

EFFECT OF SHORT-TERM CAFFEINE SUPPLEMENTATION ON STRESS RESPONSE AND IMMUNE SYSTEM OF MALE ATHLETES

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Annotation. Vigorous exercise stress might be leading cause of immune system disorders and appearance of acute and chronic inflammation in human body. Caffeine supplementation prior to exercise can be effective on body immune response. This study aimed to evaluate the effect of short-term caffeine supplementation on immune response and stress index in male athletes after an exhaustive aerobic exercise. **Materials and methods:** In a double-blind study 24 male athletes (endurance runner and triathlon) randomly divided in Caffeine supplementation (CAF) and Placebo (CON) groups. One hour prior to main exhaustive treadmill test (Bruce test) CAF group consumed caffeine (6 Mg/BW) and CON group received placebo. Blood samples were collected before and immediately after exercise test from antecubital vein. After supplying serum; Cortisol, leukocyte and serum Heat shock protein 72 (Hsp72) concentrations were determined using ELISA method. Paired and independent t student test was used for analysis of inter and intra group differences respectively. **Results:** serum cortisol and Hsp72 concentrations in CON group was significantly higher than CAF group ($P < 0.05$). However serum leukocyte changes was not statistically significant ($P > 0.05$). In addition Mean of variation in CON group was significantly higher than CAF group ($P < 0.05$). **Conclusions:** Based on study results caffeine supplementation prior to short-term exhaustive aerobic exercise has positive effect on innate immunity and body defensive system.

Keywords: caffeine, hsp72, cortisol, athlete, exhaustive aerobic exercise.

Introduction

The immune system is very complex and is essential for health. Dysfunction of this system can lead to a wide range of disorders (Brolinson, & Elliott, 2007). Positive or negative interactions between physical activity on immune system have gained scientists and sport researcher's interest (Smith, Anwar, Fragen, Rananto, Johnson, & Holbert, 2000). Researches show that moderate and light physical activity may increase the performance aspects of the immune system (Smith et al., 2000), but exhaustive exercise and long term activity such as marathon, ultra marathon, triathlon and etc. may bring about different complications in immune system function and components like antibodies and lymphocytes (Lee, Paffenbarger, & Hennekens, 1997). In addition, release of stress proteins due to vigorous exercise and proceeding stress may disturb immune system and increase possibility of acute and chronic inflammation (Smith et al., 2000; Lee et al., 1997). Cells and tissues respond to protein damaging stressors by increasing the content of highly conserved proteins known as "stress" or "heat shock proteins" (HSPs). A substantial amount of literature suggests an elevated intra-cellular HSP content confers protection to cells and tissues, including skeletal mus-cles (McArdle et al., 2004; Touchberry et al., 2012). In response to physiological stress, Heat shock proteins (Hsps) can accumulate within the cell. There is clear evidence that this proteins act as "danger" signals to alert the immune system during times of stress (Matzinger, 2002). Among heat shock proteins, Hsp72 family is the most important (Desplanches, Ecochard, Sempore, Mayet-Sornay, & Favier, 2004; Whitham, & Fortes, 2006), and in stress conditions appear in blood circulation of healthy subjects (Ganter, Ware, Howard, Roux, Gartland, Matthay et al., 2006; Lennon, Van Gammeren, Clements, Mehta, & Powers, 2002; Milne, & Noble, 2002). Hsp72 will facilitate immunity in normal physiological condition while in pathophysiological status such as atherosclerosis, Alzheimer, chronic lung disease, inflammatory disease of urinary bladder and etc, this protein can exacerbate inflammatory diseases (Fleshner, & Johnson, 2005). Thus manipulating its levels in stress conditions is considered as a therapeutic strategy.

Exercise as a stressor can activate the immune system and leave positive affect on Hsp72 (Febbraio, & Koukoulas, 2000). However, induction of Hsp72 is not only a simple answer to the cellular stress, But also it improves longevity subsequent to inflammatory response or cellular damage (Febbraio et al., 2000).

