


# Effects of mild hypohydration on cooling during cold-water immersion following exertional hyperthermia

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Received: 27 October 2015 / Accepted: 5 January 2016 / Published online: 18 January 2016  
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## Abstract

**Purpose** We investigated the effects of mild hypohydration compared to euhydration on the cooling efficacy of cold-water immersion (CWI).

**Methods** Fourteen participants (eight male, six female; age  $26 \pm 5$  years; ht  $1.77 \pm 0.08$  m; wt  $72.2 \pm 8.8$  kg;  $20.6 \pm 7.4$  % body fat) completed one euhydrated (EU) trial followed by one hypohydrated trial (HY; via 24 h fluid restriction) in an environmental chamber ( $33.6 \pm 0.9$  °C,  $55.8 \pm 1.7$  % RH). Volitional exercise was performed in a manner that matched end-exercise rectal temperature ( $T_{re}$ ) through repeating exercise mode and intensity. Participants were then immersed in ice water ( $2.0 \pm 0.8$  °C) until  $T_{re}$  reached  $38.1$  °C or for a maximum of 15 min.  $T_{re}$ , heart rate (HR), skin blood flux (SBF) and mean skin temperature ( $T_{sk}$ ) were monitored continuously during cooling.

**Results** Pre-cooling body mass was decreased in the HY trial ( $-2.66 \pm 1.23$  % body mass) and maintained in the EU trial ( $-0.66 \pm 0.44$  %) compared to baseline mass ( $P < 0.001$ ). Cooling rates were faster when EU ( $0.14 \pm 0.05$  °C/min) compared to HY ( $0.11 \pm 0.05$  °C/min,  $P = 0.046$ ). HR, SBF, and  $T_{sk}$  were not different between EU and HY trials ( $P > 0.05$ ), however, all variables significantly decreased with immersion independent of hydration status ( $P < 0.001$ ).

**Conclusion** The primary finding was that hypohydration modestly attenuates the rate of cooling in exertionally

hyperthermic individuals. Regardless of hydration status, the cooling efficacy of CWI was preserved and should continue to be utilized in the treatment of exertional hyperthermia.

**Keywords** Cold-water immersion · Hypohydration · Dehydration · Exertional hyperthermia · Exertional heat illness treatment

## Abbreviations

CWI Cold-water immersion  
EU Euhydration  
HY Hypohydration

## Introduction

Athletes and recreationally active individuals tend to report to workouts, practices and games hypohydrated (HY) (Arnaoutis et al. 2014; Godek et al. 2005; Osterberg et al. 2009; Stover et al. 2006). Hypohydration alters exercise performance (Armstrong et al. 1985; Judelson et al. 2007; Kavouras et al. 2012) and, during exercise in hot, humid environments, negatively impacts thermoregulation due to greater challenges to maintain blood flow to the skin and active musculature (González-Alonso et al. 1995, 1997). This results in a decreased ability to dissipate heat when HY, leading to increased core temperature at a given workload when compared to a euhydrated (EU) state (González-Alonso et al. 1995, 1997, 2000). These elevated core temperatures place those exercising in environments that do not permit adequate evaporative heat loss (i.e. uncompensable heat stress) at greater risk for reaching a critical core temperature of 40–41 °C (González-Alonso et al. 1999). Subsequently, exertional heat illness, such as exertional

Communicated by George Havenith.

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heat stroke (EHS), may ensue (Casa et al. 2007; Epstein and Roberts 2011).

The immediate treatment recommended for EHS is immersion in cold water, with cooling rates of at least  $0.1\text{ }^{\circ}\text{C min}^{-1}$  required to reduce core temperature below  $40\text{ }^{\circ}\text{C}$  as fast as possible (Casa et al. 2007). Several factors may influence the effectiveness of cold-water immersion (CWI), such as water temperature (Proulx et al. 2003), level of immersion (Zhang et al. 2015), and lean body mass (Friesen et al. 2014), however, the effects of hypohydration during CWI as compared to euhydration, have not been investigated. Clements et al. (2002) demonstrated that CWI was still very effective ( $0.16\text{ }^{\circ}\text{C min}^{-1}$ ) with hypohydration of  $-3.89\%$  body mass loss post exercise, however, the percent body mass loss was not compared to a controlled EU state. Furthermore, the individuals began the protocol EU and progressively dehydrated throughout the trial (Clements et al. 2002). As many athletes and recreationally active individuals report to activities in a HY state (Arnaoutis et al. 2014; Godek et al. 2005; Osterberg et al. 2009), the effects of a longer dehydration protocol are warranted. Furthermore, decreases in plasma volume associated with hypohydration from fluid restriction and exertional hyperthermia (González-Alonso et al. 1997, 2000; Zappe et al. 1993) may attenuate the capacity to carry heat away from previously active skeletal muscle.

