: body mass index, Vo_{2max}: maximal oxygen consumption

The subjects were tested in the temperature-controlled laboratory (22-25 °C, and 50-55% humidity) on 3 separate occasions. There was one week interval between the second and third session, as a washout period. The skinfold thickness, weight and height were measured in the first visit, with subjects wearing exercise clothes but no shoes using the calibrated physician scale (Harpenden Skinfold Caliper. England, Seca, Vogel and Helke, Hamburg). VO_{2max} was measured using Astrand 6-minute cycle test (Lode B.V. Medical technology Groningen-The Netherlands) and subjects were familiarized with the equipment and the protocol of Wingate test.
At the second and third sessions, the Wingate test was conducted at the same time of the day for each subject, one week apart. To eliminate any possible confounding effects of hormonal variations, all were tested on day 10 of menstrual cycle. The first blood sample was collected via venipuncture from the forearm vein while the subjects were in a seated position in the morning after an overnight fasting of 10-12 hours. The samples were then divided into two tubes, one heparinized to measure WBCs, and the other was centrifuged at 3000 rpm (revolution per minute) for 10 minutes, to separate the serum for the measurement of the MDA, TAC concentration and CK activity. The serum then was quickly frozen and stored at -70 °C. Serum concentration of MDA, as thiobarbitaric acid complexes were measured by fluorometry method. Commercial test kits from Randox Laboratories were also applied to measure the serum TAC. ABTS (2,2'-Azino-di-[3-ethylbenzthiazoline sulphonate]) is incubated with a peroxidase (metmyoglobin) and H2O2 to produce the radical cation ABTS. This has a relatively stable blue-green colour, which is measured at 600 nm. Antioxidants in the added sample cause suppression of this colour production to a degree which is proportional to their concentration. The CK activity was determined by commercial kits (Sigma Chemical Co) with automatic analyzers (RA-1000; made by America TECHNICOM Co). WBCs and differential

leukocyte levels were determined by automatic blood analyzer (Technicon H1, Technicon, Tarrytown, NY, USA).

The subjects were randomly divided into 2 groups and received a capsule, containing 5 $mg.kg^{-1}.bodyweight^{-1}$ caffeine (Merck KGaA, Germany) or dextrose as a placebo (Parsazmoon, Iran) 5 minutes after eating breakfast consisting of 200 ml pineapple juice and 30 gram biscuits, with 150 ml water. Previous studies[26] showed that after consumption of $5mg.kg^{-1}$ caffeine the urinary caffeine concentration would be less than the limitations imposed by IOC (international Olympic committee) and NCAA (national collegiate athletic association).[10,29,30] The Wingate test was performed 70 minutes following ingesting the capsules. In the present study according to the Monark ergomedic bicycle (894E, Sweden) protocol, the subjects completed a 4-min warm up consisting of both dynamic and static stretches and then cycled for 3 minutes at approximately 1-2 kg and 50-60 revolutions per minute (RPM) on an ergometer bike. After a careful adjustment of foot clips and saddle height, two prestarts were carried out, this is to ensure that the subjects know the feeling of maximal acceleration to top pedalling rate and the effect of the weight basket falling down to load the wheel. The prestarts took less than 5 seconds and were separated by a two-minutes slow pedalling without loading. After the participants reached their perceived maximal speed, the predetermined load (7.5% body weight) was dropped and the test continued at maximal effort for 30 second. The second blood sample was collected 5 minutes after finishing the test and then the participants completed 5 min cool down stretches.

Statistical Analysis

Descriptive statistics (age, height, weight, and body fat) were expressed as mean±standard deviation (M±SD). The normality test was assessed using Kolmogrov-smirnov statistic. Paired t test was used to compare WBCs (white blood cells), serum MDA (malondialdehyde) and CK activity (creatine kinase) changes. P-values of less than 0.05 were considered statistically significant.

