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Original Article

Effects of prolonged running under different environmental conditions on selected physiological markers, muscle damage and time–trial performance in recreational athletes

MUHAMMAD ZUHAILI BIN SUHAIMI[1], CHEE KEONG CHEN[2], K.N.S. SIRAJUDEEN[3]
[1]Centre for Fundamental and Continuing Education, Universiti Malaysia Terengganu, Terengganu, MALAYSIA.
[1,2]Exercise and Sports Science Programme, School of Health Sciences, Universiti Sains Malaysia, Kelantan, MALAYSIA.
[3]Department of Basic Medical Sciences, Kulliyyah of Medicine, International Islamic University Malaysia, Pahang, MALAYSIA.

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Abstract
Problem Statement: Numerous studies have been conducted to improve athletic performance, which is affected by various factors. However, studies on the exercise performance of heat-adapted athletes under different environmental condition is still limited. **Approach**: Twelve male recreational athletes took part in the present study. The participants underwent prolonged running at the intensity of 70% of their respective VO_{2max} for 60 min. It was then immediately followed by a 20-minutes time–trial performance in a randomized, cross-over trial either in the heat (32°C, 70% relative humidity) or thermoneutral (25°C, 70% relative humidity) environments. The nude body weights of the participants were measured before and after the trials. The participants' blood samples were obtained before warm-up, at the beginning of exercise, at each 20 minutes and 24 hours after exercise. These samples were examined for changes in plasma volume, plasma lactate and creatine kinase activity. **Purpose:** This study investigated the effects of prolonged exercise in a hot or thermoneutral environments on different physiological parameters and time–trial performance in recreational athletes. **Results**: There were no differences between the trials in terms of body weight, plasma lactate, or creatine kinase activity. However, as compared to a thermoneutral environment, the athletes' plasma volume changes and sweat rate were significantly ($p < 0.05$) higher in the heat trial. The thermoneutral and heat trials had running distances of 3.44 ± 0.5 and 3.13 ± 0.5 km, respectively. **Conclusion**: These findings suggest that running in the heat has no effect on plasma lactate levels or muscle damage. However, the participants' running performance was hampered by the hot and humid conditions.
Key words: Heat stress, endurance running, exercise performance

Introduction

Running is a fundamental activity required in many sports. Thus, coaches and athletes normally include running as one part of their training sessions. However, in certain events, like a marathon, running is not only part of their training but also involves a long duration to improve cardiorespiratory endurance. During marathons or prolonged running, athletes generate and dissipate large amounts of heat, which is generated from metabolic processes and subsequently increases the internal body temperature. The increase of internal body temperature is faster and higher when athletes run in a hot environment (Drust et al., 2005).

Several studies shown heat as an important factor that limits exercise performance (Ely et al., 2007; Mohr et al., 2012; No & Kwak, 2016; Donnan et al., 2021). Tatterson et al. (2000) indicated that the exercise performance of elite cyclists is affected by heat stress. According to these authors, the cycling performance of the men elite cyclists during self-paced cycling ~~was~~ in the heat declined when compared with the thermoneutral trial. In another study, Chen et al. (2004), found that heat also reduces endurance performance, even in people who are used to living and training in the heat. In addition, Zhao et al. (2013) reported that a hot environment decreased the maximum oxygen consumption (VO_{2max}) and Wingate anaerobic test performance of athletes recruited for their study.

Endurance exercise has also been linked to muscle damage (Nosaka et al., 2002). Marcora & Bosio, (2007) reported that endurance exercise that causes muscle damage significantly reduces endurance running performance by decreasing muscle and power output in humans. This type of exercise causes a damage to sarcomeres, T-tubules, sarcoplasm and sarcolemma (Proske & Allen, 2005), resulting in decreased muscle strength, power and force production (Byrne & Eston, 2002; Clarkson & Hubal, 2002). Muscle damage is indicated by the disruption of the extracellular matrix and an elevated blood levels of creatine kinase and

Corresponding Author: MUHAMMAD ZUHAILI BIN SUHAIMI, E-mail: m.zuhaili@umt.edu.my

myoglobin, as well as muscle soreness and swelling. (Chen et al., 2012; Hyldahl & Hubal, 2014). For instance, an increase in the blood creatine kinase was observed within 24 h following a high-intensity soccer match (Russell et al., 2016). According to Damas et al. (2016), muscle damage is characterized by a loss of muscle strength following eccentric exercise.

