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RESEARCH ARTICLE

SUBMAXIMAL EXERCISE ACCOMPANIED WITH MILD DEHYDRATION INCREASES OXIDATIVE LOAD ON UNACCLIMATIZED HUMANS

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ABSTRACT

Benefits of submaximal exercise in maintaining good health and in prevention of diseases are well known. However, there is conflict of studies reporting interaction between submaximal exercise and pro-oxidant/antioxidant balance. Generation of excess free radicals is known to impair many physiological and biochemical functions resulting in overall performance decrement. Therefore to address this issue, various oxidative stress parameters were estimated in plasma samples of unacclimatized human participants who were challenged with submaximal exercise accompanied with mild dehydration of 2% body weight at 45^0C in human climatic chamber. Exposure of unacclimatized human volunteers to submaximal exercise and dehydration at 45^0C in human climatic chamber resulted in significant increase in various oxidative parameters. Malondialdehyde, marker of lipid peroxidation was significantly increased (from 0.74±0.08 µmol/l to 1.01±0.11 µmol/l, $p<0.001$) post exercise. Similarly, a significant increase in hydroperoxide ($p<0.001$) and advanced oxidation protein products ($p<0.01$) was also observed. Though there was a significant increase in antioxidant level (GSH; $p<0.001$), the overall ratio of GSH/GSSG decreased implicating an increased oxidative stress. Submaximal exercise accompanied with mild dehydration in heat is associated with increased oxidative stress in unacclimatized human subjects.

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INTRODUCTION

Effects of exercise on human health and performance are a topic of debate for the last several decades. Debilitating effects of high intensity exercise are now well understood. Exhaustive or high intensity activity performed under heat has even more devastating effects. Rowell and his co-workers have shown a significantly lower cardiac output, central blood volume and stroke volume during physical exercise in a hot environment (Rowell *et al*., 1996). High intensity exercise in heat has also been known to cause muscle damage. Free radical generation has been shown to be responsible for such muscle damage (Aoi *et al*., 2004), (Alessio *et al*., 2000). Various studies have demonstrated that exercise results in generation of free radical only when it is exhaustive (Vina *et al*., 2000), (Lekhi *et al*., 2007), (Belviranli and Gokbel 2006). Since, excessive free radicals are quenched naturally by the body's antioxidant defence system, effects of exercise have also been studied on changes in the levels of enzymatic and non-enzymatic antioxidants (Vina *et al*., 2000), (Ji, 1995). The benefits of moderate or submaximal exercise in maintaining good health and in preventing various diseases are also well known for decades. Despite a large number of existing studies, there is a paradox relating to submaximal exercise and oxidative stress. The debate over oxidative stress and exercise is an outcome of many controversial results. Conflicting effects of exercise on production of free radicals have been observed in both human as well as animal model (Agarwal *et al*., 2009), (Ji, 2000), (Diaz *et al*., 2011), (Nikolaidis *et al*., 2007), (Bloomer *et al*., 2006), (Laaksonen *et al*., 1999). The inconsistency in results of earlier studies may depend on factors such as environmental conditions, training status and different exercise protocols. Moreover dehydration is one important factor has not been considered in such studies. Since alteration in hydration status alone can result in increased oxidative damage (Paik *et al*., 2009), considering dehydration as an integral part of exercise is very important. Moderate and severe dehydration (3% or more) are known to affect pro-oxidant/anti-oxidant balance.

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Whereas, there is only limited data concerning mild dehydration (<3% body weight loss) and oxidative stress (Laitano *et al*., 2012). Interestingly, heat exposure independent of exercise has also been shown to increase oxidative damage (Laitano *et al*., 2010) by reducing antioxidant defense and increasing free radical production (Hass and Massaro 1988). Thus the combined effect of heat stress, exercise and dehydration on the generation of oxidative stress is important. However, there is paucity of studies regarding this combination. Recent study (Hillman *et al*., 2011) has proposed an increased oxidative stress during dehydration in prolonged exercise in the heat, however, this study was performed on trained cyclists. Since athletes might have different response to these conditions, this study cannot be applicable to non-athletic population.

