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Effects of Prolonged Exercise in the Heat and Cool Environments on Salivary Immunoglobulin A among Recreational Athletes

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Abstract: *This study aims to determine the effects of prolonged exercise in the heat (31°C) and cool (18°C) environments on salivary Immunoglobulin A (SIgA) among recreational athletes. Thirteen healthy male participants (age: 20.9 ± 1.3 years old) were recruited and randomised in this cross-over study. In this study, participants performed the exercise trials in the heat environment at 31°C first followed by another exercise trial in the cool environment trial at 18°C or vice versa with one week of recovery period. Physiological parameters (heart rate, body weight changes and oxygen uptake) as well as room temperature and relative humidity were recorded. Cool water (3 ml.kg^{-1} body weight) was given to the participants at every 20 min during both exercise trials. Saliva samples were collected to calculate the saliva flow rate and analysed for salivary Immunoglobulin A (SIgA) concentrations and secretion rate. Paired t-test and two-way ANOVA with repeated measures were performed to analyse the data. The results revealed that saliva flow rate, SIgA concentration, and SIgA secretion rate did not significantly different between exercise trial in the heat and in the cool environments. However, prolonged exercise significantly decreased ($p < 0.05$) saliva flow rate in both trials with the values return to baseline 1 h post exercise. Salivary IgA concentration and secretion rate were not affected by prolonged running. As a conclusion, SIgA responses did not affected by ambient/room temperature. In addition, prolonged exercise with adequate fluid intake during exercise did not supress SIgA responses thus may not increase infection risk among athletes.*

Keywords: *exercise, immune function, mucosal immunity, salivary antimicrobial protein*

I. Introduction

Numerous studies have been conducted to investigate the effects of exercise on immune function. In general, it has been demonstrated that a regular moderate intensity exercise improves immune function while prolonged high intensity exercise may suppress immune function [1]. Therefore, athletes and fitness enthusiasts are very concerned about the effects of exercise on the body immune function. This is because poor health status can eventually leads to a high risk of getting infection, especially on the upper respiratory tract infection (URTI) [2, 3]. The suppression of the immune function may in turn affect the sports performance of the athletes particularly during training and competition. The depression of immune function is most pronounced when the exercise is continuous, prolonged (> 1.5 h), in a moderate to high intensity (55 – 75% of VO_{2max}) [4]. However, the suppression of immune function may return back to the baseline value within hours depending on the exercise intensity and duration [5].

With regards to environmental temperature, usually, it has been found that exercise in extreme temperature may negatively affect the immune function compared to exercise in the thermoneutral and cool condition [6, 7]. Hence, exercising in different environment has different effects on the body immune response. Nowadays, most athletes travel from one county to another country to participate in different type of competition. Even in stage world sporting events such as the Olympic Games, an athlete are required to compete in adverse environmental conditions, for instance in the extreme heat and humidity of Athens in 2004.

Therefore, it is crucial to measure the SIgA responses because it is one of the antimicrobial proteins (AMPs) which act as the body's first line of defence [8]. It involved in the immune exclusion by preventing antigens and microbes from adhering to and penetrating the epithelium, intracellular neutralization, and immune excretion by binding to antigens in the lamina propria [9]. Besides, numerous studies have observed a decline in SIgA levels after prolonged intense exercise in endurance athletes, and sometimes associated with an increased incidence of URTI symptoms [8]. Nevertheless, even though findings regarding the effects of exercise on SIgA level are numerous, but the findings are contradictory [10, 11]. In addition, studies on SIgA in saliva with regards to exercise in the heat and cool environment are also still tremendously limited.

Therefore, this study is warranted to investigate the effects of prolonged running in the heat and cold temperature on SIgA responses among recreational athletes. Most of the previous studies were carried out in room temperature, thus this study will provide useful knowledge regarding the effects of exercise in heat and cool environments on SIgA.

II. Methodology

Research Design

A randomised, cross-over trial was employed for the present study. Participants performed 2 separate trials; exercise in the heat followed by exercise in the cool environment or vice versa. Recovery period between these two trials was one week. All of the tests were conducted in the laboratory of Sports Science Unit (SSU), Universiti Sains Malaysia (USM).

