Effects of Acute Cool Water Immersion on Time Trial Performance and Exercised-Induced Oxidative Stress among Endurance Cyclists in the Heat

Kok Wai Lit*, Chee Keong Chen† and Boon Suen Ang‡

Abstract

Background: Cold water immersion has been used as a modality for recovery among elite athletes. This study investigated the effects of acute cool water immersion (CWI) at 25°C following prolonged submaximal cycling on time trial (TT) performance and oxidative stress.

Methods: Nine trained male cyclists performed a randomized crossover trial comprising of 60 minutes of cycling at 70% Vo2max followed by 30 minutes of CWI and subsequently a 20 km cycling time trial. Environmental conditions were maintained at 31.2 ± 0.3 °C and relative humidity of 72.0 ± 0.7%.

Results: Prior to the 20 km TT, rectal temperature (Trec) of the participants in the CWI trial was significantly (p<0.05) lower than the air cooling (AC) trial (36.1 ± 0.3°C vs 37.1 ± 0.3°C respectively). Similarly, resting heart rate of the participants in the CWI trial following CWI was significantly (p<0.05) lower than the AC trial (61.9 ± 10.0 beats.min⁻¹ vs 89.9 ± 8.3 beats.min⁻¹). Average cycling speed in the CWI trial was significantly (p<0.05) faster than the AC trial (27.4 ± 2.1 km.h⁻¹ vs 25.9 ± 2.4 km.h⁻¹). Time to complete the TT, Trec following CWI was significantly (p<0.05) lower than the AC trial (43.8 ± 3.3 min vs 46.4 min ± 4.5 min respectively). During the TT, Trec in the CWI trial was significantly (p<0.05) lower than the AC trial (37.8 ± 0.4°C vs 38.5 ± 0.7°C respectively). However, there were no significant differences in serum F₂-isoprostanes and GSSG/GSSG ratio during both trials.

Conclusion: CWI at 25°C lowers core body temperature effectively and improves TT performance of endurance cyclists in a hot and humid condition compared to normal air cooling.

Keywords

Cool water immersion; Endurance cycling; Time trial performance; Oxidative stress; Heat

Introduction

The importance of controlling core body temperature and whole-body cooling began to receive attention in the 80’s since high ambient temperature and humidity have detrimental effects on athletes’ performance [1]. Cooling interventions developed to lessen heat stress include cooling vest [2], head cooling [3], neck cooling [3], cold water immersion [4], whole body cryotherapy, and ice massage [5]. Various forms of cooling has been reported to improve exercise time to exhaustion [6], reduce thermal strain [7], reduce muscle soreness, improve restoration of muscle strength and recovery[8], as well as alleviating oxidative stress [9-11] and muscle damage [8]. However, data on the specific effects of cooling on the recovery profile and subsequent performance of athletes are still scarce.

Among all the cooling interventions, cold water immersion resulted in the most effective cooling strategy [12]. Most of the studies in the literature applied cold (ice or cold water, 2-20°C) and extreme cold (sub-zero°C) temperatures while investigating the effects of cooling, to ensure that it generates a thermal gradient which is steep and long lasting enough during an exercise session [4]. Taylor et al. [4] demonstrated that rapid and effective heat removal can be achieved during a temperate-water (26°C) immersion which is also known as cool water immersion. They reported that respective cooling times using water temperature of 14°C and 26°C to achieve esophageal temperature of 37.5°C were both below 4 min. Hence, a rapid, effective and comfortable cooling could still be achieved in hyperthermic individuals using temperate water temperature [4].

Exercising under hot and humid environmental will lead to hyperthermia, which in turn limits endurance performance [13] as well as causing life-threatening exertional heat illness [14]. Ryan et al. [15] reported that a body core temperature greater than 40 °C increased heat shock protein expression in leukocytes obtained from exercising individuals. McAnulty et al. [16] also showed that hyperthermia (rectal temperature of 39.5°C) enhances oxidative stress in subjects who ran on a treadmill for 50 min in the heat. The precise mechanism on how heat leads to reduction in physical performance is still unclear. Nevertheless, it was reported that there is an increased uncoupling and superoxide generation in muscle mitochondria as the temperature increases. This suggests that exercise-induced hyperthermia may lead to oxidative stress [17].