Results

The results of present study are reported for 26 athletes with the mean age of 24.22 ± 2.65 years and mean BMI (body mass index) of 21.62 ± 1.98 $Kg.(m^2)^{-1}$. No significant differences were observed in baseline WBCs count, CK activity, serum MDA and TAC levels between the caffeine and placebo treatment groups.

After Wingate test serum mean malondialdehyde concentrations (MDA) increased significantly in both groups (P <0.001). Although the increased level of MDA in placebo group was lower than in caffeine supplemented group (0.951±0.142 nm/ml versus 1.157±0.267 nm/ml respectively), the differences were not statistically significant (P>0.05), (Figure 1).

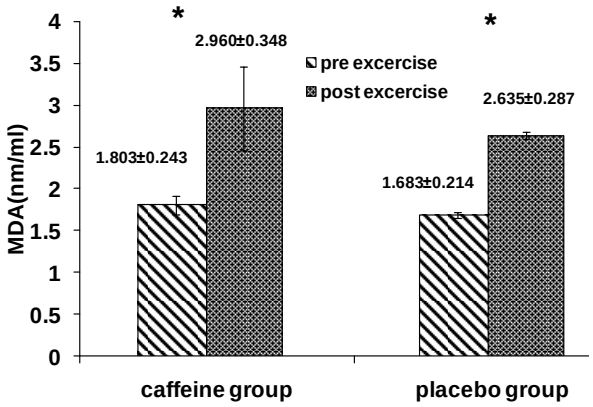

Figure 1. Comparison of serum MDA levels before and after exercise in two treatments (in both groups: n=26).
MDA: Malondialdehyde , *P<0.001

After Wingate test total antioxidant capacity levels decreased slightly in both groups (Figure 2).

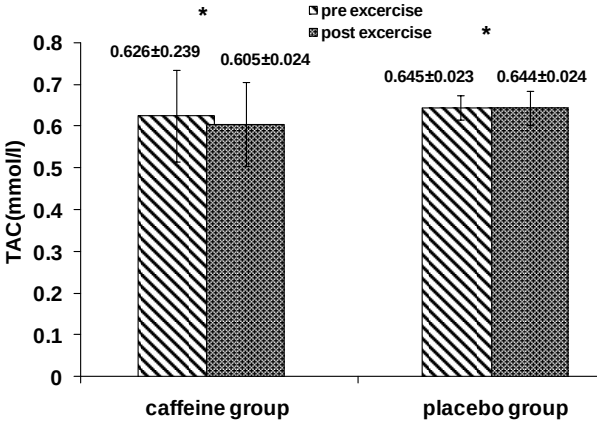

Figure 2. Comparison of serum TAC levels before and after exercise in two treatments (in both groups: n=26).
TAC: Total antioxidant capacity , *P>0.05

Serum creatine kinase activity increased insignificantly in caffeine group, from 112.27±14.80 to 125.64±15.93 IU/L (P=0.09) and placebo group from 119.50±11.90 to 123.71±11.60 IU/L, (P=0.46) (Figure 3).

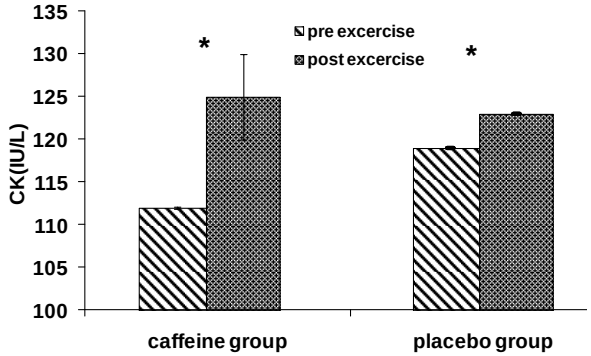

Figure 3. Comparison of serum creatine kinase levels before and after exercise in two treatments (in both groups: n=26).
CK: Creatine kinase , *P>0.05

The Wingate test significantly (P<0.001) increased WBCs count in both groups, from 5.12±0.20 to

8.08±0.32 ($\times10^3$/mm^3) in caffeine group and from 4.98±0.15 to 7.78±0.29 ($\times10^3$/mm^3) in placebo group (Figure 4). The observed difference between caffeine supplemented (2.96±0.24 $\times10^3$/mm^3) and placebo group (2.79±0.24 $\times10^3$/mm^3) was not statistically significant (P>0.05).