Participants and Sample Size Calculation

Participation in this study was in voluntary basis. This study has been approved by the Human Research and Ethics Committee, Health Campus, Universiti Sains Malaysia, Kelantan (USM/JEPeM/140361). In this study, opportunistic or convenience sampling was used whereby 13 active recreational athletes were recruited among USM students. Participants were healthy males, aged between 18 and 30 years old, non-smokers, and exercise regularly (at least 3 times per week with at least 30 min per session). Those who were having cold or respiratory tract infection at least 2 weeks prior to the study and on medication were excluded in this study. Throughout the study period, participants were required to abstain from taking any supplements that are known to affect immune function, e.g. probiotics, vitamin C, vitamin D and plant polyphenols like Quercetin.

Exercise Trials Procedures

During the first 3 visits to the laboratory, participants performed three preliminary tests which include sub-maximal test, maximal oxygen uptake (VO_{2max}; modified Astrand protocol4) test, and familiarisation trial. The preliminary tests were carried out on a motorised treadmill (TrackMaster TMX425CP, USA) to determine participant's VO_{2max}, to calculate each participant's speed at 60% VO_{2max}, and to familiarise them with the running trial protocol. The 4^{th} and 5^{th} visits to the laboratory were for carried out the actual running trial; running for 90 min at 60% of their respective VO_{2max}. Participants performed 2 running trials in 2 different environments; heat (31°C) and cool (18°C) environments. The order of the running trials was randomised. Heat environment was maintained at 31°C by using halogen lamps (Philips-500W, France) whereby cool environment was set at 18°C by adjusting the temperature on the air conditioner (York, USA). The relative humidity in both running trials was maintained at 70% by using a heated water-bath (Memment W350t, Germany).During each running trial, participant came to the laboratory in the morning after an overnight fast. Upon arrival, participants were asked to measure their nude body by using a body composition analyser (TBF-410 Tanita, Japan) in a closed room. Following that their saliva sample (2 mL) was collected by 5 min un-stimulated dribbling into a pre-weighed sterile bijou tube (Sterilin, Staffordshire, UK). They were asked to sit on a chair, lean the head forward and let the saliva passively dribble into the tube; without using their tongue or any mouth movement. Following that, the bijou tube with saliva was weighed and recorded. Then, participants were cannulated for blood drawing purposes. Blood sample (5 mL) was collected into a K_3EDTA collection tube (Sekusui Insepack, Japan). Patency of the cannula was maintained by heparinised saline whereby 0.2 ml of heparinised saline was injected into the extension tube after each blood withdrawal to avoid blood clotting. After that, participants were given a standardised breakfast; 2 pieces of white bread (Gardenia®, Malaysia) and 250 ml of cool plain water. After resting for half an hour the running trial was begun.

The running trial was begun with a 5 min warm-up at 50% of participant's respective VO_{2max} followed by 90 min running trial at 60% of participant's respective VO_{2max}. The heart rate (heart rate sensor: Sport Tester PE3000, Polar, Finland), oxygen uptake (pre-calibrated gas analyser system: VMax-SensorMedics, USA), room temperature and relative humidity (psychrometer: Extech Instruments RH305, USA) were measured before warm-up, after warm-up, at every 20 min during the running trial and at the end of the trial. During the 90 min of the running trial, participants were asked to drink 3 $mL.kg^{-1}$ body weight of cool water at every 20 min to avoid any adverse effects of dehydration. In addition, participant was directed with a standing fan with speed level 1 to mimic air flow in an open environment throughout the running trial. The second saliva sample was collected immediately at the end of the trial while the final saliva samples were collected 1 h post exercise. During this 1 h period, participants were resting in a comfortable room. Saliva samples were analysed for SIgA concentrations by using a commercially available reagent kit (LDN Labor Diagnostica Nord GmbH & Co. KG, Germany) via an ELISA (Enzyme-Linked Immunosorbent Assay) method. The calculations for determining saliva volume/weight, flow rate, and saliva antimicrobial proteins' secretion rate (SIgA and α-amylase) in this study are as follow:

