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Immune responses to exercising in a hot environment in soccer players

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Abstract

Introduction: Hot temperature and exercise independently lead to metabolic changes in the human body and depress the immune system. Changes on immunoglobulin A (IgA) and cortisol in response to an intensive exercise in hot environment especially in the soccer players are not well known. Thus, the aim of this study was to investigate the effect of an intensive exercise in thermoneutral and hot conditions on salivary IgA (s-IgA) and cortisol concentrations in soccer players.

Material & Methods: Twelve elite male soccer players (age, 21 to 34 years) participated in this study as the subject. Total unstimulated saliva samples were collected before, immediately and 30 min after the exercise training in

thermoneutral (HT: 20 °C and 20% RH) and hot environments (HT: 30 °C and 20% RH). Water was available ad-libitum.

Results: s-IgA and cortisol levels were increased after an intensive exercise at both environments and their levels were significantly higher than baseline until 30 min after the exercise ($P<0.05$). Total protein concentration was increased 30 min after the exercise in the heat ($P<0.05$), however no significant differences were observed between two occasions. Salivary flow rate was not affected by 2 conditions or differed at any time-point post-exercise. No significant differences were observed in s-IgA and cortisol levels between two environments.

Conclusions: In conclusion, enduring hot temperature intensified stressful responses elicited by intensive exercise. This study advocates that hot temperature deteriorates exercise performance under exhaustive stress and effort conditions in soccer players.

Keywords: Hot environment, Immunoglobulin A, Cortisol, Saliva, Soccer players

1. Introduction

Physical activity induces physiological adjustment to support bodily changes during exercise. This adjustment varies with the duration (1), types and intensity of exercise (2), training level (3) and environmental conditions (4). The analysis of salivary components such as total protein, α-amylase, immunoglobulin A (IgA), nitric oxide (NO) and cortisol may signify a non-invasive technique to determine the relationship of the intensity, duration, temperature, relative humidity and type of exercise with the changes that these situations could cause on the immune system and on the physical stress of the athlete (4-6). Extreme environments represent a significant source of physiological stress that can bring about a disruption of normal immune system function (7). When combined with exercise, the presence of two forms of physiological

stress may produce changes in immune cell number and function that are in excess of those observed with either stressor alone (7,8).

Several studies have investigated the effects of exercise in different situations on the immunological system by salivary IgA (s-IgA) and have reported decreased (9), increased (10) or unchanged (11) IgA levels post exercise. A scientific investigation demonstrated that a 100-km ultra-marathon induced negative immunological changes (12). As a consequence, the authors recommended that exhaustive physical exercise would cause increased vulnerability to infections (12). A decrease in the s-IgA concentration has been implicated as a possible causal factor in the increased susceptibility of athletes to upper respiratory tract infections (URTI) (13).

Cortisol has been shown to inhibit transepithelial transport of s-IgA (14), to inhibit in vivo B lymphocyte antibody synthesis (15) and has been implicated in the decreased B lymphocyte antibody synthesis after exercise (16). Prolonged exercise in the heat is associated with a greater plasma cortisol response compared with prolonged exercise in thermoneutral conditions (17). With this information in mind we hypothesised that intensive exercise in the heat would result in a reduction in s-IgA responses of greater magnitude than when the same exercise is performed in thermoneutral conditions.

Soccer is probably the most popular sport worldwide in terms of its both participation and spectator levels (18). Soccer is a sport play-able in various condition of environment, and one factor that affects this game is in heat situation. Previously Sari-Sarraf et al. (2008) indicated that IgA secretion rate, IgA and cortisol concentration had no any significant changes after repeated bouts of soccer-specific intermittent exercise (19). In the another study, Sari-Sarraf et al. (2011) found that s-IgA secretion rate were increased and cortisol concentration had not significant changes immediately after completing the soccer-specific intermittent protocol in the heat (20). The effects of hot environment on s-IgA and cortisol concentration in soccer are not well known; therefore the aim of the present study was to investigate the effects of an intensive exercise in heat on s-IgA and cortisol concentrations in soccer players.

2. Material & Methods

Subjects

Twelve, healthy elite male soccer players (Table 1) volunteered to participate in this study. All subjects were elite soccer player with a minimum of 5 years competitive experience. All subjects gave written informed consent before starting the study, which received local ethics committee approval. This investigation was approved by the Ethics Committee in Research of the Islamic Azad University, Marvdasht branch, Iran. There were no reported symptoms of infection and no subjects took medication in the 6 weeks prior to the study.