According to previous studies, a high-temperature environment has been speculated to be a significant factor that affect an athlete's performance (Donnan et al., 2021; Sebastien Racinais et al., 2014). However, research on the effects of heat on performance indicator among heat-adapted athletes is not well established. The present study was therefore conducted to determine how prolonged performance of heat-adapted recreational athletes in different environmental conditions would affect selected physiological parameters, muscle damage and time–trial performance.

Materials & methods

Research design

A randomized, crossover trial was employed as the research design for the present study. Participants took part in two trials, i.e., one in a hot (32°C, 70% relative humidity) environment and another in a thermoneutral (25°C, 70% relative humidity) environment. A one-week recovery period separated the two trials. The trials were held in the Sports Science Unit Laboratory, Universiti Sains Malaysia. To avoid the negative effects of dehydration, the participants were provided with plain water (3 $mL.kg^{-1}$.body weight of plain water) before and each 20 minutes during the trials, especially in the heat trial. This protocol is identical with the one followed in previous studies (Chen et al., 2004; Kiew et al., 2001; Muhamad et al., 2016; Ping et al., 2010). The Human Research Ethics Committee of Universiti Sains Malaysia has approved the protocol of this study (USMKK/PPP/JEPeM [293.3.(11)].

Participants

Twelve recreational athletes from Universiti Sains Malaysia (USM) were recruited. The recruited participants were healthy males (aged between 20 – 35 years), non-smokers and exercise regularly with a minimum of 30 30 minutes/session and two times/week. They were able to run for at least 60 minutes in the heat at 70% of their VO_{2max}. The informed written consent was obtained from the participants after explaining the experimental study procedures and if any possible risks about it.

Preliminary testing

Preliminary testing was carried out according to the procedure in a previous study (Chen et al., 2006). The preliminary tests included a submaximal test, maximal oxygen uptake (VO_{2max}) test and familiarization trial. The participants' heights were measured with a telescoping measuring rod (Seca 220, Germany). The weight and percent body fat of the participants were measured using an electronic body composition analyser (Tanita® TBF-410, Japan).

Following familiarization with treadmill running, the participant's maximal oxygen uptake (VO_{2max}) and the relationship between running speed and oxygen uptake were determined using submaximal and maximal oxygen consumption (VO_{2max}) test. The running speed for warm-up at 50% VO_{2max} and the endurance running performance at 70% of VO_{2max} were determined using data from the submaximal and VO_{2max} test. The familiarization trial was carried out one week before the actual experimental trials. Participants who were unable to pass this test were removed from the study.

Experimental trials

The trials were carried out in an improvised heat chamber. Halogen lamps (Philips – 500W, France) were used to maintain the environment at a temperature of 32ºC (ie. Hot environment) and the thermoneutral environment was maintained at a temperature of 25°C by adjusting air conditioner temperature (York®, Malaysia). Relative humidity (RH) of 70% in the chamber was maintained by a water bath (Memmert W350t, Germany) and to provide an airflow to the participants in an open-air environment, standing fan was used in both trials. Relative humidity and temperature of the chamber was measured using a digital psychrometer (Extech Instrument RH300, USA).

All participants kept a food diary for three days prior to the first experimental trial. They were asked to eat the same diet prior to the next session to minimize the variation in resting muscle glycogen concentrations between trials. They were also instructed to skip the workout for a day earlier to start each trial and come to the laboratory under fasting for 10 hours. On the morning of test day, all the participants were given a standardized breakfast of a slice of bread (Gardenia®, Malaysia) as breakfast and 3 $mL.kg^{-1}$ body weight of mineral water 30 minutes before the warm-up on the test day. The nude body weight of the participants was recorded using a body analyser (Tanita® TBF-410, Japan), before warm-up and at the end of each trial after emptying their urinary bladder. At the forearm vein, indwelling cannula (Vasocan® - 22 G, 1", B. Braun, Malaysia) attached with an extension tube was placed to carried out repeated blood withdrawals. A heart rate sensor (Sport Tester PE3000, Polar, Finland) was placed into the chest wall of participants to measure their heart rate during their trials.