- Saliva volume (ml) = Difference in weight (g) of bijou tube after collection of saliva assuming a saliva density of 1.0 g/ml
- Saliva flow rate (ml/min) = Saliva volume(ml)/ Collection time (min)
- SIgA secretion rate (µg/min) = Saliva flow rate (ml/min) × Saliva antimicrobial protein concentration (µg/ml)

Statistical Analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) version 22. Descriptive statistics were performed on physiological characteristics. Room temperature, relative humidity, body weight changes were analysed by paired t-test. Two-way ANOVA with repeated measures was performed to measure significant differences between trials and within trials for lysozyme responses, oxygen uptake, heart rate, and rate of perceived exertion. The accepted level of significance is set at $p < 0.05$. Results were reported as means ± standard deviation (SD).

III. Results

Mean body composition and cardiorespiratory fitness of the participants were presented in Table 1. Whereas, room temperature, relative humidity and body weight changes of the participants were presented in Table 2.

Table 1: Body composition and cardiorespiratory fitness of the participants

Variable (N=13)	
Body Composition	
Age (years)	20.9 ± 1.3
Weight (kg)	63.2 ± 7.8
Height (cm)	167.6 ± 5.0
BMI ($kg.m^{-2}$)	22.4 ± 2.1
Cardiorespiratory Fitness	
VO_{2max} ($mL.kg^{-1}.min^{-1}$)	47.0 ± 4.1
VO_2 at 60% VO_{2max} ($mL.kg^{-1}.min^{-1}$)	32.3 ± 3.4

Values are mean ± SD

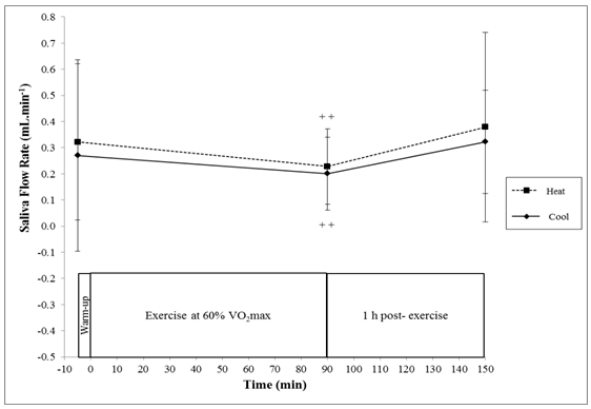
Table 2: Room temperature, humidity and body weight changes of the participants

Variable (N=13)	Heat Trial	Cool Trial
Room Temperature (ºC)	31.0 ± 0.2	18.2 ± 0.3 *
Relative Humidity (%)	70.3 ± 1.0	70.8 ± 1.0
Pre-exercise body weight (kg)	63.2 ± 7.8	63.3 ± 7.9
Post-exercise body weight (kg)	62.3 ± 7.9	62.8 ± 8.0
Body weight changes (%)	1.5 ± 0.6	0.7 ± 0.5 *

Values are mean ± SD.

*significantly different from the heat trial ($p < 0.05$)

Heart rate was significantly increased over time ($p < 0.001$) in both trials. However, it was significantly higher ($p < 0.001$) in the heat trial compared to cool trial. Post-exercise heart rate of the participants for heat and cool trials was 167.2 ± 12.1 and 142 ± 3.3 $beats.min^{-1}$ respectively. Similarly, the oxygen uptake during exercise was significantly increased ($p < 0.001$) from baseline value until end of warm-up session at approximately 50% VO_{2max}, but then it was relatively stable throughout exercise at approximately 60% VO_{2max} with a significant difference ($p = 0.027$) found between trials. There was a significant main effect of time on saliva flow rate during both trials ($p = 0.025$) (Fig. 1) whereby it was significantly decreased post-exercise. Nevertheless, it was increased to approximately baseline values at 1 h post-exercise in both trials. However, there was no significant difference ($p > 0.05$) on saliva flow rate between trials. Besides, there were also no significant main effects of time ($p > 0.05$) on SIgA concentration (Fig. 2) and secretion rate (Fig. 3) in both trials. In addition, there was no significant difference ($p > 0.05$) on SIgA concentration and secretion rate between trials.