The lacunae in understanding of exercise and dehydration in heat resulted in design of present study. To our understanding, this is the first study that aimed to investigate the effects of submaximal exercise induced mild dehydration in heat on oxidative stress markers and antioxidant status in unacclimatized human volunteers. To provide a complete depiction, our protocol included session of submaximal exercise till the subjects reached a mild dehydration of about 2% of body weight loss. The inconsistent factors were restricted by performing the study in controlled, simulated conditions. Various oxidative stress parameters were analyzed before and after the completion of exercise protocol.

MATERIALS AND METHODS

Participants

Ten healthy males volunteered to participate in the study but due to certain unavoidable reasons four of them could not complete the study and therefore their data is not included. Rest six volunteers (age 31 ± 2 years, height 170.4 ± 7.5 cm, body mass 76.8 ± 5.1 kg, body surface area 1.8± 0.1m^2) completed the study. Inclusion criteria consisted of a medical history free of musculoskeletal, cardiac, endocrine, and heat-related illnesses. Participants were briefed about the experimental procedures and possible hazards before the commencement of the actual experimental protocol.

Ethics, consent and permission

All protocols and procedures were approved by Institutional Human Ethical Committee. Informed written consent was obtained from the participants.

Experimental protocol

The participants were made to perform submaximal exercise till they reached a level of 2% dehydration in human climatic chamber simulated at 45^0C and 30% RH. All the participants were instructed not to engage in any vigorous physical activity for at least 24 hours prior to exercise session. The subjects were asked to ingest 5ml of water per kg body weight 2 hours before reporting to the laboratory to attain a euhydrated state (Montain and Coyle 1992). After reporting to the laboratory, the subjects rested for 60 minutes in a thermoneutral room following which, blood samples were collected, plasma was obtained and stored at -80°C for further analysis. Oral temperature was recorded using YSI electrodes. Initial nude body weight was measured using digital human weighing machine (model PFPF 100K, make PERFECT) before putting the subjects to continuous submaximal work. Participants were subjected to physical exercise of 50 watt intensity (Gaebelein and Senay 1982) until targeted hypohydration level was attained. After the completion of exercise in HCC, the subjects were instructed to exit the chamber following which post exposure data was recorded and blood samples collected and stored at -80°C for further analysis.

Oxidative stress markers

Lipid peroxidation: Malondialdehyde (MDA) was measured in plasma samples as marker of lipid peroxidation (Yagi, 1982).

Protein carbonyl: Protein carbonyl content was estimated using 2,4-DNPH as described earlier (Levine *et al*., 1990).

Hydroperoxide: It was estimated by FOX1 assay (Wolff, 1994). Concentration of hydroperoxide was calculated using extinction coefficient of 1.5 x 10^4 M^{-1} cm^{-1} at 560 nm wavelength.

Advanced oxidation protein products: Spectrophotometric determination of AOPP plasma levels was performed by Witko-Sarsat's method in a micro-plate reader (Witko-Sarsat *et al*., 1998).

Antioxidant status

TAS: Total antioxidant status was measured in plasma as an 2,2'-Azino-di-3-ethylbenzthiazoline sulphonate (ABTS) radical cation decolorizing assay using commercially available kit (Randox Laboratories, UK).

Uric Acid: It was measured in plasma using commercially available kit (Randox Laboratories, UK).

Reduced and oxidized glutathione (GSH and GSSG): Both forms of glutathione were estimated according to the method described earlier (Hissin and Hilf 1976).

GPx: Glutathione peroxidase activity was determined using commercially available kit (Randox Laboratories, UK).

GR: Glutathione reductase activity was measured as described by (Carlberg and Mannervik 1985). Glutathione reductase catalyzes the reduction of GSSG by oxidizing NADPH to NADP+. The activity was measured at 340 nm. The activity was expressed as the amount of NADPH (in micromole) oxidized per minute per ml of plasma.