CWI is also a popular recovery aid from exercise routines and athletic practices (Versey et al. 2013). Post-exercise CWI leads to decreases in muscle temperature and skin blood flow (Mawhinney et al. 2013), as well as mitochondrial biogenesis (Ihsan et al. 2015), and improved subsequent performance bouts (Peiffer et al. 2010; Versey et al. 2013). Cardiovascular recovery is also enhanced as immersion leads to fluid shifts, increasing central blood volume and stroke volume (Wilcock et al. 2006). This is followed by reductions in heart rate (HR) and cardiovascular strain (Wilcock et al. 2006).

Hypohydration impedes post-exercise recovery, particularly following exertional hyperthermia, through impacting mean arterial pressure (Gagnon et al. 2012), HR (Charkoudian et al. 2003), baroreflex sensitivity (Charkoudian et al. 2003), and neuromuscular control (Distefano et al. 2013). This in turn may have negative impacts on future performance or subsequent exercise bouts (McDermott et al. 2013). The effects of CWI on cardiovascular variables following exertional hyperthermia in EU and HY states have not been exclusively investigated. When immersed while HY, fluid shifts into the plasma volume to improve cardiac filling pressure and increase stroke volume (Stachenfeld et al. 1997). Therefore, CWI while HY may ameliorate these negative aspects and allow for improved recovery. Thus, the purpose of this study was to determine the physiological effects of mild hypohydration during

CWI following exertional hyperthermia. Based on the evidence from Clements et al. (2002), it was hypothesized that CWI would provide acceptable rectal temperature ( $T_{re}$ ) cooling rates ( $\geq 0.1\text{ }^{\circ}\text{C min}^{-1}$ ) during mild hypohydration and ameliorate cardiovascular impairments elicited from the HY state compared to the EU state.

## Methods

### Participants

Fourteen healthy participants (eight male, six female; age  $26 \pm 5$  years, ht  $1.77 \pm 0.08$  m; wt  $72.2 \pm 8.8$  kg;  $20.6 \pm 7.4\%$  body fat; lean body mass  $55.41 \pm 9.29$  kg; body surface area  $1.88 \pm 0.15\text{ m}^2$ ; body surface area-to-lean body mass,  $345.61 \pm 40.86\text{ cm}^2\text{ kg}^{-1}$ ) volunteered to participate. Informed consent was obtained from all participants prior to the experimental procedures, which were approved by the University's Institutional Review Board. All participants were physically active (at least three times per week for at least 30 min), had no history of chronic disease or illness, were not suffering from injury or current illness, and had not experienced heat exhaustion or EHS in the past 3 years. All participants were advised to refrain from alcohol use and exercise for 24 h, and caffeine use for 12 h prior to each trial. For 24 h prior to each trial, participants recorded their food/fluid intake on a standard diet log.

All participants underwent a dual energy X-ray absorptiometry scan (DXA, Lunar Prodigy; General Electric<sup>®</sup>, Madison, WI, USA) to assess body composition. Body surface area was calculated via Du Bois and Du Bois (1989) and used to assess the body surface area-to-lean body mass ratio.