Table 1. Anthropometric characteristics of the subjects (Mean ± SD) [n = 12].

Age (y)	25.5 ± 4.5
Height (cm)	171.7 ± 6.7
Body mass (Kg)	66.3 ± 6.8
BMI ($Kg.m^{-}$)	22.5 ± 1.8

Experimental procedures

During the 24 h prior to each exercise trial subjects were required to refrain from training. On two occasions separated by at least 7 days, subjects reported to the laboratory at 1200 hours following a 4 h fast. The exercise trials were performed at 12:00 when s-IgA concentration appears to stabilise after a decrease during the morning (21). Subjects drank only water from 8:00. Subjects run for 40 min on a treadmill at 70 to 80 % of VO_{2max} in an environmental chamber on one occasion at a temperature and relative humidity of 30 °C and 20% (HOT) and on another occasion at a temperature and relative humidity of 20 °C and 20% (NORMAL). Each participant was equipped with a heart rate monitor (Polar, FS3c, Finland) to ensure accuracy of the exercise level. The trials were completed in a randomised order. On arrival at the laboratory subjects were asked to empty their bladder and bowels and nude body mass was obtained (Seca 705; Hamburg, Germany). The 40 min exercise bout was performed with a fan placed 1 m in front of the treadmill with the wind speed set at 2.0 m/s (4). Water consumption was permitted ad libitum throughout the trials with exception of the 15-min period before a saliva collection.

Saliva collection and analysis

In order to minimise the effects of circadian variation and habitual activity known to cause alterations in salivary immunoglobulin and cortisol levels (22,23), saliva samples were collected at the same time of the day and on the same day of the week. Subjects were seated for 10 min prior to the resting and 30 min post-exercise sample collection. Unstimulated saliva samples were collected over a 2-min period into pre-weighed tubes (Sarstedt, Leicester, UK) at pre-exercise, post-exercise, and 30 min post-exercise. Each subject was asked to swallow in order to empty the mouth before a saliva sample was collected. The saliva sample was collected by the subject placing the polyester salivette swab (diameter 1 cm, length 2.5 cm) under the tongue for exactly 2-min. The polyester swab was then replaced in the inner snap seal container and back in the salivette outer centrifuge tube. Whole saliva samples were collected while the subject sat quietly in the laboratory in temperate conditions with minimal orofacial movements during the collection. Saliva volume was estimated by weighing to the nearest mg and saliva density was assumed to be 1.00 g/ml (24). From this, salivary flow rate was determined by dividing the volume of saliva by the collection time. Total protein concentration in saliva was analysed using albumin as standard (25). Saliva samples were stored in their plastic containers at –40°C prior to analysis. After thawing, the swabs were spun at 3,000 g for 5 min at room temperature allowing the collection of saliva in the bottom of the salivette centrifuge tube for analysis. The s-IgA and cortisol concentrations were determined using an enzyme-linked immunosorbent assay (ELISA) kits (Diametra Italy, Inc). The intra and inter-assay coefficients of variation for s-IgA were 8 and 5% respectively and a sensitivity of 0.5 μg/ml. The intra and inter-assay coefficients of variation for cortisol were 9.3 and 7% respectively and a sensitivity of 0.05 ng/ml.

Statistical analysis

Data in figures are presented as mean (SEM). The sample size was estimated to be n = 10 (26) using previous data examining the effects of prolonged exercise on saliva parameters (27). We estimated the effect size for saliva flow rate, s-IgA and cortisol concentration to be ~ 0.8.

Alpha and power levels were set at 0.05 and 0.8, respectively, both of which are standard estimates. To allow for dropout we recruited n = 12 subjects. The data were examined using a two-factor (2 trial·3 time measurements) repeated measures ANOVA design. Assumptions of homogeneity and sphericity in data were checked and, where appropriate, adjustments to the degrees of freedom were made. Significant differences were analysed using post hoc Tukey's HSD test. Statistical significance was accepted at $P<0.05$.