Statistical analysis

All the results are presented as mean ± SD. The experiments were conducted on two different occasions and the data was analyzed using paired t-test. Significance level was set at p

<0.05. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS Inc version 15.0)

RESULTS

Submaximal exercise in heat increases core body temperature

A significant increase in core body temperature ($p<0.01$) was observed following submaximal exercise at 45^0C, 30% RH (Fig. 1).

Submaximal exercise increases levels of oxidative stress markers

Table 1 depicts changes in levels of oxidative markers following submaximal exercise in HCC. A significant increase in Lipid peroxidation, as indicated by MDA ($p<0.001$), hydroperoxide ($p<0.001$) and AOPP ($p<0.01$) was observed post exercise. After exercise, protein carbonyl also increased but the increase was not significant.

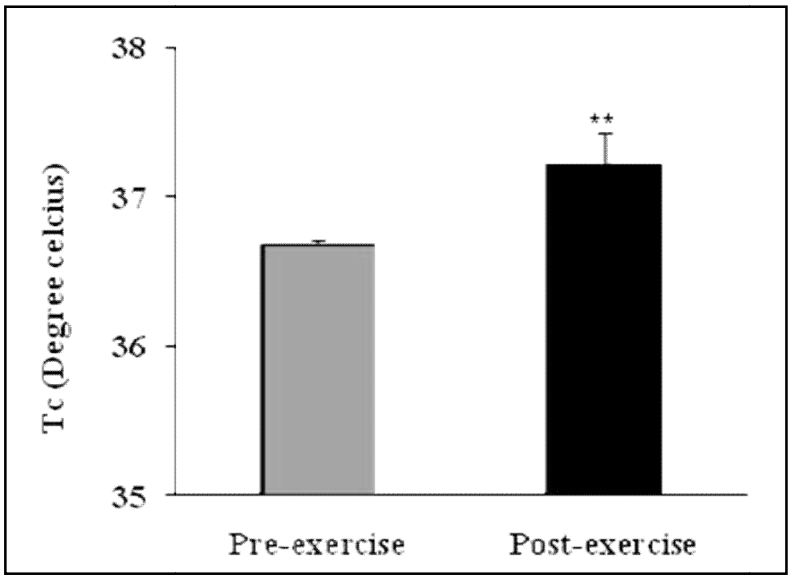
Submaximal exercise alters antioxidant status

As depicted in Fig. 2A, total antioxidant status of plasma increased significantly ($P<0.01$) after exercise. A similar increase in uric acid ($p<0.001$) was also observed (Fig.2B). Submaximal exercise also resulted in significant increase in GSH and GSSG levels (Fig. 3A). However, the percentage increase was higher in GSSG (20% > control) as compared to GSH (12% > control) resulting in decreased GSH/GSSG ratio (Fig. 3B). Following exercise, a significant increase in GPx ($p<0.05$) was also observed (Fig. 4A). In addition, a non-significant increase in GR was also observed (Fig. 4B).

Table 1. Effects of submaximal exercise accompanied with mild dehydration in heat on various oxidative parameters