### Hydration protocols

Each participant completed one EU trial followed by one HY trial. Each trial was separated by at least 1 week to prevent residual heat acclimation and habituation to CWI (Tippton et al. 2000, 2013). Further, the ordering of trials was utilized to ensure that participants would attain similar  $T_{re}$ , as HY has been shown to cause elevations in esophageal temperature when compared to EU conditions (González-Alonso et al. 1995, 1999). Thus, EU trials were completed first to guarantee participants would reach the same  $T_{re}$  in subsequent trials. To ensure participants arrived in a hydrated state for the EU trial, they were instructed to consume an additional 470 mL of water the night before and morning of their trial. Following the EU trial, the participants were provided a scale (BalanceFrom High Accuracy Bathroom Scale, BalanceFrom LLC, China) to take home and instructed to record nude body masses for 3 days during

the week prior to the HY trial. Prior to recording each body mass, participants were instructed to consume 470 mL to ensure a EU state. The mean of the 3-day body mass was utilized as the baseline body mass to compare for % body mass loss (an indicator of hydration status). The dehydration protocol then consisted of 24-h fluid restriction prior to the start of their HY trial during which they consumed no fluids and were instructed to avoid water-dense foods.

### Exercise protocol

Upon arrival to the laboratory, participants completed a nude body mass and provided a urine sample to assess hydration status (Armstrong et al. 2010). Urine specific gravity (USG) and urine osmolality ( $U_{\text{osm}}$ ) values were required to be  $<1.020$  and  $<700$  mOsm/kg, respectively, for EU trials and above these values for HY trials. Participants were then instrumented and entered an environmental chamber ( $33.6 \pm 0.9$  °C,  $55.8 \pm 1.7$  % RH) to complete a 10-min seated rest period during which baseline physiological measures were taken. Participants then completed self-selected, volitional exercise (treadmill running or cycling) until a  $T_{\text{re}} \geq 39.0$  °C was reached. Exercise type, intensity (i.e. speed, grade, and resistance) and end  $T_{\text{re}}$  were recorded and repeated during the following trial such that the end of exercise metabolic heat production was similar between trials. This was accomplished by matching at least the last 10 min of exercise the previous trial. Water (warmed to estimated body temperature;  $\sim 38$  °C) was consumed ad libitum during exercise in the EU trial while water was withheld during the HY trial. During the exercise protocol  $T_{\text{re}}$ , skin temperature ( $T_{\text{sk}}$ ), and HR were continuously recorded.

### Immersion protocol

Immediately following exercise, participants removed their shoes, obtained a body mass after being towed dry, and were transferred to the CWI area inside the environmental chamber. Transfer time was standardized to 5 min to mimic transfer of an athlete off the field of play, as well as to allow proper time to instrument participants with a blood pressure cuff and forearm skin blood flux (SBF; via Laser Doppler) and collect pre-cooling measures. Once these measures were completed, a standard amount of 151.4 L of ice water ( $2.0 \pm 0.8$  °C) was poured into the cooling container and continuously oscillated around the participant resting in a semi-recumbent position. Water circulation was standardized by utilizing the same core researchers to move the water in a repeated fashion. As this was part of a larger validation study for a modified CWI technique, this quantity of water was sufficient to immerse participants between the iliac crest and xiphoid process. Participants

remained immersed until a  $T_{\text{re}}$  of  $\sim 38.1$  °C was reached ( $n = 9$ ), or a maximum of 15 min elapsed ( $n = 5$ ), at which time they were removed and monitored inside the environmental chamber. A maximum cooling time of 15 min was pre-determined using after-drop  $T_{\text{re}}$  values demonstrated in previous CWI protocols (Proulx et al. 2003, 2006) and adjusting the recommended safe-cooling limit (between 37.8 and 38.6 °C) due to the reduced surface area utilized with the immersion in the current study. Additionally, 15 min was used to prevent further participant discomfort. During cooling  $T_{\text{re}}$ ,  $T_{\text{sk}}$ , HR, and SBF were measured every 2 min while blood pressure was measured at pre-cooling and end of cooling. To minimize measurement error, water oscillation was paused while measurements were being attained. During recovery, maximal SBF values (later used to normalize SBF data) were obtained by locally heating the skin to 44 °C for 30 min or until a plateau was achieved (Bruning et al. 2012). Following recovery, participants provided a final urine sample to assess hydration status.