Major outcomes of Hsp are regulated by the sympathetic nervous system (Peker, Gören, Çiloglu, Karacabey, Ozmerdivenli, & Saygin, 2005). This course can be influenced by various stimulants such as caffeine (Whitham, Walker, & Bishop, 2006). Caffeine as a synergistic factor via releasing of catecholamines in physical activity improves the performance of athletes (Whitham et al., 2006). Researchers believe that caffeine increases blood content of free fatty acids and decreases glycolysis and lactate and delays exhaustion threshold in intense physical activity (Peker et al., 2005). Caffeine as well has been reported to protect cells from oxidative damage (Whitham et al., 2006). The possibility that caffeine could improve sports performance without oxidative cell damage has become scientists' interest (Peker et al., 2005; Machado, Breder, & Ximenes, 2009). In this context Olcina et al. revealed that caffeine intake during a progressive exercise could restrain oxidative damage (Olcina, Muñoz, Timón, Caballero, Maynar, Córdova et al., 2006).

Short-term and severe muscular activity increase number of blood stress hormones such as cortisol. Changes in level of stress hormones may alter the immune response (Cinar, Mogulkoc, Baltaci, & Polat, 2008). Cortisol is released specially in response to the stress and its alteration is concurrent with the HSP72 response as innate immune response

(Whitham et al., 2006). **Peker** in a study on 20 endurance runners in supplement and control groups showed that the Caffeine induces blood cortisol increment after exercise, But the percentage of increase in caffeine group were lower than the control group (Peker et al., 2005). Moreover, Vimercatti in a study showed that the caffeine intake during aerobic exercise is associated with an increase or no change in leukocyte count (Vimercatti, Zovico, Carvalho, Barreto, & Machado, 2008).

Considering the potential relationship among vigorous physical stress, hormones, Hsp changes and ensuing complications with regarding the disparity in many investigations, necessity of new extensive studies are undeniable. The present study attempted to clarify the effect of caffeine supplementation prior to exhaustive aerobic exercise on the immune system and serum concentrations of cortisol and Hsp72.

Methods

Subjects: The study group included 24 male endurance runners and triathlon athletes between 19–24 years of age, healthy, non-smokers, who used no drugs or dietary supplements, or anabolic steroids, and participated voluntarily. Subjects were instructed to the procedures of the study and written consent was obtained. Because the habituation can alter the caffeine effects (Graham, 2001), we select athletes who reported no usage of caffeine. All subjects submitted their written consents to participate and the study was approved by the local committee of ethics.

Experimental protocol: In a randomized double-blind, placebo-controlled design, the subjects were divided into 2 groups: experimental (CAF; n=12) and control (CON; n=12). No caffeine, xanthines, or other substance that could mask the results were ingested by the athletes for 12 h before blood collection. A morning blood specimen was collected 1h (PRE) after a standardized breakfast. Ten minute of warm up (jogging, joint mobilization and stretching) was carried out 35 minute after receiving the supplement.

Diet supplementation: The different supplements were in identical capsules so that the subjects were not aware of which substance they were ingesting. Caffeine (Merck, Germany) was given to the group CAF at a dose of 6 mg·kg⁻¹ in one 500 mg capsule (Dose confirmation WADA), which also contained enough cellulose to fill the capsule. This dose was chosen because it is within the supplementation range shown (3.0-9.0 mg·kg⁻¹ body weight at 30-60 minute prior exercise ingestion) to improve athletes' performance (Graham, 2001; Jones, 2008). The control group (CON) received one capsule with 500 mg cellulose only. The supplements were ingested immediately after the blood sample collection.

Test protocol: all subjects performed exhaustive aerobic tests (Bruce test) on treadmill and were continued to reach exhaustion. The intensity of exercise was controlled by coaches in accordance with protocol of Bruce test. The athletes were allowed to ingest water ad libitum throughout their test.

Data collection: Venous blood samples were collected from the forearm while the subjects were in a seated position. The first sample (PRE, 0) was collected in the morning and other sample immediately after the test. After collection, the blood samples were divided in two tubes (one heparinised tube for hematological measures and the other for serum). After centrifugation, the separated serum was quickly frozen and stored at -70°C. From each heparinised sample the following hematological measures were obtained; Heat shock protein 72 (eHsp72) and Cortisol levels were measured by ELISA method and with kits (stress gene - Canada) and (IBL - Germany), respectively. In addition, Changes of leukocytes were determined with cell counter (Mindray-USA) by H1 method.