Oxidative stress has damaging effects on lipids, proteins, and DNA as well as pro-inflammatory responses, which can cause cellular damage [18]. It has been reported that free radical production during exercise is one of the factors that may contribute to muscle fatigue [18,19]. Furthermore, free radical-induced fatigue is also classified under muscle trauma model of fatigue among the eight linear models of fatigue reviewed by Abbiss and Laursen [20]. The mechanism by which free radicals induce muscular fatigue remains unclear. However, some investigators have postulated that free radicals might damage the sarcoplasmic reticulum resulting in reduced calcium release during depolarization of the muscle [21,22] and consequently lead to decreased muscle performance and muscular fatigue [23,24].

Studies on changes in core body temperature during recovery, fatigue, oxidative stress and time trial performance using temperate water temperature (25-26°C) are still lacking in the literature. Application of cool or extreme cold water immersion has given rise to many logistic issues to the athletes and the entire sports team during the actual event such as the supply of cold water, ice cubes, electrical supply (for specific cooling device) and space [1]. Thus, further exploration and validation on the effectiveness of using temperate water temperature on cooling rate, fatigue, oxidative stress and...
exercise performance are warranted. Hence, this study investigated the effects of cool water (25°C) immersion on time trial performance and oxidative stress among trained cyclists the heat.

Materials and Methods

Research design

A randomized cross-over design was employed in this study. Two separate experimental trials were performed by the subjects with a rest interval of 7 days between each trial. These 2 trials consisted of cycling at 70% VO$_{2\text{max}}$ followed by a recovery period of either cool water immersion (CWI) or air cooling (AC) at 25°C and a subsequent 20 km time trial on an indoor bike trainer.

Subjects

Nine healthy trained male cyclists representing Kelantan state were recruited for this study (age: 18.9 ± 4.5 years; body weight: 52.9 ± 4.2 kg; height: 164.4 ± 3.3 cm; VO$_{2\text{max}}$: 57.6 ± 3.6 mLkg$^{-1}$min$^{-1}$ and body fat percentage 16.7 ± 1.8%). They were given explanation to the nature and risks of the experiment procedures and a written informed consent were obtained from them. This study protocol was approved by the Human Research Ethics Committee of Universiti Sains Malaysia.

Procedures

Four pre-trial visits were conducted at the Sports Science Laboratory, School of Medical Sciences, Universiti Sains Malaysia to: 1) determine the maximal oxygen consumption (VO$_{2\text{max}}$) 2) establish the relationship between oxygen uptake and cycling workload via a sub-maximal oxygen consumption test; 3) familiarize with the experimental protocol and 4) familiarize with the time trial performance. VO$_{2\text{max}}$ and sub-maximal oxygen consumption tests were performed on a cycle ergometer (Lode Excalibur Sport, Nederland). Appropriate cycling workload during warm-up at 50% VO$_{2\text{max}}$ and endurance cycling at 70% VO$_{2\text{max}}$ in the actual experimental trials were established from the results of these two tests. Each subject performed their individual time trial by riding on the road bike (Trek 1200, USA) suspended on an indoor bike trainer (JetFluid™ Pro Trainer, USA). During the actual experimental trials, subjects were required to cycle in the heat (31°C, 70% relative humidity) at 70% VO$_{2\text{max}}$ on a cycle ergometer for 60 minutes, followed by a cooling period of 30 minutes by either immersing in cool water (25°C) or resting in an ambient environment at 25°C. Subsequently, they were required to perform a 20 km time trial.

On the day prior to the experimental trial, participants were required to undergo an overnight fast of 10-12 hours. Upon arrival at the laboratory, participants were asked to void their bladder before nude body weight was measured using body composition analyser (Tanita® TNF-410, Japan). Core body temperature was monitored throughout the test, by inserting a rectal probe to a depth of 10 cm beyond anal sphincter. Subjects were given 500 ml of water and a standardized breakfast of 2 slices of bread one hour prior to the trials.