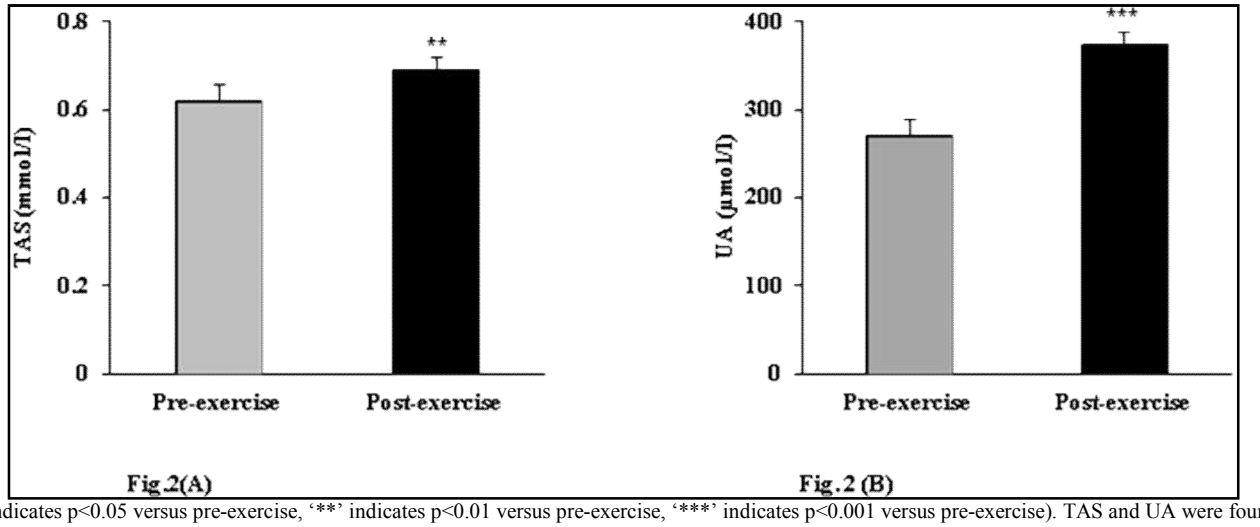
Parameter	Pre-exercise	Post-exercise
Lipid peroxidation (µmol/l)	0.74±0.23	1.01±0.11***
Hydroperoxide (µmol/ml)	428.4±15.7	518.6±12.9***
Protein Carbonyl (nmol/mg protein)	0.526±0.03	0.582±0.07
AOPP (nmol/ml)	82.25±8.6	100.03±5**

('*' indicates $p<0.05$ versus pre-exercise, '**' indicates $p<0.01$ versus pre-exercise, '***' indicates $p<0.001$ versus pre-exercise). Various oxidative stress parameters such as MDA, PC, hydroperoxide and AOPP were found to be significantly elevated following submaximal exercise accompanied with mild dehydration of 2 % body weight loss in heat.



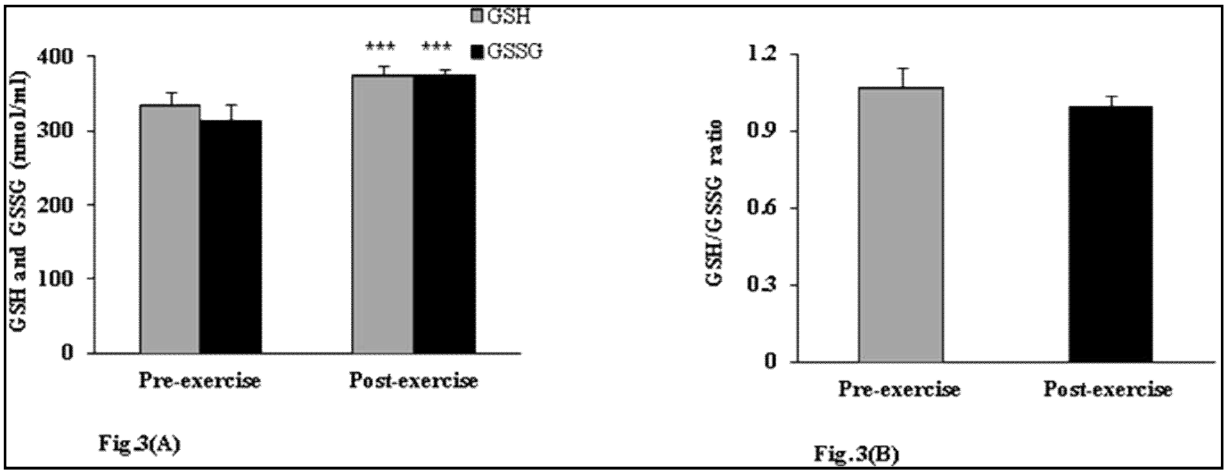
('*' indicates $p<0.05$ versus pre-exercise, '**' indicates $p<0.01$ versus pre-exercise, '***' indicates $p<0.001$ versus pre-exercise). A significant increase in core body temperature was observed following submaximal exercise.