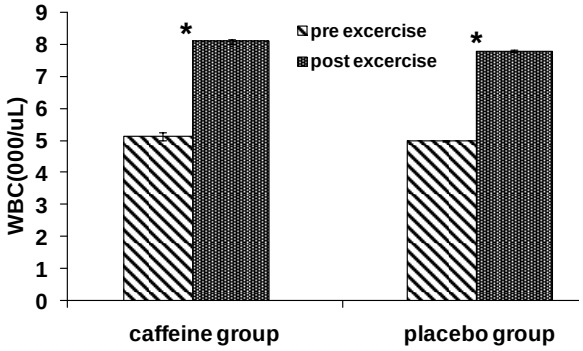

Figure 4. Comparison of serum white blood cells levels before and after exercise in two treatments (in both groups: n=26).
WBC: White blood cells
* P<0.001

Lymphocyte (LYM) count also increased significantly (P<0.001) after Wingate test in both groups (from 2.11±0.12 to 3.64±0.15 ($\times10^3$/mm^3) in caffeine group and from 2.10±0.10 to 3.51±0.19 ($\times10^3$/mm^3) in placebo group). The comparison of the elevated levels between two groups (1.53±0.12 ($\times10^3$/mm^3) in caffeine versus 1.40±0.14 ($\times10^3$/mm^3) in placebo group) were not statistically significant (P>0.05), (Figure 5).

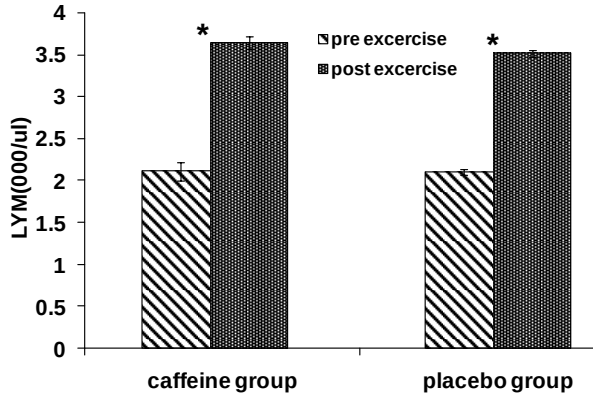

Figure 5. Comparison of serum lymphocyte level before and after exercise in two treatments (in both groups: n=26).
LYM: Lymphocyte
* P<0.001

Granulocytes (GRA) count were higher after Wingate test (P<0.001) (Figure 6), and caffeine supplementation had no significant effect on increased granulocyte level versus placebo group (P>0.05). Granulocyte count increased from 2.86±0.19 to 4.14±0.28 ($\times10^3$/mm^3) in caffeine supplemented group and from 2.68±0.15 to 4.06±0.23 ($\times10^3$/mm^3) in placebo group. The difference of increased levels of GRA in both groups (1.27±0.14 in caffeine group versus 1.38±0.15 in placebo group), was not statistically significant (P>0.05).

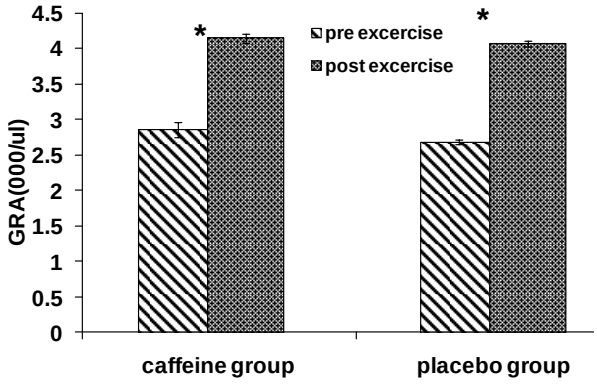

Figure 6. Comparison of serum granulocyte level before and after exercise in two treatments (in both groups: n=26).
GRA: Granulocyte
* P<0.001