Heart rate monitor (Polar S710, Finland) was fitted onto the chest of the subjects, right below their pectoral muscles. An indwelling cannula (Voscan Brannule Indwelling Cannula, Braun, Germany) was inserted into subcutaneous forearm vein for blood sample collection. Ten milliliters of blood was taken for baseline measurement. A head gear and the breathing apparatus were fitted to the participant and then connected to the gas analyzer for determining the oxygen consumption. Each trial consisted of 4 stages: a) Warm up at 50% VO$_{2\text{max}}$ for 5 minutes, b) Cycling at 70% VO$_{2\text{max}}$ for 60 minutes (31°C, relative humidity 70%), c) Cooling period of 30 minutes and d) 20 km time trial.

For the air-cooling trial, each subject rested for 30 minutes in an air-conditioned room maintained at 25°C, whereas in the cool water immersion trial, the participants were required to submerge their body in a tank filled with cool water (25°C) until the neck region for 30 minutes. Total time taken to complete the time trial of 20 km was used as the indicator of endurance cycling performance after the 30 minutes of cooling period. Core body temperature was monitored throughout the whole session. Blood samples collected at each stage were analyzed for oxidative stress markers. A rest interval of 7 days between each trial was given to ensure that the participants have fully recovered before they go through the next trial.

Blood sample collection and analysis

Six milliliters of blood samples were drawn just before the experimental trials (resting), the end of 1 hr cycling at 70% VO$_{2\text{max}}$, after 30 minutes of cooling recovery, end of 20 km time trial and 24 hr post-exercise.

For the analysis of hematocrit fraction and hemoglobin concentration, 3 ml of blood was aliquoted into EDTA tubes. The remaining 3 ml of blood was aliquoted into plain tubes for the analysis of serum F$_2$-isoprostanes and GSH / GSSG ratio. For the post-24 hr samples, 4 ml of blood was taken and aliquoted into plain tubes for the analysis of F$_2$-isoprostanes and GSH / GSSG ratio.

Statistical analysis

Statistical analysis was carried out using the SPSS version 19.0. Two-way repeated-measures analysis of variance (ANOVA) followed by paired t-test was used to analyse the blood parameters. Paired t-test was used to compare the performance between trials. The criterion for statistical significance was set at p<0.05. The results were reported as means ± S.D. (Standard deviation).

Results

There were no significant differences in mean room temperature (31.2 ± 0.3 vs. 31.2 ± 0.4°C) and relative humidity (71.9 ± 0.9 vs. 72.1 ± 0.6%) during the 1 hr cycling at 70% VO$_{2\text{max}}$ and time trial between AC and CWI trials. Time trial performance was significantly (p<0.05) better in the CWI trial compared to the AC trial (Table 1). On average, the subjects completed the 20 km time trial in a time of 44.0 minutes during the CWI trial, which was 2.7 ± 1.9 minutes faster than the AC trial. Average speed was also significantly (p < 0.05; 25.9 ± 2.4 km.h$^{-1}$ vs. 27.4 ± 2.1 km.h$^{-1}$ in the AC and CWI trials respectively) higher in the CWI trial compared to the AC trial (Table 1).

Heart rate response was similar in both trials during the one hour of cycling (Figure 1). Heart rate achieved at the end of 60 minutes of cycling for AC trial and CWI trial were168.4 ± 4.8 and 166.33 ± 10.4

Table 1: Time taken and average cycling speed during 20 km time trial in both trials.

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Mean ± S.D</th>
<th>AC</th>
<th>CWI</th>
</tr>
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<tbody>
<tr>
<td>Time (min)</td>
<td>46.7 ± 5.4</td>
<td>44.0 ± 2.7*</td>
<td></td>
</tr>
<tr>
<td>Average speed (km/h)</td>
<td>25.9 ± 2.4</td>
<td>27.4 ± 2.1*</td>
<td></td>
</tr>
</tbody>
</table>

* significantly different from AC trial at p<0.05.
beats.min⁻¹ respectively. However, during the 30 minutes of cooling, heart rate recovered significantly faster in the CWI compared to the AC trial. At the end of 30 minutes cooling period, the heart rate in the CWI trial was significantly lower than AC trial (61.9 ± 10.0 beats.min⁻¹ vs. 89.9 ± 8.3 beats.min⁻¹, p<0.001).