$^{++}$ significantly different from respective resting value ($p < 0.01$)

Figure 1: Saliva flow rate ($mL.min^{-1}$) in the heat and cool trials

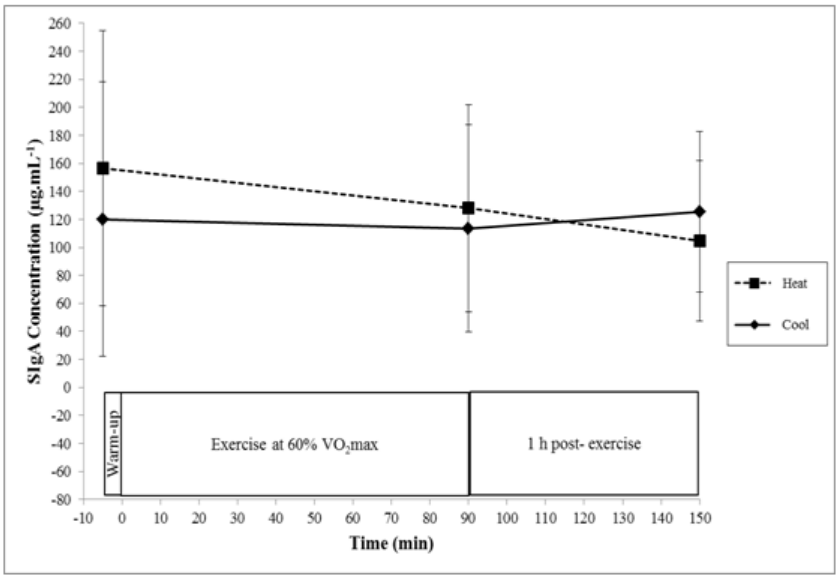
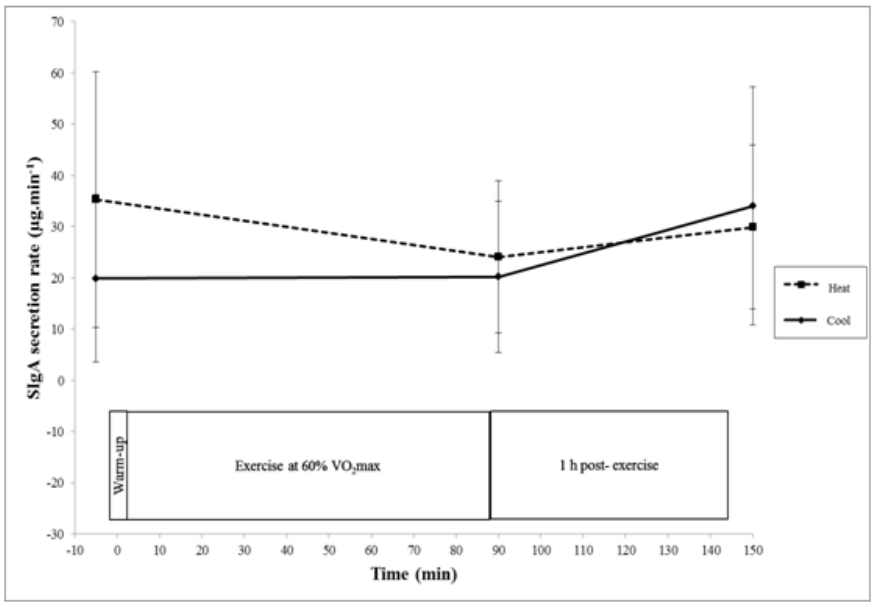


Figure 2: SIgA concentrations (µg.mL^{-1}) in the heat and cool trials.



+ Significantly different from respective resting value ($p < 0.05$).