Discussion

Previous studies in physically active university students have shown that 30 seconds of high intensity exercise, such as Wingate test, resulted in increment of plasma MDA concentration which was due to lipid peroxidation.[31,32] Although some previous studies reported antioxidant activity of caffeine,[12-14] since no significant differences were observed between two groups we concluded that 5 mg/kg caffeine supplementation did not affect the MDA level. Our findings were in accordance with the findings of Olcina et al., who reported that 5 mg/kg caffeine supplementation had no significant effect on plasma MDA levels immediately after maximum incremental effort until exhaustion.[16] While in another study Olcina et al., showed that caffeine supplementation caused lipid peroxidation and raised plasma MDA levels immediately after 30 minutes aerobic exercise at 75% $Vo_{2max,}$ in active males.[17] In present study TAC levels were slightly decreased in placebo and caffeine supplemented groups, with no significant differences between the two groups. Although caffeine supplementation did not have any significant effect on serum MDA levels, the increased levels in caffeine supplemented group were insignificantly higher than the placebo group. The insignificant effect of caffeine on serum MDA level may be due to the dose which is used in this study. It is possible that higher doses of caffeine may have significant effect on MDA production. Since the altered levels were similar in both groups we concluded that 5 mg/kg of caffeine supplementation did not have significant effect on MDA and TAC levels after a 30 second supramaximal exercise.

To the best of our knowledge, there was no study about the effect of caffeine supplementation on creatine kinase levels in short-term exercises. Concerning long-term exercises, our findings (non significant increased levels of CK activity) were consistent with the findings

of Marco Machado, Vimercatti et al and Bassini-Cameron. Marco Machado reported that serum CK levels increased significantly after exercise tests in soccer players in both caffeine (4.5 and 5.5 mg.kg) supplemented and placebo groups and the increased level were not different between groups.[20,33,34] Vimercatti et al[21] also indicated that serum CK levels increased immediately after 60-min treadmill exercise at 65%Vo_{2max}, however 4.5 or 5.5 mg/kg supplementation did not have any significant effect on the increased levels of CK. In contrast Bassini-Cameron showed that consumption of 5 $mg.kg^{-1}$BW caffeine significantly increased serum Ck levels after 45 minutes of variable distance run protocol.[25] Since these authors did not indicate whether their results were adjusted for the hemoconcentration effect, the discrepancy between our results and their findings may be due to the hemoconcentration changes.

It has been suggested that caffeine ingestion has hypoalgesic effect on muscle during high-intensity exercise[18,19] and enhances the risk of muscle damage.[20,21] Since anaerobic power in this study increased insignificantly in caffeine supplemented group (data not reported yet) the more increment in MDA and CK in this group is probably due to decrement in muscular pain perception and effort perception of caffeine.

Many previous studies have shown that anaerobic exercise can increase WBC concentration.[21,35] In the present study the white blood cell population, lymphocyte and granulocyte count were increased after Wingate test in both groups without any significant differences between the two groups. These results were consistent with the report of other previous studies.[21,24] Walker et al., showed that leukocyte count was increased immediately following 120 min exercise at 65% VO_{2max} and caffeine supplementation (6 $mg.kg^{-1}$BW) did not significantly affect it. Vimercatti also indicated that leukocyte count increased after a 60-min treadmill exercise at 65% Vo_{2max} in both placebo and caffeine supplemented groups, however 4.5 or 5.5 mg/kg caffeine supplementation did not have any significant effect. In contrast to our findings and two above mentioned studies, Bassini-Cameron demonstrated that lymphocyte level was increased after 45 minutes of intense distance run protocol in both groups, and caffeine had a significant synergistic effect on blood lymphocyte count[25], which may be due to more increment of muscle injury and creatine kinase in caffeine ingested group.

Conclusion

The results of the present study indicated that 5 mg/kg caffeine supplementation, 1 hour prior to 30 second Wingate test did not have any significant effect on oxidative estress, exercise-induced muscle damage and inflammatory factors in female basketball players.