Rectal temperature (T_{rect}) gradually increased throughout the one hour cycling at 70% VO_{2max} in both trials but no significant differences were observed between trials (Figure 2). However, during the cooling period, T_{rect} of CWI trial was significantly lower than that of AC trial (61.9 ± 10.0 beats.min⁻¹ vs. 89.9 ± 8.3 beats.min⁻¹, p<0.001).

Serum F₂-Isoprostanes concentration was not different between AC and CWI trials (Figure 3). Serum F₂-Isoprostanes concentration significantly (p<0.05) increased in both AC and CWI trials after 1 hour of cycling at 70% VO_{2max}. Following 30 minutes of cooling, F₂-Isoprostanes concentration returned to resting levels (p<0.05) in both trials. Subsequently, F₂-Isoprostanes concentration significantly (p<0.05) increased in both trials following the 20 km time trial. At 24 hr post-exercise, F₂-Isoprostanes concentrations returned to respective resting values in both trials.

There was no significant difference in GSH/GSSG ratio between AC and CWI trials (Figure 4). GSH/GSSG ratio significantly (p<0.05) decreased for AC and CWI trials after 1 hour of cycling at 70% VO_{2max}. Following 30 minutes of cooling, GSH/GSSG ratio increased significantly (p<0.05) in both trials. After 20 km of time trial, GSH/GSSG ratio decreased significantly (p<0.05) in both trials and returned to pre-exercise levels at 24 hours post-exercise in both trials.

Discussion

The most notable finding in the present study was recovery with CWI elicited an improved 20 km time trial (TT) performance. Average speed during the 20 km TT was also 6% (p<0.05) faster than that of AC trial (Table 1). It has been well documented by other researchers that cooling elicited improvement in endurance cycling performance, particularly in cycling speed [7], and also delay the onset of fatigue and thus extend the time to exhaustion of endurance cyclists [6,25]. However, most of the data in the literature available seem to emphasize on using an intense cooling temperature ranging from 2°C-10°C [1,12]. Although most of these studies reported...
improvement in endurance cycling performance, limited research findings are available for water temperature of 25°C. Our current findings demonstrated that cool water immersion (25°C) for 30 minutes not only reduce core body temperature effectively but also significantly (p<0.05) improved 20 km TT performance of endurance cyclists.

Increases in heart rate have a linear relationship with rectal temperature, and it is attributed to the increase in metabolic and blood circulatory demands. Heart rates began to differ significantly (p<0.05) between both trials when participants underwent the 30 minutes of cooling at respective cooling methods. Although the cooling temperature used was the same (25°C) for both trials, CWI elicited a more rapid and effective cooling rate than air cooling during acute recovery. Heart rate of CWI trials was significantly lower than AC trials just after 5 minutes of cooling. As a result, rectal temperature in CWI trial was significantly lower than the AC trial (36.2 ± 0.1°C vs. 37.1 ± 0.1°C; p<0.05) following 30 minutes of cooling. It is well documented that hyperthermia will induce an increase in skin blood flow and sweat rate [26], and shunts systemic blood flow to the skin for heat dissipation rather than supplying blood to active muscles [27]. Since rectal temperature in CWI trial rapidly decrease back to resting levels (Figure 2), it indicated that there was less cardiovascular strain for heat dissipation. After 5 km into the time trial, heart rates in AC trial was higher than CWI trial (167.6 ± 2.7 beats.min⁻¹ vs. 157.1 ± 6.0 beats. min⁻¹) even though the average cycling speed during the first 5km time trial of CWI trials was faster than the AC trial (27.6 ± 2.3 km.h⁻¹ vs. 27.0 ± 2.9 km.h⁻¹). It was postulated that the participants in CWI trial were able to cycle faster and yet have a lower exercise heart rate than participants in the AC trials because the rectal temperature was lower in the CWI trials and thereby lessen the burden on the cardiovascular system for heat dissipation.

