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Effects of prolonged running in the heat and cool environments on selected physiological parameters and salivary lysozyme responses

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ABSTRACT

Introduction: Lysozyme is one of the salivary antimicrobial proteins which act as the "first line of defence" at the mucosal surface. The effects of prolonged exercise in the hot and cool environments among recreational athletes on salivary lysozyme responses are very limited in the literature, especially in the Asian countries.

Objective: To determine the effects of prolonged running in the hot and cool environments on selected physiological parameters and salivary lysozyme responses among recreational athletes.

Methods: Randomised and cross-over study design. Thirteen male recreational athletes (age: 20.9 ± 1.3 years old) from Universiti Sains Malaysia participated in this study. They performed two separate running trials; 90 min running at 60% of their respective maximum oxygen uptake ($\dot{V}O_2$max) One running trial was performed in the hot (31°C) while the other was in the cool (18°C) environment and this sequence was randomised. Each running trial was started with a 5 min warm-up at 50% of participant's respective $\dot{V}O_2$max Recovery period between these two trials was one week. In the both trials, saliva samples, blood samples, heart rate, ratings of perceived exertion, skin and tympanic temperatures, oxygen consumption, nude body weight, room temperature, and relative humidity were collected.

Results: Participants' skin temperature, tympanic temperature, body weight changes, heart rate, ratings of perceived exertion, and plasma volume changes were significantly higher ($p < 0.05$) in the hot trial compared to the cool trial. Saliva flow rate was not significantly ($p = 0.949$) different between the hot (0.32 ± 0.08 ml/min) and cool (0.27 ± 0.05 ml/min) trials. However, in each trial, it significantly decreased ($p < 0.05$) at post-exercise as compared to pre-exercise but it returned to baseline value at 1 h post-exercise. In addition, there were no significant differences between and within hot and cool trials in salivary lysozyme concentration ($p = 0.925$; 4.79 ± 1.37 and 4.44 ± 1.11 μg/ml respectively) and secretion rate ($p = 0.843$; 1.67 ± 1.1 and 1.17 ± 1.0 μg/min respectively).

Conclusion: This study found similar lysozyme responses between both hot and cool trials. Thus, room/ambient temperature did not affect lysozyme responses among recreational athletes. Nevertheless, the selected physiological parameters were significantly affected by room temperature.

Introduction

In recent years, many researchers started to study the effects of exercise on the body immune functions. In general, regular exercise at a moderate intensity improves immune function whereas prolonged exercise at high intensity may suppress immune function.[1] Depression of immune function is most pronounced when the exercise is continuous, prolonged, of moderate to high intensity, and performed without food intake.[1] Suppressed immune function may lead to risk of infection, especially the upper respiratory tract infection (URTI). It is believed that athletes who are free from illness prior to and during the competition are likely to perform better than their peers with illness.[2] This is because infection/illness may result in fever and dehydration, decrease energy and protein storage, decrease muscle strength, less motivation, and lead to stress. Therefore, athletes, coaches, and fitness enthusiasts are very concerned regarding this matter.

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To our knowledge, investigations regarding the effects of exercise on innate mucosal immune secretions, specifically on antimicrobial proteins (AMPs) are scarce. It has been well-documented that reduced levels of salivary AMPs in athletes can contribute to increasing risk of URTI.[4] Lysozyme is one of the salivary AMPs which serve as the "first line of defence" at the mucosal surface. Salivary lysozyme is produced by epithelial cells and salivary glands, and also localised in granules of neutrophils.[6] It exerts immunological action via enzymatic effects on the peptidoglycan layer of gram-positive bacterial walls. It hydrolyses the bonds (beta 1-4 glucosidic linkages) in exposed bacterial cell walls and causes cell lysis and death.[7] It has been reported that salivary lysozyme concentration may be reduced by an intense physical exercise where it is significantly reduced after exercise in elite swimmers.[8]

However, the effects of prolonged exercise in the hot and cool environments among recreational athletes on salivary lysozyme responses are very limited in the literature especially in the Asian countries. Exercising in extreme environmental temperature can stress the body's thermoregulatory capacities and compromise cardiovascular responses which in turn, can influence the immune function.[9] It has been reported that performing the exercise in hot conditions with associated elevated circulating stress hormones and catecholamines, would cause greater immune disturbance compared with exercise in thermoneutral conditions.[10] Similarly, it has been found that performing the exercise in cold temperature results in attenuated leukocytosis.[11] Therefore, the purpose of this study is to investigate the effects of prolonged running in the hot and cool temperatures on salivary lysozyme responses among recreational athletes.

