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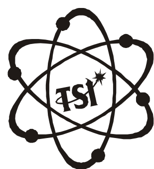

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Effect of wet cupping on antioxidant status in peripheral and cupping blood of non-athletes and athletes

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ABSTRACT

Objective: ROS are highly reactive molecules that react with cellular components, resulting in oxidative damage. They are involved in many degenerative conditions. In other side, wet cupping is an effective method of treating radical-related disorders. This study is aimed to evaluate the effect of wet cupping together with sporting on antioxidant defense system.
Subjects and Methods: In this study 40 healthy, non-smoker men were assigned into non-, amateur, and professional athlete groups. They were subjected to two samplings with one week interval. The levels of antioxidant enzyme activities, oxidative damage biomarkers, and vitamins C and E were evaluated in blood samples.
Results: We observed increased antioxidant enzyme activities with sporting activity in both peripheral and cupping blood. Low enzyme activities and high level of oxidative damage biomarkers were observed in cupping blood with respect to peripheral blood samples. In addition, the level of enzyme activities elevated considerably in second sampling with respect to first sampling in both peripheral and cupping blood. The level of oxidative damage biomarkers in second sampling was significantly lower than first sampling. In both kind of blood, we did not observe any significant difference in vitamin C and E at first and/or second sampling.
Conclusions: Sporting activity together with wet cupping induced adaptive responses in antioxidant and repair systems, increased protection of cells against ROS and decreased the accumulation of oxidative damage biomarkers.
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KEYWORDS

Wet cupping;
Antioxidant defense;
Oxidative damage;
Vitamin;
Sporting.

INTRODUCTION

Free radicals are highly reactive and unstable naturally occurring molecules that have an unpaired electron in their outer shell[1]. Reactive oxygen species (ROS) are a kind of such free radicals which are formed during mitochondrial electron transport chain as well as by various environmental stressors[2, 3]. As a source, it has been known that oxygen consumption and metabolic activity are elevated due to muscle contraction during physical activity and sporting, resulting increase in the electron leakage from the mitochondrial trans-

port system leading to the formation of ROS[4]. ROS as causative factors are involved in many diseases and degenerative conditions[5, 6]. Free radicals have been implicated in the pathogenesis of many diseases such as diabetes, inflammation, cancer, cardiovascular disorders, and process of ageing[7]. They react with cellular components such as proteins, nucleic acids, fatty acids, and complex lipids[1,8], leading to production of oxidative damage biomarkers. Evaluation of these biomarkers is commonly used to monitor the occurrence of oxidative stress in health related diseases[9]. Reactive carbonyl derivatives (RCD) are an important detectable biomarker of protein oxidation. RCD are also formed by ROS-mediated oxidation of side chains of some free amino acid residues within cells[10]. The interaction of $HO^{\bullet}$ with the nucleobases of DNA strand, such as guanine, forms 8-hydroxy-2'-deoxyguanosine (8-OHdG) that has been widely used as a biomarker for oxidative stress[11]. Malondialdehyde (MDA) is primarily of interest as a product of in vivo lipid peroxidation[9]. Under physiological conditions formation of ROS is normally balanced by rate of antioxidant enzymes activities, including catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPX)[1, 12]. SOD is the major ROS detoxifier of the cell[13] and catalyzes the dismutation of the superoxide radical anion to hydrogen peroxide and oxygen. CAT catalyzes the dismutation of hydrogen peroxide to O_2 and H_2O[6]. GPX is an erythrocyte enzyme that specifically reduces H_2O_2 by GSH, reduces a broad scope of organic hydroperoxides and protects biomembranes against lipid peroxidation[14].

On the other hand, non-enzymatic defenses, predominantly ascorbic acid in the aqueous and vitamin E in lipid media are the major chain-breaking antioxidants in living systems[12, 15, 16]. These systems scavenge radicals in order to terminate free radical chain reactions[16]. Regular physical training causes an increase in the antioxidant system and a reduction in lipid peroxidation. It has been suggested that individuals who exercise regularly, placing a constant oxidative stress on the muscles and other cells, have an augmented antioxidant defense system to reduce exercise-induced oxidative risk[4].

In recent years, Alternative and complementary therapies (ACTs) are gaining use in the general population and in chronic conditions, such as cancer, asthma and arthritis[17, 18]. In other side, bloodletting and cupping are effective methods of treating radical-related disorders including fevers, local inflammatory[19], reduction in cardiovascular events, and disorders caused by iron overloading such as polycythemia, hemochromatosis[20]. Cupping mainly described as treatment for chronic pain including lower back pain, and headache, it has also been used to treat other non-specific disease processes including indigestion and menstrual disturbance[21].