In the present study, there was a significant difference (p<0.05) in the rectal temperature between AC and CWI trials from minute 15 onwards of cooling period. Water has a much higher heat storage capacity than air, thus water was able to exert a much rapid and effective cooling rate as compared to air. Taylor and colleagues (2008) have shown that a cooling rate of esophageal temperature (Teₚ) of 0.10 ± 0.04°C.min⁻¹ using air temperature of 20-22°C; 0.88 ± 0.06°C.min⁻¹ using water temperature of 14°C and 0.71 ± 0.02°C.min⁻¹ using water temperature of 26°C. These differences in cooling rate could be due to the difference in heat storage capacity between water and air, and thermal gradient applied in the cooling protocol. In another similar study, a cooling rate of Tₑₚ of 0.15 ± 0.06°C.min⁻¹ was reported using water temperature of 14°C [12]. The current guidelines on treatment for hyperthermic individuals states that any treatment modality should achieve a Tₑₚ cooling rate of at least 0.10°C.min⁻¹, which is most effectively achieved in cold water immersion [28]. In the present study, highest Tₑₚ cooling rate of 0.06°C.min⁻¹ was achieved, where Tₑₚ decreased from 38.2°C to 37.6°C in the first 10 minutes of CWI. However, this figure may not be comparable with other previous studies as it differs with other studies in the aspect of associated heating factors (exercise intensity and environmental temperature) and end point body temperature after the exercise session.

Serum F₂-isoprostanes was elevated during 1 hour cycling at 70% VO₂max and 20 km time trial in both trials (Figure 4). Escalating oxygen consumption during prolonged and exhaustive exercise can result in an increased leakage of reactive oxygen species (ROS) as by-products of the mitochondrial electron transport chain [29,30]. F₂-isoprostanes has been reported to increase following exhaustive exercise in several studies [23,31-33]. Isoprostane is a stable end product of peroxidation of arachidonic acid, and is widely accepted as reliable indicator of oxidative stress [34].

The intracellular glutathione (GSH) redox cycle between reduced GSH and oxidized GSH is an effective mechanism in protection against intracellular oxidative damage. The ratio of reduced-oxidized GSH thus can be used as another index of intracellular oxidative stress [35]. The surge in blood GSSG induced by bicycle exercise at various range of intensities has been reported to return to pre-exercise concentrations after 24 hours [36]. Blood GSH was decreased and GSSG increased during the first 15 minutes of 90-minute cycle ergometer exercise at 65% VO₂max. GSH and GSSG did not change further during the remaining 75 minutes of exercise, returning to pre-exercise levels after 15 minutes of recovery [37]. This findings support the observation from the present study and it was found that level of GSH was back to near resting levels within 30 minutes of recovery after 1hr cycling at 70% VO₂max, as well as 24 hr post-exercise (Figure 3). In the present study, CWI did not elicit any significant difference in GSH/GSSG ratio following prolonged endurance cycling.

The data from serum F₂-isoprostanes and GSH/GSSG ratio in this study seem to demonstrate that ROS produced during prolonged cycling in the heat were being rapidly mopped up by the action of antioxidants in our body. When the participants stopped exercising or underwent recovery period, the removal or scavenging of oxidants by antioxidants seems to have overwhelmed the production of oxidants and thus serum F₂-isoprostanes and the GSH/GSSG ratio were back to near resting levels. However, the levels of both these oxidative stress markers following the 20 km time trial performance indicated that the participants were predisposed to oxidative stress in both trials (Figure 4). At 24 hour post exercise, both these markers were back to baseline levels indicating that the oxidative stress experienced during the experimental trials were transient. Nevertheless, more studies are warranted to provide evidence that oxidative stress during prolonged cycling in the heat has a role in inducing muscular fatigue among trained cyclists.

**Conclusion**

Data from the present study indicated that time trial performance of the participants were significantly improved in the CWI trial. Hence, we conclude that cool water immersion (25°C) for 30 minutes as a recovery modality enhanced time trial performance of endurance cyclists. It has been shown that a rapid, effective and comfortable cooling could still be achieved in hyperthermic individuals using temperate water temperature of 25°C. 30 minute of cool water immersion significantly lowered the resting heart rate and core body temperature prior to the subsequent bout of exercise. However, CWI also did not seem to attenuate the oxidative stress markers. Hence, the precise mechanism of cooling intervention on improving sports performance requires further investigation.

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**Conflict of Interests**

No Conflicts of interests reported.
References


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