### Instrumentation

$T_{\text{re}}$  was measured via rectal thermocouple inserted to a depth of at least 15 cm past the anal sphincter (Physitemp Instruments Inc, Clifton, NJ). HR was measured by heart rate monitor (Polar Inc, Lake Success, NY) and arterial blood pressure was collected via auscultation of the left brachial artery using an automated sphygmomanometer (Tango+; SunTech Medical, Inc., Morrisville, NC, USA). Skin thermochrons (iButton, Maxim Integrated, San Jose, CA) were placed on the right anterior thigh, lateral calf, chest, and upper arm to assess  $T_{\text{sk}}$ . Laser Doppler flowmetry probes (Laser Doppler Perfusion Monitor and Probe 2b, Moor Instruments Ltd., UK) were used to assess the red blood cell flux on the dorsal forearm held in place by a local heater (Periflux System 5000, Perimed, Ardmore, PA, USA). The SBF values are expressed as a percentage of the maximum value collected at the end of the trials. The urine samples provided were assessed for  $U_{\text{osm}}$  (freezing point depression; Model 3250, Advanced Instruments Inc., Norwood, MA) and USG using a handheld refractometer (Master-SUR/NM, Atago, Japan).

### Data analysis

Data were sampled at 50 Hz and stored using computer software for further analysis (LabChart 7; AD Instruments, Colorado Springs, CO, USA).  $T_{\text{re}}$  during CWI was used to calculate cooling rates via the equation ( $\Delta T_{\text{re}}/\text{time}_{\text{cooling}}$ ). Blood pressure measurements were used to calculate mean arterial pressure (MAP) while accounting for changes in HR (i.e. changes in systolic/diastolic period) using the equation ( $\text{MAP} = \text{diastolic}$

**Table 1** Rectal temperature and heart rate measures at baseline and end of exercise

	EU		HY	
	Baseline	End of exercise	Baseline	End of exercise
Rectal temperature, °C	37.1 ± 0.4	39.3 ± 0.3 <sup>†</sup>	37.2 ± 0.3	39.3 ± 0.4 <sup>†</sup>
Heart rate, beats/min	69 ± 11	159 ± 15 <sup>†</sup>	79 ± 9*	166 ± 15 <sup>†</sup>

EU euhydrated, HY hypohydrated

<sup>†</sup> Significant difference from pre-exercise ( $P < 0.05$ )

\* Significant difference from EU ( $P < 0.05$ )

BP + fraction of systole × pulse pressure) where (fraction of systole =  $0.01 \exp(4.14 - 40.74/HR)$  (Moran et al. 1995). The  $T_{sk}$  are reported as mean-weighted  $T_{sk}$  using the equation  $[(0.3 \times T_{chest}) + (0.3 \times T_{arm}) + (0.2 \times T_{thigh}) + (0.2 \times T_{leg})]$  (Ramanathan 1964). SBF values were analyzed every 2 min during cooling and divided by the maximum SBF value attained at the end of each respective trial to be reported as a percent of maximum. Equipment malfunction limited the ability to record blood pressure throughout cooling, thus SBF was not able to be reported as cutaneous vascular conductance.

### Statistical analysis

Statistical analysis was conducted using SPSS v. 22.0 (IBM Corporation, Somers, NY). Dietary intake of kilocalories, protein, carbohydrate, fat, and sodium were analyzed via two-tailed, paired-samples  $t$  tests. Cooling rates between EU and HY trials were compared using a two-tailed, paired-samples  $t$  test. All other variables were compared by 2 (hydration state) × 4 (time) repeated-measures analysis of variance. Post-hoc analyses of significant main and interaction effects were conducted using proper Bonferroni corrections. A Pearson's product moment correlation was utilized to analyze the relationship between the body surface area/lean body mass ratio and cooling rate. An alpha of 0.05 was set a priori. With the exception of  $T_{re}$ , no statistical differences in gender were identified ( $P > 0.05$ ), thus all male and female data were pooled for analyses. All variables are reported as mean ± SD.

## Results

### Hydration measures

There were no differences in dietary intake of kilocalories ( $P = 0.16$ ), protein ( $P = 0.27$ ), carbohydrate ( $P = 0.79$ ), fat ( $P = 0.47$ ), or sodium ( $P = 0.32$ ) between trials. Urine parameters (USG [EU  $1.011 \pm 0.008$  vs HY  $1.025 \pm 0.004$ ],  $U_{osm}$  [EU  $461 \pm 288$  vs HY