Since we did not achieve any remarkable changes with this supplemented dose, it is suggested that more research should be carried out with higher doses of caffeine in order to learn about the precise effect of caffeine on supramaximal exercise.

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References

1. Belviranlı M, Gökbel H. Acute exercise induced oxidative stress and antioxidant changes. *Eur J Gen Med* 2006;3(3):126-31.
2. Vincent HK, Bourguignon CM, Vincent KR, Weltman AL, Bryant M, Taylor AG. Antioxidant Supplementation Lowers Exercise-Induced Oxidative Stress in Young Overweight Adults. *Obesity* 2006;14(12):2224–35.
3. Fisher-Wellman K, Bloomer RJ. Acute exercise and oxidative stress: A 30 year history. *Dyn med* 2009;8(1):217-42.
4. Deaton CM, Marlin DJ. Exercise-associated oxidative stress. *Clin Tech Equine Pract* 2003;2(3):278-91.
5. Bloomer RJ, Falvo MJ, Fry AC, Schilling BK, Smith WA, Moore CA. Oxidative stress response in trained men following repeated squats or sprints. *Med Sci Sports Exerc* 2006;38(8):1436-42.
6. Viitala PE, Newhouse IJ, LaVoie N, Gottardo C. The effects of antioxidant vitamin supplementation on resistance exercise induced lipid peroxidation in trained and untrained participants. *Lipids Health Dis* 2004;3:14.
7. Bassini-Cameron A, Sweet E, Bottino A, Bittar C, Veiga C, Cameron LC. Effect of caffeine supplementation on haematological and biochemical variables in elite soccer players under physical stress conditions. *Br J Sports Med* 2007;41(8):523-30
8. Kyröläinen H, Takala TE, Komi PV. Muscle damage induced by stretch-shortening cycle exercise. *Med Sci Sports Exerc* 1998; 30(3):415-20.
9. Tidball JG. Inflammation in skeletal muscle regeneration. In: Schiafano S and Partridge T (Eds). Skeletal muscle repair and regeneration. New York: Springer;2007. P.243-68.
10. Woolf K, Bidwell WK, Carlson AG. The effect of caffeine as an ergogenic aid in anaerobic exercise. *Int J Sport Nutr Exerc Metab* 2008;18:412-29.
11. Goldstein E, Jacobs PL, Whitehurst M, Penhollow T, Antonio J. Caffeine enhances upper body strength in resistance-trained women. *J Int Soc Sports Nutr* 2010;7:18.
12. Devasagayam TP, Kamat JP, Mohan H, Kesavan PC. Caffeine as an antioxidant: inhibition of lipid