Statistical analyses: Statistical analysis was performed on SPSS version 15.0, performing the two way ANOVA test, in which the within group factor corresponds to the exhaustive aerobic tests and the between group factor corresponds to the caffeine effect. The p value of < 0.05 was used to determine statistical significance.

Results

The anthropometric and performance characteristics of groups are identical (Table 1). Normal distribution of data was confirmed by Kolmogorov-Smirnov test (Table 2). statistical analysis showed that leukocyte count increased after exercise in CON and CAF groups (Table 3), however this increment in caffeine treated group was not statistically significant (P>0.05) (Table 4).

Exhaustive exercise caused significant elevation in Serum Hsp72 and cortisol levels in both groups (Table 3). However, according to the t test results, average of this increase in the CON group was significantly higher than CAF group (P <0.05) (Table 4).

Table 1

Participant characteristics

Age (years)	CON Group (n=12)		CAF Group (n=12)	
	22.8±2.3		21.9±3.6	
Height (cm)	175.6±3.8		173.3±5.2	
Weight (kg)	70.8±4.7		72.7±3.4	
VO ₂ max(ml·kg ⁻¹ ·min ⁻¹)	61.5±1.8		60.1±2.3	
Systolic blood pressure(mm Hg)	120± 12		116± 17	
Diastolic blood pressure(mm Hg)	82± 0.7		83± 0.9	
Heart rate (per minute)	52± 5		54±4	

Data are mean ± S

Table 2

Results of Kolmogrov - Smirnov test

	CON Group (n=12)		CAF Group (n=12)	
	Statistic Z	Sig	Statistic Z	Sig
Hsp72 (ng/ml)	1.203	0.110	1.204	0.110
Cortisol (pg/ml)	0.503	0.962	0.654	0.786
Leukocyte (ml/1000)	0.557	0.893	0.557	0.916

(P>0.05)

Table 3

Mean changes of serum Hsp72, cortisol and leukocytes during an exhaustive aerobic exercise

	CON Group			CAF Group		
	Pre test	Post test	Sig	Pre test	Post test	Sig
Hsp72 (ng/ml)	0.27±0.12	0.62±0.44	*0.003	0.28±0.09	0.36±0.06	0.014*
Cortisol (pg/ml)	54.18±11.1	114.8±15.2	0.022*	55.47±23	98.15±27.2	0.036*
Leukocyte(ml/1000)	7.11±2.04	12.78±3.13	0.035*	7.23±1.24	11.62±2.43	0.046*

*significant compared to pre test values (P> 0.05)

Table 4

Comparison of mean serum Hsp72, cortisol and leukocyte levels between the CAF and CON groups.

	Group	df	Sig
Hsp72 (ng/ml)	CAF	22	0.021 *
	CON		
Cortisol (pg/ml)	CAF	22	0.039 *
	CON		
Leukocyte (ml/1000)	CAF	22	0.063
	CON		

*(P> 0.05)

Discussion

Results of present study showed that leukocyte count increased significantly after exhaustive exercise in both groups, but no difference was observed between groups. Scientists believe that leukocyte count is elevated with physical activity-induced muscle damage. Then exhaustive physical activity causes cellular damage, stimulates the immune system and consequently leukocyte accumulation, especially neutrophils (Machado et al., 2009; Olcina et al., 2006). Therefore less muscle damage in supplement group could be a possible mechanism involved in lower leukocyte increment.

Caffeine consumption is accompanied by further oxidation of fatty acids in muscle cells, maintained intramuscular amino acid stores and reduced protein catabolism rate in this stance (Peker et al., 2005). Subsequently cellular and muscular damage Indexes release such as creatine kinase in blood stream is reduced which pledge less stimulation of immune system and less activity-induced leukocytosis (Machado et al., 2009). Decrease of muscular

damage Indexes in the supplement group are in agreement with some reports (Vimercatti et al., 2008; Bassini-Cameron, Sweet, Bottino, Bittar, Veiga, & Cameron, 2007).