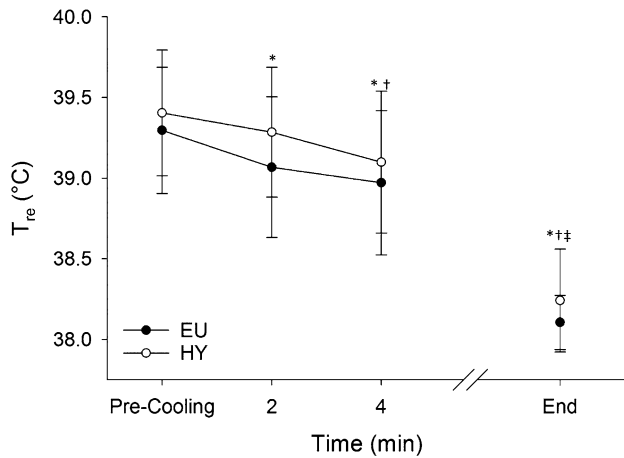
$988 \pm 116$  mOsm/kg], and  $U_{color}$  [EU  $3 \pm 2$  vs HY  $4 \pm 1$ ]) indicated that the dehydration protocol was successful as all HY values were above the standard cutoff criteria and significantly greater than in EU ( $P < 0.05$ ) (Armstrong et al. 2010). Post-trial urine parameters (USG [EU  $1.011 \pm 0.007$  vs HY  $1.024 \pm 0.003$ ],  $U_{osm}$  [EU  $419 \pm 225$  vs HY  $870 \pm 99$  mOsm/kg], and  $U_{color}$  [EU  $3 \pm 1$  vs HY  $5 \pm 1$ ]) also indicated that the EU trial maintained hydration status given standard cutoff criteria (Armstrong et al. 2010) and were significantly lower than the HY trial ( $P < 0.05$ ). Euhydration was maintained, as assessed via body mass changes ( $\Delta BM$ ), in EU trials ( $-0.66 \pm 0.44$  %) while the HY trial further dehydrated participants from pre-exercise ( $-1.65 \pm 1.23$  %) to pre-cooling ( $-2.66 \pm 1.23$  %,  $P < 0.001$ ).

### Exercise

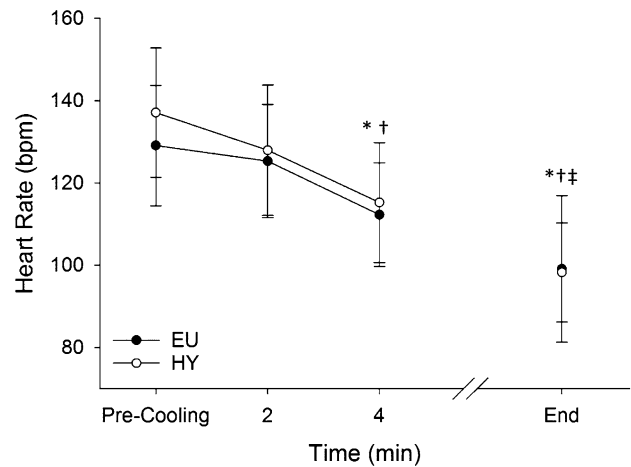
Baseline and end of exercise  $T_{re}$  and HR values are shown in Table 1. There were no differences in  $T_{re}$  between EU and HY ( $P = 0.19$ ) or an interaction between time and hydration ( $P = 0.12$ ), but there was a main effect over time ( $P < 0.001$ ) as end exercise  $T_{re}$  was increased above baseline. HR demonstrated a significant interaction between time and hydration ( $P = 0.03$ ) with baseline HR significantly higher in HY vs EU ( $P = 0.003$ ).