Cupping involves the application of a vaccum to closed system cup on a speci?c area of skin[22]. Two styles of cupping therapy are used today, dry cupping and wet cupping. Dry cupping simply pulls the local underlying tissue up into the suctioning cup[23]. Wet cupping includes some scarification of the skin before applying the cupping glasses[24] and is an ancient medical technique, with documented use dating to several ancient cultures and contemporary practice in many parts of the European and Eastern world, including Iran[25].

This study was conducted to investigate the effects of wet cupping on enzymatic and non-enzymatic antioxidant system within athletes and non-athletes.

MATERIALS AND METHODS

This study was performed on forty healthy volunteer men aged between 20 and 45 years. All volunteers were tested and their healthiness was confirmed by an expert physician. They gave their informed consent to perform wet cupping on them. Based on data, they all were non smoker and we assigned them into three groups including; non-athletes (group 1; N = 13), amateur athletes (group 2; who exercise 2 hours per day and 2 days per week; N = 14), and professional athletes (group 3; who exercise 3 hours per day and 3 day per week; N = 13). All volunteers were considered for wet cupping on Jun. to Dec. 2010. Blood sampling was done at Iranian Institute of Hejamat Research and related institutions. Seven days after first sampling second sampling was done on the same volunteers.

In each case, a blood sample was taken from capital vein. In addition, the position on the vertebrae T2-T5 was disinfected with betadine. A glass cup was applied to the skin and a partial vacuum created inside the cup via electrical suction. The cup clung on the skin and

left for a period of 5 min. Then super?cial incisions were made to the skin with sterile surgical blades. The cup placed back on the skin, until it is ?lled with blood from the capillary vessels[25] Then the cup was removed and 5 ml of collected cupping blood used for biochemical measurements.

After each sampling, heparinized whole blood samples were immediately centrifuged at 3000 rpm in 4 °C for 10 minutes. The supernatant was aspirated and collected as serum and stored at -70 °C. To separate erythrocytes, the buffy coat was drained and the sediment was washed four times with 3 ml of 0.9% sodium chloride solution, and was centrifuged under the same conditions. Aliquots of the washed erythrocytes were lysed by freezing (-20°C) for 24 h and then they were used for measurements.

Antioxidant enzymes assay

The SOD activity in erythrocytes was measured according to the method of Misra and Fridovich[26] on the basis of their ability to inhibit free radical chain oxidation in which O_2 was a chain-propagating radical and the autooxidation of epinephrine was included. Human erythrocyte SOD was used as a standard and the activity was expressed in Unit/g.Hb.

CAT activity was determined by monitoring the disappearance of H_2O_2 at 240 nm. CAT activity was expressed as unit/mg.protein. One unit of enzyme was the amount necessary to decompose 1 µmol of H_2O_2 per min at 25°C[27].

GPX activity was measured by the method of Paglia et al[28]. Heparinized whole blood (0.05 ml) was diluted with 1 ml diluting agent, incubated for 5 min, and then 1 ml of double strength Drabkin's reagent was added and mixed well. GPX catalyses the oxidation of glutathione (GSH) by cumene hydroperoxide. In the presence of GSH reductase and nicotinamide adenine dinucleotide phosphate (NADPH), the oxidized GSH was immediately converted to the reduced form with the concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance at 340 nm was measured by a spectrophotometer.

The assay of oxidative damage biomarkers

MDA concentration in plasma was measured by the high-performance liquid chromatography technique (model 4225; Unicam, LCD/Analytical) in which the MDA–thiobarbituric acid (TBA) adduct was separated[29]. Briefly, plasma lipoperoxides were hydrolyzed by boiling in diluted phosphoric acid. MDA was reacted with TBA to yield the MDA–TBA adduct. The protein-free extract was fractionated on a C18 column of octadecyl silicagel to separate the MDA–TBA adduct by elution with methanol/phosphate buffer and quantified by a spectrophotometer at 532 nm (model: crystal 200; Unicam LCD/Analytical Inc.).

8-OHdG levels were measured essentially as described previously[30]. Briefly, an automated column switching LCEC method for 8-OHdG was based on the unique selectivity of the integral porous carbon column for purines.