In the present study, after exhaustive aerobic exercise significant increase was observed in serum HSP72 concentration of male athletes in both groups and amount of increase in control group was higher than the supplement group. However, it is clear that exercise and physical activity as a stress factor has an important role in stimulating the immune system (Campisi, & Fleshner, 2003). Therefore, HSP72 after exercise and physical activity as the most sensitive response to the body's innate immune system for protection of stress injuries begins to increase (Febbraio et al., 2000). Researchers believe that there are certain thresholds for this protein in response to stress. Hence, optimal increment of this protein as a danger signal leads to augmented inflammatory agents summon and environmental immune components whereas its amplification leads to Apoptosis and cell death, especially in tumor species (Matzinger, 2002; Febbraio et al., 2000). Therefore, modulation of HSP72 response to stress condition is a considerable approach that interests many scientists (Febbraio et al., 2000). In accordance with other studies (Whitham et al., 2006) our results demonstrate that caffeine supplementation modulates serum HSP72 response to physiological stress and exhaustive aerobic exercise. However, less increased percentage of this protein was observed in the supplement group in comparison to control group (26/66% vs. 33/33%). It can be elucidated that less increase in HSP72 response succeeding physical activity in supplement group is due to caffeine in comparison with control group. In another study, **Whitham** reported increased HSP72 levels in both control and supplement groups following exercise on ergometer. They observed that immediately after exercise concentration of this protein was significantly higher in caffeine group (Whitham et al., 2006). It should be mentioned that **Whitham** carried out a long-term exercise (90 minutes) and subjects were accustomed to caffeine consumption. Furthermore, majority of studies declare that athletes versus non-athletes express more response to caffeine (Peker et al., 2005; Machado et al., 2009). **Olcina** reported that after a progressive exercise on ergometer caffeine supplementation reduces cellular damage (Olcina et al., 2006). Due to mechanism of action on catecholamine release, caffeine can improve performance and endurance. As well, antioxidant trait is attributed to caffeine which vindicates its protective effect on cellular damage (Whitham et al., 2006). The present study confirms that accompanying caffeine and exercise could lower HSP72 concentrations during short term activities and is beneficial for cells. However, caffeine consumption during long-term aerobic activity have been associated with high cellular damage; because this supplement with increasing the longevity of exercise could increase oxidative damage (Whitham et al., 2006).

It has been reported that exhaustive exercise with activating the hypothalamic-pituitary-adrenocortical axis could has direct influence on central nervous system (Karcz-Kubicha, Antoniou, Terasmaa, Quarta, Solinas, Justinova et al., 2003). Physical activity with increasing the production of adrenocorticotrophic hormone from pituitary leads to increased cortisol secretion (Karcz-Kubicha et al., 2003). Cortisol secretion during exercise is largely dependent on exercise intensity and individual's capacity. Moreover, athletes response to glucocorticoids and exercise differs especially in intensive training (Gleeson, 2007). In agreement with similar studies (Peker et al., 2005; Nieman, Henson, Smith, Utter, Vinci, Davis et al., 2001) our data showed that plasma cortisol concentration after exhaustive aerobic activity had increased in both groups, although this increase in caffeine group was lower than the control group. Less increment in cortisol secretion might be the reason for immunity and long-term health effects (Lovallo, Al'absi, Blick, Whitsett, & Wilson, 1996). The possible mechanism of caffeine in **Beaven et al.** opinion is that supplementation prior to endurance exercise prevents the protein catabolism and consequently by inhibiting the cortisol secretion, concentrations of growth hormone is increased which cause testosterone levels maintenance. Therefore anabolic processes will continue (Beaven, Hopkins, Hansen, Wood, Cronin, & Lowe, 2008).

Conclusion

Results of present research indicate that ensuing exhaustive aerobic exercise, fluctuations of innate immune system markers have been mitigated in the caffeine group. It is supposed that caffeine consumption preceding exhaustive aerobic exercise would reduce the susceptibility to immune challenges.

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Conflict of interests

The authors declare no conflict of interests.

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