### Cold-water immersion

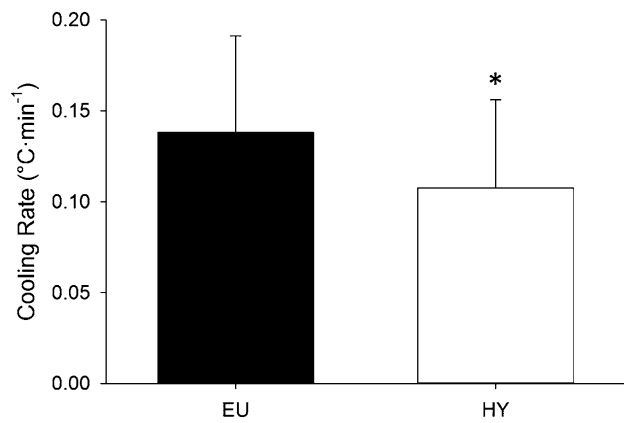
The interaction of gender and time ( $P = 0.001$ ) for  $T_{re}$  was a consequence of female participants reaching volitional fatigue before male participants, thus female  $T_{re}$  were significantly lower only at pre-cooling (male  $39.53 \pm 0.41$  °C, female  $39.12 \pm 0.18$  °C,  $P = 0.02$ ). When gender  $T_{re}$  data were combined there was a main effect of time ( $P < 0.001$ ) but no main effect of hydration status ( $P = 0.17$ ) or interaction of time and hydration ( $P = 0.34$ , Fig. 1). Mean cooling rates utilizing the change in  $T_{re}$  over the duration of each participant's cooling time are presented in Fig. 2 ( $P = 0.046$ ). There was only a tendency for differences in cooling rates to reach the first 0.5 °C drop (EU,  $0.14$  °C min<sup>-1</sup>, vs HY  $0.09$  °C min<sup>-1</sup>,  $P = 0.06$ ) and first



**Fig. 1** Rectal temperature ( $T_{re}$ ) responses to cold-water immersion following exercise in euhydrated (EU, closed circles) and hypohydrated (HY, open circles) conditions. \*Significant difference from pre-cooling ( $P < 0.05$ ). †Significant difference from 2 min ( $P < 0.05$ ). ‡Significant difference from 4 min ( $P < 0.05$ )



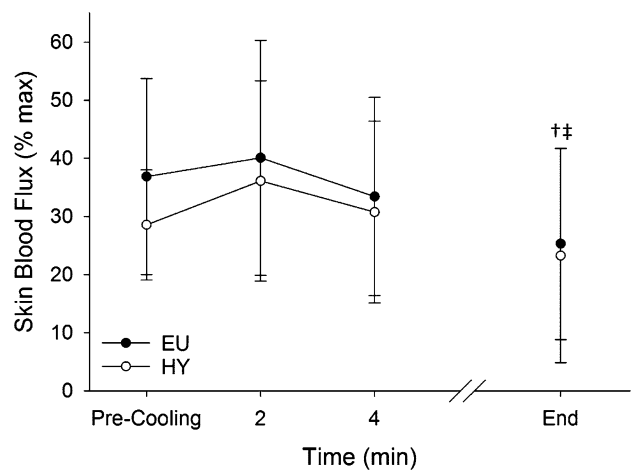
**Fig. 3** Heart rate responses in euhydrated (EU, closed circles) and hypohydrated (HY, open circles) conditions during cold-water immersion following exercise. \*Significant difference from pre-cooling ( $P < 0.05$ ). †Significant difference from 2 min ( $P < 0.05$ ). ‡Significant difference from 4 min ( $P < 0.05$ )



**Fig. 2** Cooling rate during cold-water immersion in euhydrated (EU, dark bar) and hypohydrated (HY, light bar) conditions. \*Significant difference from EU ( $P = 0.046$ )

1  $^{\circ}\text{C}$  drop (EU, 0.14  $^{\circ}\text{C}\cdot\text{min}^{-1}$ , vs HY 0.10  $^{\circ}\text{C}\cdot\text{min}^{-1}$ ,  $P = 0.06$ ) in  $T_{re}$ . Interestingly, there was no difference in mean cooling time between EU (9.6  $\pm$  3.6 min) and HY (11.8  $\pm$  3.4 min,  $P = 0.09$ ). Further, there was a non-significant correlation between EU cooling rate and BSA/LBM ratio ( $r = 0.14$ ,  $P = 0.65$ ).