After getting ready the participants entered the chamber to perform the running performance either in a hot (32°C) or in a thermoneutral (25°C) environment. A blood sample of 0.8 mL was collected. Then the participants ran for 5 minutes as warm-up on the treadmill with 50% of VO_{2max}. After warm-up, they ran for 60 minutes at 70% of their VO_{2max}, subsequently by a 20-minute time-trial performance test. By regulating the treadmill's pace, the participants were urged to run the longest distance feasible in 20 minutes. At the start of the experiment, the participants were given plain water (3 $mL.kg^{-1}$ body weight), and which they were given every 20 minutes during the trials and after they finished. The participants were given 5 min to calm down after completing the trials. After towelling off, the participants' post-exercise nude body weight was assessed to calculate the percentage of body weight loss. The participants performed the same protocol in a different environment (in either hot or thermoneutral environment) 1 week after the first trial.

Analysis of blood parameters

The blood samples from the participants were taken at different time periods of the experimental study (ie. Before as well as after warm-up, each 20 minutes during the trials, and after 24 hours of exercise) and analysed for plasma volume changes, plasma lactate and creatine kinase activity.

Statistical analysis

Statistical Package for Social Sciences (SPSS) version 25 was used to analyse the collected data. The significant difference in time–trial performance between trials was determined by using the Student's paired t-test. The significance of the differences in physiological parameters between two trials over time was determined using ANOVA with repeated measures. P value of < 0.05 considered statistically significant. Data are presented in term of means ± standard deviations (SD).

Results

The result of physiological parameters/baseline characteristics of participants are shown in **Table 1.**

Table 1. Participants' baseline characteristics

Parameters	Mean ± Standard Deviation
Age (years)	23.1 ± 2.1
Height (cm)	171.2 ± 5.4
Body Weight (kg)	64.0 ± 8.7
Body Mass Index ($m.kg^{-2}$)	21.7 ± 2.1
Body Fat (%)	18.1 ± 3.7
VO_{2max} ($mL.kg^{-1}.min^{-1}$)	54.3 ± 4.6

Room temperature and relative humidity

The mean (SD) of room temperature as well as relative humidity in the heat (H) and thermoneutral (N) environments observed to be 31.9 ± 0.1ºC, 70.3 ± 0.1% and 25.0 ± 0.1ºC, 70.5 ± 0.1%, respectively. The room temperature as well as relative humidity maintained constant throughout the trial's periods.

Body weight changes

Baseline and after exercise body weights of the participants were similar for both trials (Table 2). However, body weight loss was higher ($p<0.001$) in the heat trial when compared to thermoneutral trial (2.0% vs. 1.2%).

Table 2. Baseline body weight (kg), after exercise body weight and body weight loss of the participants in both trials

	Thermoneutral (N)	Heat (H)
Baseline body weight (kg)	64.3 ± 8.4	64.4 ± 8.2
After exercise body weight (kg)	63.5 ± 8.1	63.1 ± 7.9
Body weight loss (%)	1.2 ± 0.5	2.0 ± 0.4***

Data as mean ± SD.
*** $p<0.001$ when compared to N trial

Fluid intake and sweat rate

The individuals' fluid intake for both trials did not differ significantly. (Table 3). However, in comparison to the thermoneutral trial, the sweat rate was significantly higher in the heat trial ($p<0.001$).