3. Results

Changes of salivary flow rate, total protein, s-IgA and cortisol concentration in the hot and thermoneutral conditions are presented in the Figure 1-4. Salivary flow rate was not affected by 2 conditions or differed at any time-point post-exercise (Figure 1).

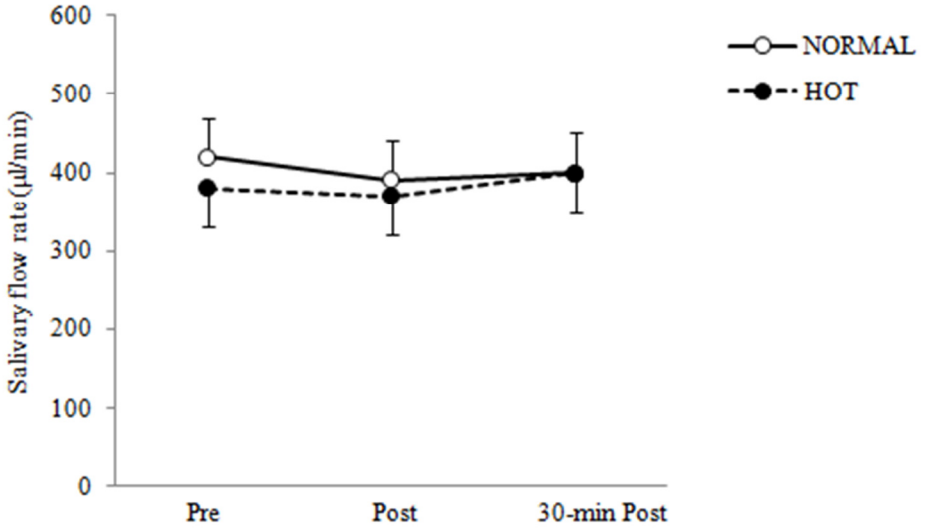

Figure 1. The effect of intensive exercise in the hot and normal conditions on the salivary flow rate. Values are means (± SD).

Total protein concentration was increased 30 min after the exercise in the heat ($P<0.05$), however no significant differences were observed between two occasions (Figure 2).

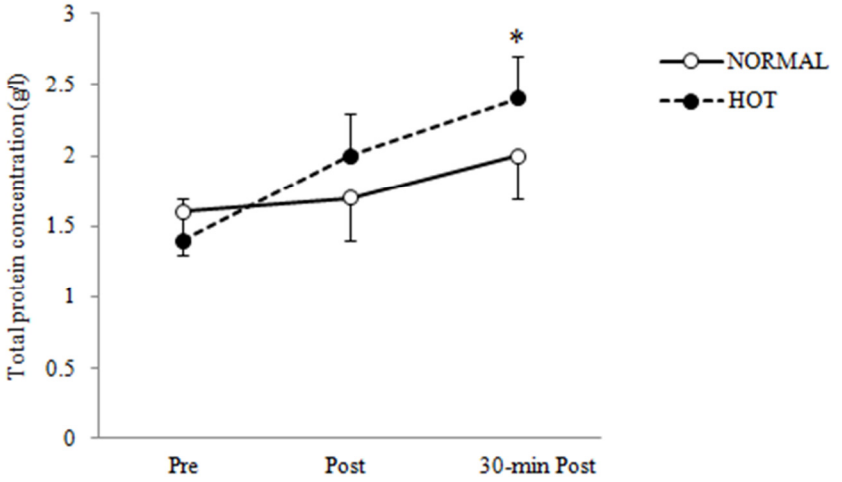

Figure 2. The effect of intensive exercise in the hot and normal conditions on total protein concentration. Values are means (± SD). Significantly higher than pre-exercise, * P < 0.05.

The repeated measures ANOVA indicated that s-IgA and cortisol levels were increased after an intensive exercise at both environments and their levels were significantly higher than baseline until 30 min after the exercise (P<0.05). No significant differences were observed in s-IgA and cortisol levels between two environments (Figure 3 and 4).