Samples were injected onto a C8 column and the band containing 8-OHdG was then quantitatively trapped on a carbon column. The selectivity of the carbon column for 8-OHdG allowed elimination of interfering peaks by washing the column with a second mobile phase and then eluting 8-OHdG to an analytical C18 column with an identical mobile phase containing adenosine to displace 8-OHdG. Detection with series colorimetric electrodes provides qualitative certainty for 8-OHdG peak by response ratios.

RCD were detected by their reactivity with DNPH to form protein hydrazones and their amount evaluated at 370 nm and expressed in nmol/mg.protein[31].

Vitamins

The evaluation of vitamin C was performed based on the method of Jacques-Silva et al. Plasma was precipitated with one volume of a cold 5% trichloroacetic acid solution and by centrifuged. An aliquot of 300 mL of the supernatants was mixed with 2,4-dinitrophenylhydrazine (4.5 mg/mL), $CuSO_4$ (0.075 mg/mL) and trichloroacetic acid 13.3% (final volume 1 mL) and incubated for 3 hours at 37 °C. Then, 1 mL of H_2SO_4 65% (v/v) was added to the medium. The level of vitamin C was calculated using a standard curve and expressed as mg vitamin C/ml of plasma[32].

Determination of plasma vitamin E was performed by HPLC(model 4225; Unicam, LCD/Analytical). To precipitate proteins, aliquots (200 µl) of freshly plasma samples were mixed with 200 µl of HPLC-grade water and 400 µl of ethanol. For the vitamin extraction, 800

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μl of hexane were added, and mixed for 30 min. Then, 600 μl of upper layers (hexane) were collected, dried in Speed-Vac system, and dissolved in 150 μl of mobile phase with 0.5% (m/v) BHT for the vitamin stabilization. An aliquot of 20 μl of this solution was injected into the HPLC system[33].

Statistical analysis

All data were expressed as mean ± SEM (standard error of mean). The results were analyzed by one-way analysis of variance (ANOVA) followed by Paired-Samples T Test to evaluate the significance of the difference between groups using Statistical Package for Social Science (SPSS 16.0 for windows). A significant change was accepted at $p < 0.05$.

RESULTS

TABLE 1 demonstrates clinical characteristics of cases at first and second monitoring in this study. All subjects were at the same range of age with mean of 32.2 to 33.5 years. There were no significant differences between groups for hematocrit (Hct) and blood pressure as they were evaluated at first and/or second time.

TABLE 1 : Clinical descriptive data of the studied groups. Data are expressed as mean ± SD.

studied groups	blood pressure		Hct	Age (year)
	diastolic	systolic		
group1(N=13)	8.82±0.87	13.54±0.84	51.29±5.36	32.76±8.55
group2(N=14)	9.20±0.46	14.16±1.45	49.02±7.26	32.21±7.95
group3(N=13)	9.15±0.89	13.80±1.06	48.86±5.23	33.53±8.51

Antioxidant enzyme activities in peripheral and cupping blood samples at first and second samplings are presented in TABLE 2. In both kinds of blood, higher activities were found for SOD, CAT, and then GPX in each studied group respectively. On the other hand, activities of three studied enzymes increased in group 2 and 3 respectively. However, the increases in SOD and GPX activities for group 2 were significant with respect to group 1 as they were assayed in cupping or peripheral blood at first sampling. In both peripheral and cupping blood, differences between 1 and 3 as well as 2 and 3 groups were significant at first or second sampling. In each studied group, the activities of three enzymes in peripheral blood were significantly higher than cupping blood at first or second sampling. Comparison of second with first sampling showed that the activities of all three enzymes in peripheral and/or cupping blood were higher than the same kind of blood at first sampling. Among these enzymes, there was only insignificant increase in CAT activity in cupping and peripheral blood samples at second sampling with respect to the same kind of blood collected at first sampling.