Fig. 1. Effects of submaximal exercise accompanied with mild dehydration in heat on core temperature (Tc)



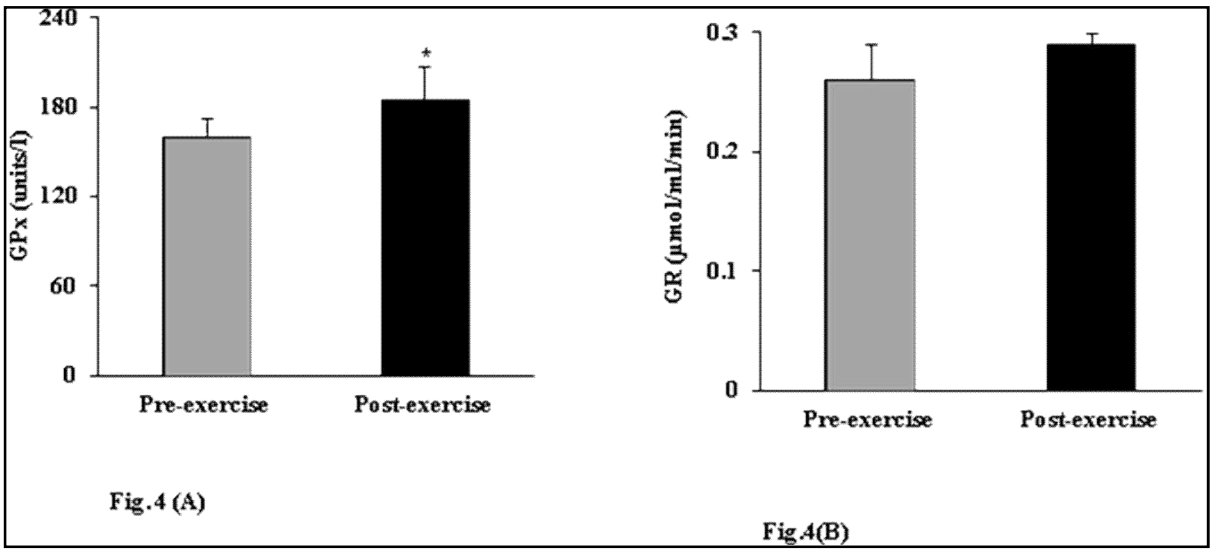
('*' indicates p<0.05 versus pre-exercise, '**' indicates p<0.01 versus pre-exercise, '***' indicates p<0.001 versus pre-exercise). TAS and UA were found to be significantly increased following exercise.

Fig. 2. Effects of submaximal exercise accompanied with mild dehydration in heat on (A) TAS and (B) UA



('*' indicates p<0.05 versus pre-exercise, '**' indicates p<0.01 versus pre-exercise, '***' indicates p<0.001 versus pre-exercise). Decreased GSH/GSSG ratio was obtained post exercise in heat.

Fig. 3. Effects of submaximal exercise accompanied with mild dehydration in heat on (A) GSH, GSSG and (B) GSH/GSSG ratio



('*' indicates p<0.05 versus pre-exercise, '**' indicates p<0.01 versus pre-exercise, '***' indicates p<0.001 versus pre-exercise). Submaximal exercise in heat resulted in significant increase in GPx.

Fig. 4. Effects of submaximal exercise accompanied with mild dehydration in heat on on (A) GPx and (B) GR