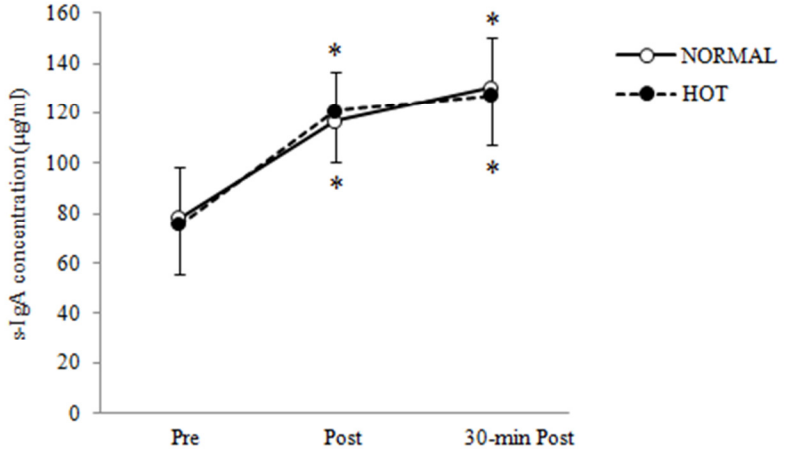

Figure 3. The effect of intensive exercise in the hot and normal conditions on the s-IgA concentratipon. Values are means (± SD). Significantly higher than pre-exercise, * P < 0.05.

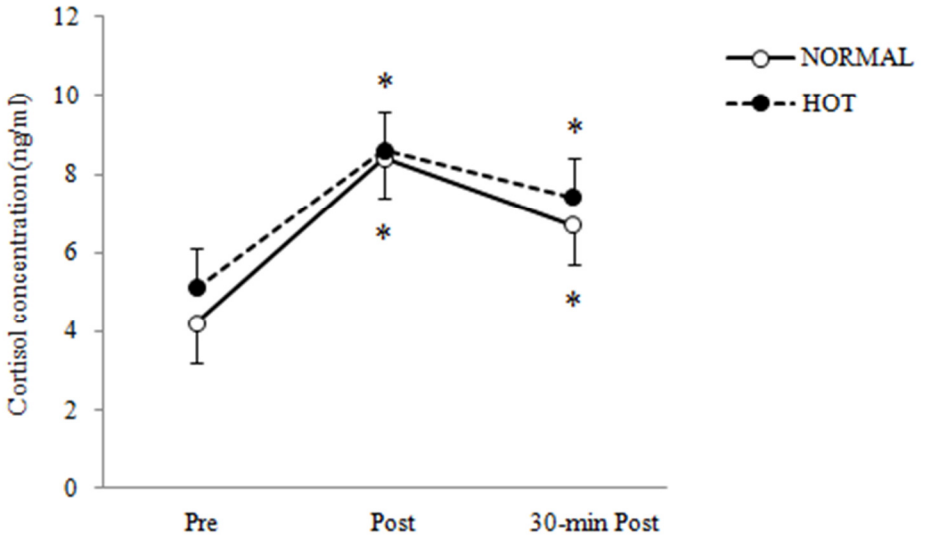

Figure 4. The effect of intensive exercise in the hot and normal conditions on the cortisol concentration. Values are means (± SD). Significantly higher than pre-exercise, * $P < 0.05$.

4. Discussion

Soccer players must train hard and may also be at increased risk of URTI and compromised immune function (28). Changes in s-IgA have been coincident with or preceded the appearance of URTI in collegiate soccer players (29). Soccer is a sport play-able in various condition of environment, and one factor that affects this game is in heat situation. Exercise in a hot environment produced a greater degree of heat-induced physiological stress than exercise in a cold environment (30). The aim of this study was to investigate the effect of an intensive exercise in normal and hot environments on s-IgA and cortisol concentrations in soccer players.

Our data revealed that salivary flow rate was not affected by 2 conditions or differed at any time-point post-exercise. Laing et al. (2005) reported that salivary flow rate was decreased after prolonged exercise in a hot environment in trained cyclists (4). They argued an increase in sympathetic nervous system (SNS) activity, fluid availability and body

fluids during dehydration may explain the decrease in salivary flow rate during prolonged exercise (4). Increases in SNS activity cause vasoconstriction of the blood vessels to the salivary glands which in-turn may limit water availability for saliva production (31).

On the other hand, research results have shown that performing prolonged exercise with sufficient fluids to offset fluid losses prevents the decrease in salivary flow rate but does not prevent the increase in plasma catecholamines (32). This suggests that fluid availability *per se* has a greater involvement in the decrease in salivary flow rate during prolonged exercise than neuro-endocrine regulation (32). During prolonged exercise with restricted fluid intake, plasma volume change has been shown to correlate strongly with saliva flow rate (9). Water was available ad-libitum in the present study thus the unchanged of salivary flow rate may due to body rehydration during the intensive exercise.