TABLE 3 shows the levels of oxidative damage biomarkers; MDA, RCD, and 8-OHdG in both peripheral and cupping blood at first and second sampling. Blood samples collected at first sampling revealed significant higher levels of MDA and RCD as compared with the same blood from second sampling in all three groups. In this sampling time, the concentration of MDA and RCD decreased significantly from group 1 to 2 and then 3 in both peripheral and cupping blood. At

TABLE 2 : The levels of antioxidant enzyme activities in peripheral and cupping bloods at first and second sampling.

enzyme		first sampling		second sampling	
		peripheral	cupping	peripheral	cupping
SOD (U/g.Hg)	Group1	375.45 ± 8.40*	353.45 ± 8.08	424.00 ± 13.00*	406.00 ± 12.84
	Group2	415.77 ± 8.64*®¶	403.83 ± 9.46®¶	463.57 ± 13.14*	444.21 ± 12.59
	Group3	455.47 ± 10.59*®	441.17 ± 10.48®	504.53 ± 11.90*®	484.61 ± 12.31®
GPX (U/g.Hg)	Group1	101.37 ± 5.35*	91.87 ± 5.13	121.00 ± 7.70*	110.00 ± 7.15
	Group2	133.44 ± 4.48*®¶	123.33 ± 4.37®¶	145.71 ± 5.80*®¶	130.92 ± 5.45¶
	Group3	184.29 ± 6.19*®	170.17 ± 5.43®	199.61 ± 7.20*®	180.69 ± 6.43®
CAT (U/g.Hg)	Group1	155.45 ± 4.09*	151.75 ± 4.35	164.46 ± 5.51*	158.53 ± 5.76
	Group2	170.38 ± 6.52*¶	166.77 ± 6.48¶	181.85 ± 7.96¶	170.64 ± 12.18¶
	Group3	195.29 ± 8.96*®	190.88 ± 9.35®	212.15 ± 10.90*®	206.30 ± 9.87®

***Differed significantly as compared with cupping blood ($p < 0.05$). ® Differed significantly with respect to group 1 ($p < 0.05$). ¶ Differed significantly in regard to group 3 ($p < 0.05$).**

TABLE 3 : The levels of oxidative damage biomarkers in peripheral and cupping bloods at first and second sampling.

enzyme		first sampling		second sampling	
		peripheral	cupping	peripheral	cupping
MDA (µmol/ml)	group 1	37.20 ± 2.37*	39.83 ± 2.48	32.92 ± 2.92*	35.76 ± 2.86
	group 2	32.66 ± 1.71*¶	35.66 ±1.71¶	30.07 ± 1.94*¶	32.42 ± 2.17¶
	group 3	22.23 ± 1.52*®	24.70 ± 1.66®	19.30 ± 1.82*®	22.46 ± 1.98®
RCD (nmol/mg.pro)	group 1	57.45 ± 2.35*	60.12 ± 2.45	46.30 ± 2.98*	49.69 ± 3.40
	group 2	52.88 ± 1.70*¶	56.38 ± 1.66¶	44.50 ± 1.81*¶	46.57 ± 2.03¶
	group 3	42.88 ± 1.68*®	44.52 ±1.69®	32.69 ± 1.93*®	34.92 ± 1.99®
8-OHdG (nmol/mg.pro)	group 1	7.75 ± 0.10*	7.94 ± 0.12	7.59 ± 0.11*	7.90 ± 0.17
	group 2	8.47 ± 0.18*®	8.56 ± 0.20	8.34 ± 0.21*®	8.77 ± 0.22
	group 3	8.26 ± 0.25*	8.54 ± 0.27	8.01 ± 0.28	8.18 ± 0.33

*Differed significantly as compared with cupping blood ($p < 0.05$). ® Differed significantly with respect to group 1 ($p < 0.05$). ¶ Differed significantly in regard to group 3 ($p < 0.05$).

TABLE 4 : The levels of vitamin C and E activities in peripheral and cupping bloods at first and second sampling.

vitamin		first sampling		second sampling	
		peripheral	cupping	peripheral	cupping
vitamin C (mg/dL.10^{-2})	group 1	45.58 ± 1.45	43.98 ± 1.46	45.92 ± 1.81	45.00 ± 1.37
	group 2	42.74 ± 0.75	43.92 ± 1.41	45.10 ± 1.99	44.70 ± 1.41
	group 3	43.40 ± 1.38	44.74 ± 1.13	46.16 ± 0.99	42.76 ± 1.47
vitamin E (mg/dL.10^{-2})	group 1	70.94 ± 1.12	71.74 ± 2.19	72.74 ± 3.84	71.30 ± 1.45
	group 2	70.44 ± 1.46	72.76 ± 2.31	71.12 ± 2.14	75.72 ± 1.04
	group 3	71.40 ± 2.74	71.78 ± 3.65	71.10 ± 3.51	69.26 ± 2.67

second sampling, the levels of MDA and RCD fall down insignificantly from group 1 to 2 and decreased significantly from group 2 to 3 in both cupping and peripheral blood. However, the level of 8-OHdG elevated significantly from group 1 to 2 and decreased insignificantly from group 2 to 3 as evaluated in peripheral or cupping blood whether in first or second sampling. In both kinds of blood, significantly higher levels of these three indexes were found in cupping blood as compared with peripheral blood.