Table 3. Participants' total volume of fluid intake (mL) and estimated sweat rate ($L.h^{-1}$) in both trials

	Thermoneutral (N)	Heat (H)
Fluid intake (mL)	965.1 ± 126.2	965.8 ± 122.6
Sweat rate ($L.h^{-1}$)	1.3 ± 0.4	1.7 ± 0.3***

Data as mean ± SD.
*** $p<0.001$ when compared to N trial

Endurance running performance

Endurance running performance of the participants in the heat (H) trial was significantly lower compared to that in the thermoneutral (N) environment (Fig. 1). The mean distance covered in both heat (H) and thermoneutral (N) trials was 3.13 ± 0.50 and 3.44 ± 0.50 km, respectively. The distance achieved by the participants in the heat trial was approximately 310 m shorter than in the thermoneutral trial.

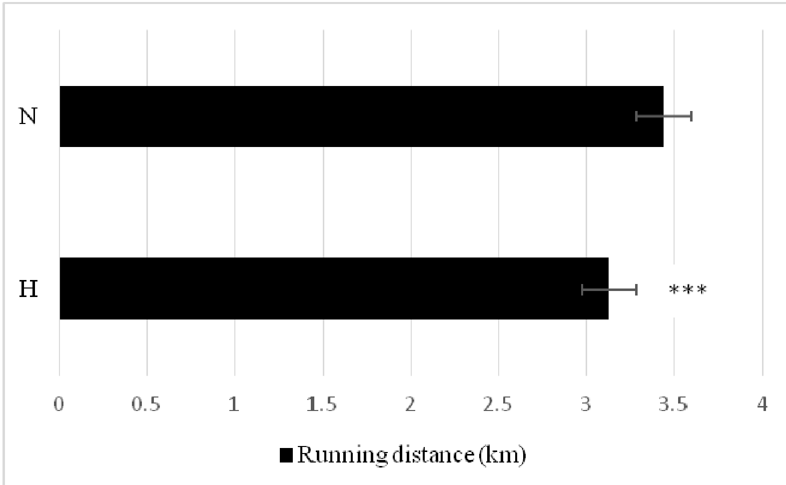


Fig. 1. Endurance running performance (km) of the participants in the heat (H) and thermoneutral (N) environments
*** Indicates a significant difference from the N trial (*p*<0.001)

Plasma lactate

Plasma lactate concentration significantly increased in both trials. In general, the plasma lactate concentrations in the heat trials were significantly higher from their baseline value during the time trial compared to the thermoneutral environment (N) (Fig. 2). But on the completion of the time trials, the participants in N and H trials had mean plasma lactate concentrations of 7.4 ± 3.1 and 5.3 ± 2.1 $mmol.L^{-1}$, respectively.

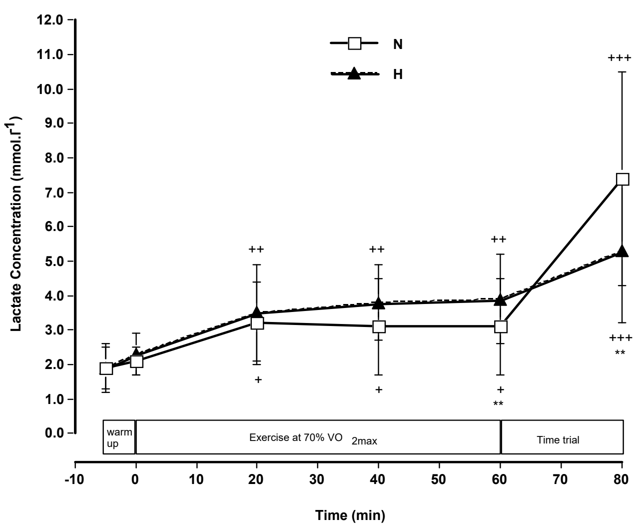
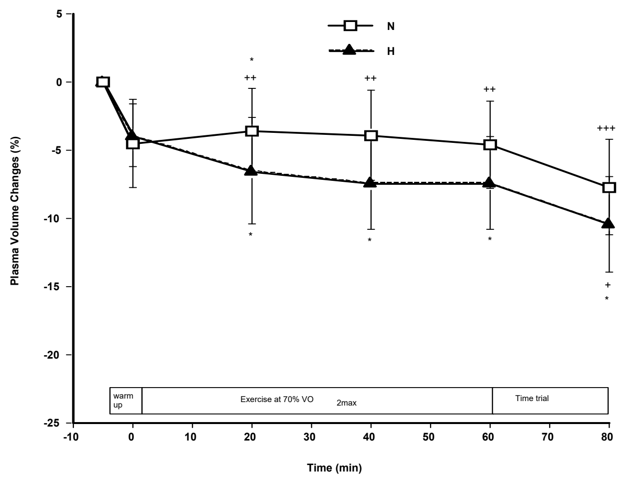