Figure 3: SIgA secretion rate (µg.min^{-1}) in the heat and cool trials.

IV. Discussion

The main finding in this study is to investigate the effects of prolonged running in the heat and cool temperature on SIgA responses among recreational athletes. In the present study, SIgA concentration (Fig. 2) and secretion rate (Fig. 3) were not significantly different during exercise between the heat and cool trials which is in agreement with the previous studies [12, 13]. However, the previous and the present study also found that within each trial, the prolonged exercise was significantly decreased only in SIgA secretion rate (Fig. 3) [14, 15] which was contrary to the SIgA concentration (Fig. 2). Nevertheless, there were also contradicting study which reported that cold temperature has been associated with the increased [16], no changed [17] and decreased [18, 19] in SIgA responses. Other investigators also reported there were actually a significant increase [20, 21] in SIgA after exercise.

Theoretically, SIgA is secreted by both acinar and ductal units under the stimulation of α- and β-adrenergic and peptidergic receptors where, stimulation of β-adrenoreceptors increased SIgA secretion rate. However, prolonged β-adrenoreceptors stimulation appeared to reduce the replenishment of SIgA into the glandular pool [22]. The inconsistency of the secretory immune response of SIgA concentration and secretion rate may be attributed to the interaction between different types of stimulation and their receptors during

exercise. This discrepancy may also be attributed to the differences during the time of the saliva collection [23] and due to the different methods of expressing SIgA, nutritional status of the individual, and the exercise protocol employed.

Moreover, the saliva flow rate during exercise in the heat and cool trials was not significantly difference (Fig. 1) and was similar to the previous study [24-26]. This was associated with the stimulates sympathetic nervous system due to performing exercise [27]. Therefore, it was suggested that sympathetic nervous system activity influenced the decreased of saliva flow rate [28]. Hence, this explains the reduction on the saliva flow rate found in the present study.

Overall, participants recruited was within the range of Asian populations' BMI [29] which were not obese and was considered 'average' in term of cardiorespiratory fitness [30] (Table 1). While, the room temperature of 31°C and relative humidity of 70% were selected in the present study based on the numerous studies conducted in the heat had set the room temperature and relative humidity at about these values [31-33]. This is also similar to the cool trial which 18°C was selected as the temperature for the cool environment [34-36] (Table 2). Besides, the protocol was chosen as it was intended to suppress the immune function temporarily in order to determine the effects of heat and cool environments on the immune function [1]. This is due to the depression of immune function is most prominent when exercising in continuous and prolonged (> 1.5 h) at moderate to high intensity. The VO_2 values (Table 2) were also being measured to avoid bias between trials. Therefore, based on the present study, both VO_2 values were found with no significantly difference. Hence, the participant performed the exercise at the same intensity as suggested by previous study in both of the trials.

Furthermore, heart rate in the present study was significantly increased during exercise and higher when exercising in a hot temperature compared to cool environment (Table 2)which is consistence with the previous study [6, 36]. Other than that, the body weight changes (Table 2) were also found higher in during the heat compared to the cool trial. Thus, based on the present study, it was found that there were significantly difference between heat and cool trial and it was predictable as in the previous finding [6]. The measurement of body weight changes was crucial because performance can actually alter when dehydration exceeds 2% of body mass [37, 38]. In the present study, the amount of fluid given during both trials was $3mL.kg^{-1}$ body weight at every 20 min. This amount of fluid has been used in previous studies to rehydrate the participants during exercise longer than 1 h [31-33]. Since the body weight changes in both trials were less than 2%, it is considered that the amount of fluid ingested was sufficient to avoid the adverse effects of dehydration in this study.

V. Conclusion

The present study showed that room or ambient temperature (31°C vs 18°C) does not affect the changes in SIgA responses during prolonged exercise among recreational athletes. However, several physiological parameters did affected by the heat temperature. Thus, fluid intake during exercise especially while performing exercise in the heat is warranted. Future studies should measure other parameters which include other salivary antimicrobial proteins to clearly understand their responses in different temperatures.

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