According to the current results, a significant increase of s-IgA in saliva post-test was observed. Yet, there was no significant impact of hot temperature on s-IgA responses. As the levels of s-IgA are reflective of the ability of immune system to protect (10), the results of this study suggested no decline in the immune capacity of saliva post-test performed at different levels of thermal stress. Nevertheless, generalization of the complete immune system based only on s-IgA should not be assumed. s-IgA has been previously investigated in other studies that exposed increased levels of s-IgA after performing progressive exercise to exhaustion at different intensities and in the normal and hot environments (4,10). Until now, a decrease was conveyed in the levels of s-IgA in a moderate environment after performing a triathlon (33). Taken together, the current findings suggest that hot temperatures do not intensify s-IgA reduction in response to exhaustive exercise. It is of interest that such inconsistencies may be attributable to deviations in hydration status of athletes. Additionally, Blannin et al. (1998) reported that s-IgA concentration and ratio to osmolality simultaneously increased during exhaustive exercise on an electrically braked cycle ergometer (10). Sari-Sarraf et al. (2011) also found that s-IgA secretion rate were increased a immediately after completing the soccer-specific intermittent protocol in the heat (20). As an important

conclusion, Laing et al. (2005) indicated that exercise impacts the quantity but not the quality of saliva (4). Equally, decreased s-IgA and salivary flow has been previously observed during prolonged exercise in hot environments, and this was described as because of increased SNS activity (34). These deliberations predict the existence of an inverse connection between the levels of cortisol and s-IgA, which was unnoticed in this study, since the elevation in the levels of s-IgA was lagged by increased levels of cortisol. Our results agree with Laing et al. (2005), who found no inverse association between s-IgA and cortisol levels (4).

It has been reported that cortisol concentrations are elevated above normal during a soccer match (35). Most likely the psychological stress of a real match provides an additional stimulus for cortisol secretion besides the physiological stress of exercise. Performing exercise in hot conditions with elevated salivary cortisol concentration should result in a decrease in s-IgA concentration, since cortisol has been shown to inhibit transepithelial transport of s-IgA (36), and to have a delayed inhibitory effect on in vivo B lymphocyte antibody synthesis (37). Nevertheless, a reduction was not observed in s-IgA concentration after intensive exercise in either condition due to the increase of cortisol concentration post-exercise. In agreement with the previous studies (4,20,38), it seems that a short-term effect of cortisol at the concentrations may not have an inhibitory effect on the transepithelial transport of s-IgA. As cortisol has been shown to rapidly inhibit transepithelial transport of s-IgA (36), and to have a more delayed inhibitory effect (taking many hours to days) on in vivo B lymphocyte antibody synthesis (37), we hypothesized that performing intensive exercise in hot conditions (with elevated plasma cortisol response) would result in a decrease in s-IgA concentration of greater magnitude than prolonged exercise in thermoneutral conditions. In contrast, we did not observe a reduction in s-IgA concentration after intensive exercise on either trial (at least within 30 min after exercise) despite a significant increase in plasma cortisol concentration post-exercise. Therefore, these results do not support a short-tem inhibitory effect of elevated cortisol concentration on transepithelial transport of s-IgA after intensive exercise in soccer players. However, it is possible that we may have missed a more delayed effect of elevated cortisol concentration on s-IgA as prolonged exercise

has been shown to cause a reduction in s-IgA level between 2 and 24 h after exercise (21). The results of this investigation draw attention to playing soccer in hot environments. Considering that it increased the physiological stress, the sports medicine clinical team should be vigilant in hot temperature situations throughout competitions.

5. Conclusion

Hot temperature intensified responses of cortisol concentration induced by intensive exercise, signifying more intense stressful responses elicited by the heat in soccer players. Yet, there was no significant influence on s-IgA. These findings suggest that hot temperatures reduce exercise performance in soccer players and increase the probability of disorders caused by maximal effort. It is therefore necessary to highlight the significance of this information to clinical sport coaches responsible for soccer players during competition in hot environments.

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Conflict of interests: None of the authors declare competing financial interests.

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