Fig. 2. Changes of plasma lactate concentration in both trials
+, ++ and +++ represent a significant difference from respective baseline values (*p*<0.05, *p*<0.01 and *p*<0.001 respectively).

Plasma volume changes (PVC)

Plasma volume significantly decreased over time in both trials (Fig. 3). When compared to their respective resting volumes, plasma volume was significantly lower in both trials. At the end of the N and H trials, the mean change in the volume of plasma of the participants was −7.7 ± 2.3 and −10.4 ± 3.5% respectively.



+, ++ and +++ represent significant differences from the respective baseline values ($p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively).** $p<0.01$ when compared to N trial

Fig. 3. Changes of plasma volume in both trials

Plasma creatine kinase (CK)

The mean plasma creatine kinase concentration of the participants increased at 24 h post-exercise in both trials, where the mean plasma creatine kinases for the N and H trials were 510.9 ± 94.6 and 429.1 ± 267.1 $U.L^{-1}$ respectively (Fig. 4). However, there were no significant difference observed between the trials.

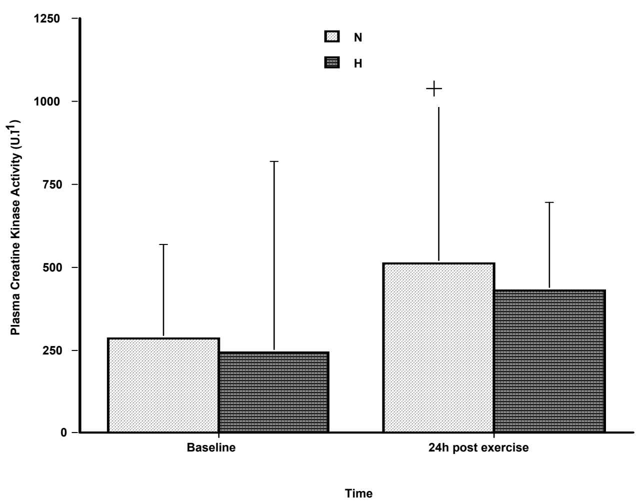


Fig. 4. Differences of plasma creatine kinase activity between two trials
+ Indicates a significant difference from respective resting values ($p < 0.05$).

Discussion

In this study, the mean BMI of the participants was within the normal classification of BMI, which is from 18.5 to 24.9 $kg.m^{-2}$ (Shiwaku et al., 2004). In terms of cardiorespiratory fitness, the mean VO_{2max} of the participants (54.3 ± 4.6 $mL.kg^{-1}.min^{-1}$) is considered excellent when referring to the norm of the age group (McArdle et al., 2007). These values indicate that the participants were fit enough and able to complete the experimental protocol. While the room temperature was maintained at 25°C and 32°C for the N and H trials, respectively, throughout the experimental trials, the relative humidity of the room was kept stable at 70% for both trials. The temperature and relative humidity of the chamber for both trials were established using Malaysia's daily temperature and humidity, as well as those utilized in other previous studies (Chen et al., 2006; McAnulty et al., 2005).

In the present study, the hot and humid environment affects the participants running performance. It was better in the N trial when compared with the H trial (Fig. 1). In comparison to the hot environment, the participants ran 310 m further in the thermoneutral environment. This finding is supported with the other studies (McAnulty et al., 2005; Walters et al., 2000). Most of those studies, however, were conducted on humans adapted to living in a cooler environment and who were either non-acclimatized or partially acclimatized to the heat. Our study demonstrated that exercising in the heat significantly reduces endurance performance in participants who have always lived and exercised in a hot as well as humid environment. However, exercising under this environment did not affect plasma lactate or the marker for muscle damage among the heat-adapted participants.