Vitamin C and E activities in peripheral and cupping blood samples at first and second samplings are presented in TABLE 4. In both kind of blood, we found no tangible differences in vitamin C and E activities at first and/or second sampling.

DISCUSSION

Interest in complementary and alternative medicine (CAM) has recently grown in many countries around the World[34]. Today, wet cupping, as a CAM method, is used mainly in Asia for a variety of illnesses[35]. On other hand, sporting is an important component of a healthy life style and is recommended by clinicians and scientists[36]. Many people try it to improve the body's strength and recovery from fatigue.

Several studies documented the effect of sporting on antioxidant enzyme activities. Most of them reported the increase in antioxidant capacities as a result of exhaustive exercise or exercise with moderate intensity[37-39]. On the other hand, sporting increases oxygen consumption, of which 2% may be converted to ROS[36, 37, 40]. The generated ROS during exercise may influence the redox-regulated processes by which the adaptive responses to exercise occur[37]. In addition ROS may activate redox sensitive NF-κB signaling and increase gene expression of antioxidant enzymes[41, 42]. In agreement with these documents, our finding showed significant increase in antioxidant enzyme activities in both peripheral and cupping bloods of professional athletes with respect to non-athletes at first sampling. After then, the activities of these enzymes increased remarkable at second sampling in both peripheral and cupping bloods in all studied groups.

It is our conviction that wet cupping is a causative factor for expression of antioxidant enzymes within the period time between first and second sampling, leading to elevation in antioxidant enzyme activities. In addition, this process may promote the observed effect of sporting on our studied enzyme activities. Accordingly, we found increasing effect of sporting on these enzymes at second sampling in both peripheral and cupping bloods with respect to the same kind of blood collected at first sampling.

Vitamin E may be the last line of defense against membrane phospholipids peroxidation[15,16]. The main function of vitamin E is protector of the polyunsaturated fatty acids of cell membranes against free radical damage[43]. Also Vitamin C is one of the most important biomolecules, which acts as antioxidant and radical scavenger. The antioxidant behavior of vitamin C is due to its ability to terminate the radical chain reactions[44]. In this study, we found no significant differences between studied groups as they were evaluated for vitamin E and/or C. It can be referred to effective role of enzymatic defense activity in neutralizing ROS. DNA can undergo rapid repair and it is better protected against oxidative damage[45]. Our results revealed that 8-OHdG concentration, as a biomarker of DNA damage, increased in amateur athletes and decreased in professional athletes with respect to non-athletes and amateur athletes, respectively. This is in agreement with other studies[38,46] and may be due to activation of DNA repair systems in professional athletes. Regular training protects DNA against exercise-induced damage, probably via adaptive responses such as upregulation of cellular proteolytic enzymes and repairing systems[46]. In addition we found that 8-OHdG amount in second sampling was lower in respect to first sampling, and this decline in professional athletes was more remarkable as compared with non-athletes. Thus, wet cupping augments exercise-induced adaptive responses to oxidative stress.

MDA is the most sensitive biomarkers of oxidative stress[40]. Jennifer M. Sacheck et al. showed that MDA increased immediately post exercise and returned to baseline levels 6 hour after exercise[47]. But Richard J. Bloomer et al. indicated that MDA level is lower in trained individuals in regard to untrained individuals[45]. Our data, also, indicated decreased MDA in athletes with respect to non-athletes and in second sampling was lower than first sampling.

RCD is the most used biomarker for determination of oxidative damage of proteins[48]. Some studies suggested increase in RCD with exercise-induced oxidative stress[37,40]. We found lower RCD in athletes in regard to non-athletes in agreement with Richard J. Bloomer's study[45], and this decrease was more remarkable in second sampling. Exercise may increase the activity of proteasome complex, which is responsible for degradation of modified and damaged proteins[49,50]. It is likely that wet cupping act as procedure to increase activity of these complexes.

In conclusion this study suggests that sporting activity together with wet cupping induces adaptive responses in antioxidant and repair systems. The effects of these methods increase the protection against ROS and decrease the accumulation of oxidative damage biomarkers. Our study suggests that wet cupping together with sporting is more effective on antioxidant defense as they were considered alone.

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