Methods

Overview

A randomised, cross-over design was employed for this study. Participants performed two running trials; one trial in the hot environment (Hot trial) followed by another in the cool environment (Cool trial) or vice versa. Recovery period between these two trials was one week. Flowchart of the study protocol was summarised in Fig. 1. All the tests were conducted in the Sports Science Laboratory, Universiti Sains Malaysia (USM). Participation in this study was on a voluntary basis. This study was been approved by the Human Research Ethics Committee, USM (USM/JEPeM/14100164). The Committee adopts research ethics guidelines outlined by the Helsinki Declaration agreed by the World Medical Association and Council for International Organizations of Medical Sciences (CIOMS).

Participants

Opportunistic or convenience sampling was used, whereby 13 active recreational athletes were recruited among USM students. Participants were healthy males, aged 18-30 years old, non-smokers, and exercising regularly (at least three times/week with at least 30 min/session). Those who were having cold or respiratory tract infection at least two weeks prior to the study and on medication were excluded from 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 quercetin.

Exercise protocols

During the first three visits to the laboratory, participants performed three preliminary tests which included sub-maximal test, maximal oxygen uptake ($\dot{V}O_2max$) test, and familiarisation trial. The preliminary tests were carried out on a treadmill (TrackMaster TMX425CP, USA) to establish a relationship between speed and oxygen uptake, determine participant's $\dot{V}O_2max$, calculate each participant's speed at 60% $\dot{V}O_2max$, and familiarise them with the running trial protocol.

For the actual running trial, participants were randomised to run for 90 min at 60% $\dot{V}O_2max$; either in the hot (31°C) or cool (18°C) environments. The order of the running trials in the two different environments was randomized. Hot environment was maintained at 31°C 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% using a heated water-bath (Memment W350t, Germany).

During each running trial, participants came to the laboratory in the morning after an overnight fast. Upon arrival, participants were asked to measure their nude body using a body composition analyser (TBF- 410 Tanita, Japan) in a closed room. Participants were given a standardised breakfast; two pieces of white bread (Gardenia®, Malaysia) and 250 ml of cool plain water.

The running trial was started by a 5 min warm-up at 50% of participant's respective $\dot{V}O_2max$ followed by 90 min running at 60% $\dot{V}O_2max$. During the 90 min of exercise, participants were asked to drink 3 ml/kg body weight of cool water at every 20 min to avoid any adverse effects of dehydration. Heart rate (Heart rate sensor: Sport Tester PE3000, Finland), oxygen uptake (gas analyser system: VMax-SensorMedics, USA), Borg's rating of perceived exertion (RPE), skin temperature (thermistors: Yellow springs Instrument, USA), tympanic temperature (ear thermometer: Microlife 1R1DB1, Switzerland), room temperature and relative humidity (psychrometer: Extech Instruments RH305, USA) were measured before warm-up, after warm-up, at every 20 min during exercise and post-exercise. After exercise, participants were asked to rest in a comfortable room for 1 h before dismissed.

Participant's saliva sample (~2 ml) was collected by 5 min of unstimulated dribbling into sterile bijou tube (Sterilin, 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. The bijou tube with the saliva collected was weighed and recorded. Saliva samples were collected before breakfast (baseline), immediately post-exercise, and 1 h post-exercise. Saliva samples were centrifuged (centrifuge: Hettich Zentrifugen, Germany) at 12,000 rpm for 5 min. Then, the upper layer (liquid part) was transferred into eppendorf tube and kept in the freezer (Acson® ACF 300, Malaysia) at -20°C until further analysis were carried out. Saliva samples were analysed in duplicates for lysozyme concentrations via an enzyme-linked immunosorbent assay (ELISA) method using a commercially available reagent kit (AssayMax Human Lysozyme ELISA kit, USA). Before analysis was carried out, saliva samples were diluted 1000 times with diluent solution. The microplate used was read using a microplate reader (Molecular Devices; $Versa_{max}$ tunable microplate reader, USA) at a wavelength of 450 nm. The intra- and inter-assay coefficients of variability were 4.2% and 8.2% respectively.

Blood samples (5 ml) were collected into a K_2EDTA collection tube (Sekusui Insepack, Japan) before breakfast (baseline), after warm-up, at every 30 min during the running trial, immediately post exercise, and 1 h post-exercise. All blood samples were taken in standing posture. Participants were cannulated for blood withdrawal purposes. 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. Blood samples were analysed for haemoglobin (Hb) concentration whereby the values were used to calculate plasma