In both trials, the plasma volume was decreased significantly, but was significantly higher in the H trial than in the N trial with respect to their baseline values (Fig. 3). After warming up, the reduction in plasma volume was slightly similar with both trials. However, the decrease in plasma volume from 20 min of exercise to the end of the time trial was greater in the H trial than in the N trial. The greater decrease in plasma volume in the H trial compared to the N trial was most likely because of increased fluid loss through sweating during heat-induced exercise. This finding was consistent with the findings of other studies (Fritzsche et al., 1999; Sawka & Montain, 2000). Homeostatic mechanisms such as peripheral vasodilation and increased sweat rate are used to dissipate heat from the human body and maintain internal body temperature (S. Racinais et al., 2015). The increased sweat rate during exercise causes a loss of body fluid and subsequently reduced plasma volume in the body. Excessive body fluid loss can also lead to dehydration and consequently affects exercise performance (Menon et al., 2015; Fortes et al., 2018; Wittbrodt & Millard-Stafford, 2018; McCarthy et al., 2020).

During exercise, plasma lactate concentration increased in both trials, but was higher during the course of H trial than in the N trial (Fig. 2). Other studies (Hargreaves et al., 1996; McAnulty et al., 2005) also shown the similar findings in which plasma lactate accumulation was elevated when people were exercising in the heat compared to in a thermoneutral environment. However, a study conducted by Tatterson et al. (2000) reported that plasma lactate levels were not significantly different following endurance cycling in heat and thermoneutral environments. In general, hyperthermia and elevated amount of hydrogen are two side effects of vigorous and prolonged exercise that might impair performance (Coyle, 1999). The accumulation of hydrogen ions in the blood and muscle is known as lactic acidosis, and it produces a dramatic shift in the acid–base balance (Kemp et al., 2005). Plasma lactate reflects a lactate production and clearance balance that favours the former (Phypers & Pierce, 2006). The plasma lactate concentration in the N trial was considerably greater at the completion of trial than in the H trial. This was probably attributed to a faster pace that the participants were running in the cooler environment.

Plasma creatine kinase (CK) activity in the N trial was significantly higher 24 h after exercise compared to resting level, but not that in the H trial. However, the plasma CK activity between pre-exercise and 24 h post-exercise of H and N trials did not differ statistically (Fig. 4). The post-exercise rise in plasma CK activity in the N trial was similar to that observed by other investigators, following a range of exercise regimens (Clarkson et al., 2006; Magal et al., 2010; K. Nosaka et al., 2002), and the peak production of CK was after 24 to 96 h post-exercise (Brancaccio et al., 2007). This suggests that the occurrence of muscle damage is caused by sources other than heat. There is no clear mechanism for causing muscle damage, but mechanical factors may be responsible for the workout. Mechanical disruption in the muscle is one of the basic mechanisms that cause muscle damage (Hyldahl et al., 2014). Chen et al. (2012) reported that exercise-induced muscle damage was evident after isometric and eccentric muscle contractions. Many factors, including intensity, speed, number of contractions, muscle length, muscle group, exposure to eccentric exercise, age and sex influenced the extent to which muscle injury was affected (Nosaka & Aoki, 2011). Muscle damage caused by eccentric exercise causes dramatic and long-lasting changes in motor unit recruitment, which likely affects muscle strength and exercise performance. (Semmler, 2014).

Conclusion

In this study, the prolonged running performance of heat-adapted recreational athletes has been affected by a hot and humid environment. The recreational athletes ran a longer distance in the thermoneutral environment as compared to in a hotter environment. However, prolonged running in the heat did not affect plasma lactate or muscle damage. Thus, it is advisable for all coaches and athletes to consider the adverse effects of heat stress as a critical factor that may affect endurance performance. They should take any relevant strategies or approaches, such as heat acclimatization or pre-exercise cooling intervention, to maintain or improve endurance performance in hot and humid environments.

Conflict of interest

There were no conflicts of interest in the publication of this manuscript.

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