peroxidation induced by reactive oxygen species. *Biochim Biophys Acta* 1996;1282(1):63-70.
13. Shi X, Dalal NS, Jain AC. Antioxidant behavior of caffeine: efficient scavenging of hydroxyl radicals. *Food Chem Toxicol* 1991;29(1):1-6.
14. Nikolic J, Bjelakovic G, Stojanovic I. Effect of caffeine on metabolism of L-arginine in the brain. *Mol Cell Biochem* 2003;244(1-2):125-8.
15. Dianzani MU, Muzio G, Biocca ME, Canuto RA. Lipid peroxidation in fatty liver induced by caffeine in rats. *Int J Tissue React* 1991;13(2):79-85.
16. Olcina GJ, Timon R, Munoz D, MAynar JI, Caballero MJ, Maynar M. Caffeine ingestion effects on oxidative stress in a steady-state test at 75% Vo_{2max}. *Sci sports* 2007;23:87-90.
17. Olcina GJ, Munoz D, Timon R, Caballero MJ, Maynar JI, Córdova A, et al. Effect of caffeine on oxidative stress during maximum incremental exercise. *J Sports Sci Med* 2006;5:621-8.
18. Motl RW, O'Connor PJ, Dishman RK. Effect of caffeine on perceptions of leg muscle pain du ring moderate intensity cycling exercise. *J Pain* 2003;4(6):316-21.
19. Kalmar JM, Cafarelli E. Caffeine: A valuable tool to study central fatigue in humans? *Exerc Sport Sci Rev* 2004;32(4):143-7.
20. Machado M, Breder AC, Ximenes MC, Simões JR, Vigo JFF. Caffeine Supplementation and muscle damage in soccer players. *Braz J Pharm Sci* 2009;45:257-61.
21. Vimwecatti NS, Zovico PV, Carvalho AS, Barreto JG, Machado M. Two doses of caffeine do not increase the risk of exercise-induced muscle damage or leukocytosis. *Phys Educ Sport* 2008;52:96-9.
22. Kalmar JM. The influence of caffeine on voluntary muscle activation. *Med Sci Sports Exerc* 2005;37(12):2113-9.
23. Tsigos C, Chrousos GP. Hypothalamic–pituitary–adrenal axis, neuroendocrine factors and stress. *J Psychosom Res* 2002;53(4):865-71
24. Walker GJ, Finlay O, Griffiths H, Sylvester J, Williams M, Bishop NC.Immunoendocrine Response to Cycling following Ingestion of Caffeine and Carbohydrate. *Med Sci Sports Exerc* 2007;39(9):1554-60.
25. Bassini-Cameron A, Sweet E, Bottino A, Bittar C, Veiga C, Cameron LC. Effect of caffeine supplementation on haematological and biochemical variables in elite soccer players under physical stress conditions. *Br J Sports Med* 2007;41(8):523–30.
26. Carrillo JA, Benitez J. CYP1A2 activity, gender and smoking, as variables influencing the toxicity of caffeine. *Br J Clin Pharmacol* 1996;41(6):605-8.
27. Ascherio A, Weisskopf MG, O'Reilly EJ, McCullough ML, Calle EE, Rodriguez C, et al. Coffee consumption, gender, and Parkinson's disease mortality in the cancer prevention study II cohort: the modifying effects of estrogen. *Am J Epidemiol* 2004;160(10):977-84.
28. Hartley TR, Lovallo WR, Whitsett TL. Cardiovascular effects of caffeine in men and women. *Am J Cardiol* 2004;93(8):1022-6.
29. Pasman WJ, van Baak MA, Jeukendrup AE, de Haan A. The effect of different dosages of caffeine on endurance performance time. *Int J Sports Med* 1995;16(4): 225-30.
30. Beck TW, Housh TJ, Schmidt RJ, Johnson GO, Housh DJ, Coburn JW, et al. The acute effects of caffeine-containing supplement on strength, muscular endurance, and anaerobic capabilities. *J Strength Cond Res* 2006;20(3): 506-10.
31. Baker JS, Bailey DM, Hullin D, Young I, Davies B. Metabolic implications of sesistive force selection for oxidative stress and markers of muscle damage during 30 second of high-intensity exercise. *Eur J Appl Physiol* 2004;92(3):321-7.
32. Groussard C, Rannou-Bekono F, Machefer G, Chevanne M, Vincent S, Sergent O, et al. Changes in blood lipid peroxidation markers and antioxidants after a single sprint anaerobic exercise. *Eur J Appl Physiol* 2003;89(1):14-20.
33. Machado M, Antunes WD, Tamy ALM, Azevedo PG, Barreto JG, Hackney AC. Effect of a single dose of caffeine supplementation and intermittent-interval exercise on muscle damage markers in soccer players. *J Exerc Sci Fit* 2009;7(2):91–7.
34. Machado M, Zovico PVC, da Silva DP, Pereira LN, Barreto JG, Pereira R. Caffeine does not increase resistance exercise-induced microdamage. *J Exerc Sci Fit* 2008;6(2):115-20.
35. Yalcin O, Erman A, Muratli S, Bor-Kucukatay M, Baskurt OK. Time course of hemorheological alterations after heavy anaerobic exercise in untrained human subjects. *J Appl Physiol* 2003;94(3):997-1002.