DISCUSSION

The relationship between submaximal exercise and oxidative stress has been a topic of debate for the past few years. Though the beneficial effects of exercise on physical health have been well established, its role in increased oxidative stress cannot be neglected. Increased oxygen supply during exercise is considered as the primary reason of free radical generation in muscle. Though a limited supply of free radicals is required for many cellular and molecular pathways, excessive free radical generation has detrimental effects on antioxidant system (Yu, 1994). As shown in our results, combination of heat, mild dehydration and submaximal exercise resulted in significant increase in oxidative markers such as lipid peroxidation, hydroperoxides, protein carbonyl and AOPP. Hydroperoxides interact with proteins to result in formation of reactive carbonyl derivatives in the peptide side chain of arginyl, aspartyl, glutamyl, lysyl, prolyl, and threonyl residues. The formation of carbonyl groups in proteins leads to inactivation of the proteins and increases their susceptibility for proteolytic degradation (Stadtman, 1990). Accumulation of oxidatively modified adducts is dependent upon the rate of free radical generation and their removal by body's defense mechanism. In accordance to another recent study (Diaz *et al*., 2011), our results showed that following submaximal exercise in heat there was a significant increase in the antioxidant status as revealed by increased levels of TAS and UA. Uric acid plays a dual role in human body. At lower concentration, uric acid acts as a powerful scavenger of free radicals and provides about 2/3rd of free-radical scavenging capacity in plasma (Ames *et al*., 1981), (de Oliveira and Burini 2012), (Maxwell *et al*., 1997). However, uric acid values higher than 7.0 mg/dl is a risk factor for the development of many problems such as renal calculi and gout (Roddy and Doherty 2010). A recent study (Knez and Periard 2014) has indicated that high intensity exercise in hot condition increased antioxidant defences rather than exacerbating oxidative stress. They proposed that a critical body core temperature or rate of change in core temperature might be responsible for a significant upregulation in antioxidants, limiting the production of ROS and the subsequent production of oxidative stress. In partial contrast to this study we have observed a significant increase in both the antioxidants as well as oxidants resulting in overall increased oxidative stress.

Another significant endogenous defense system against oxidative stress in body is glutathione system. GSH plays a key role in protection against free radicals due to the high reaction rate of thiols with free radicals. Oxidative conditions result in conversion of GSH to its oxidized form (GSSG). Interestingly, we also showed that submaximal exercise alters glutathione metabolism by increasing both GSH as well as GSSG level. This finding is in accordance with a recent study (Hillman *et al*., 2011). However, the GSH/GSSG ratio was found to be decreased which implies an increased oxidative stress. These changes in GSH and GSSG levels could be attributed to similar changes in GR and GPx levels. GR and GPx act in cycling manner to maintain glutathione homeostasis. Our results indicate increase in glutathione reductase and glutathione peroxidase levels. But the increase was much more in GPx as compared to GR which might explain the decreased GSH/GSSG ratio. Thus, the results of our study provide clear evidence of increased oxidative markers along with increased antioxidant levels but the increase in antioxidants is not sufficient to protect against the oxidative damage. This result is in contrast to a recent study (Knez and Periard 2014) which showed that short duration bouts of submaximal aerobic exercise are sufficient to induce reactive oxygen and nitrogen species but the antioxidant defense system is capable of protecting against enhanced RONS mediated oxidative damage (lipid peroxidation and protein carbonyl). The difference in results could be due to fact that earlier studies did not consider the dehydration that is accompanied with exercise. It is well known that thirst sensation appears once the body has reached a mild dehydration of 1-2% body weight reduction (Swaka and Montain 2000). Since it is a practical habit of people drinking water on perceived thirst sensation, individuals working under hot conditions usually under consumes fluids and remain dehydrated. Therefore, it is of utmost importance to know the effects of hydration status and intensity of exercise for people engaged in physical activities in hot environment.

Conclusion

In conclusion, the results of our study have demonstrated that submaximal exercise accompanied with mild dehydration in heat alters pro-oxidant/antioxidant ratio in unacclimatized volunteers. Thus, people undergoing mild dehydration during submaximal exercise in heat develop oxidative stress which has been known to exert negative effects on overall health and physical performance of the individual. The present study is beneficial to a larger section of population, since exercise and dehydration are very important aspects determining the health and performance of athletes, occupational workers and people who are engaged in physical activities under hot environment.

Competing interest

The authors declare that they have no competing interests.

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Consent to publish

Consent of participants was obtained for publishing this data.

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