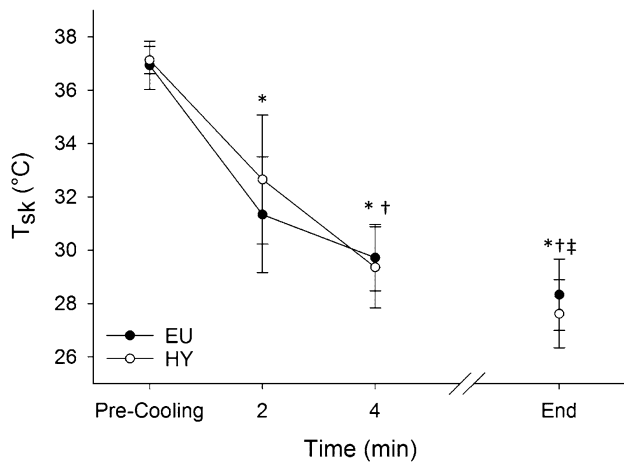
HR decreased significantly over time ( $P < 0.001$ ), however, there was not a main effect of hydration status ( $P = 0.10$ ) nor an interaction ( $P = 0.07$ , Fig. 3). Due to equipment error, MAP measurements were recorded for 11 participants for pre—(EU 84.2  $\pm$  13.5, vs HY 82.7  $\pm$  10.2 mmHg) and end (EU 90.1  $\pm$  6.0, vs HY 91.6  $\pm$  15.9 mmHg) cooling measures with no effect of



**Fig. 4** Skin blood flux responses in euhydrated (EU, closed circles) and hypohydrated (HY, open circles) conditions during cold-water immersion following exercise. †Significant difference from 2 min ( $P < 0.05$ ). ‡Significant difference from 4 min ( $P < 0.05$ )

time ( $P = 0.052$ ), hydration status ( $P = 0.88$ ) or interaction ( $P = 0.46$ ).

SBF was also reduced over time ( $P < 0.001$ ), regardless of hydration status ( $P = 0.35$ ) with no interaction ( $P = 0.21$ , Fig. 4). Mean-weighted  $T_{sk}$  decreased over time ( $P < 0.001$ ) independent of hydration status ( $P = 0.77$ , Fig. 5). The chest  $T_{sk}$  was significantly reduced over time ( $P < 0.001$ ) as well, however, there was no effect of hydration status ( $P = 0.24$ ) nor interaction ( $P = 0.41$ ). Similarly, thigh  $T_{sk}$  was decreased over time ( $P < 0.001$ ) independent of hydration status ( $P = 0.93$ ).



**Fig. 5** Mean weighted skin temperature ( $T_{sk}$ ) during cold-water immersion in euhydrated (EU, closed circles) and hypohydrated (HY, open circles) conditions following exercise. \*Significant difference from pre-cooling ( $P < 0.05$ ). †Significant difference from 2 min ( $P < 0.05$ ). ‡Significant difference from 4 min ( $P < 0.05$ )

## Discussion

The primary finding in this study was that mild hypohydration attenuates the rate of cooling in exertionally hyperthermic individuals. As hypothesized however, CWI elicited acceptable cooling rates ( $>0.1$  °C  $\text{min}^{-1}$ ) in both hydration states (EU,  $0.14$  vs HY,  $0.11$  °C  $\text{min}^{-1}$ ) (Casa et al. 2007; Zhang et al. 2015). Thus, the utilization of CWI to treat exertional hyperthermia is recommended, regardless of hydration state. A previous study demonstrated a cooling rate of  $0.16$  °C  $\text{min}^{-1}$  during ice water immersion and CWI to neck level, despite hypohydration of  $-3.89$  and  $-3.61$  % for body mass change, respectively (Clements et al. 2002). The participants, however, began the protocol EU and progressively dehydrated throughout the exercise trials (Clements et al. 2002). Furthermore, the authors did not include a EU trial, thus limiting the ability to determine the effects of the progressive dehydration on the cooling efficacy. It should be noted that the level of hypohydration in the present study ( $-2.66$  %) was less than that of Clements et al. (2002), thus a greater degree of exercise dehydration may have allowed for further divergence of cooling rates.

There are several physiological and physical factors that alter the cooling effectiveness of CWI. Water temperature (Proulx et al. 2003, 2006), depth of immersion (Zhang et al. 2015), body surface area and lean body mass (Friesen et al. 2014) all impact cooling rates, however, this is the first study, to our knowledge, to compare EU and HY states during CWI. There may have been several mechanisms attributed to the decrease in cooling rate with hypohydration that occurred. When immersed in ice water, cold-induced cutaneous vasoconstriction reduces the demand for blood

flow to the skin (Mawhinney et al. 2013). Thus, a thermal gradient between the water and skin relies upon the ability to deliver blood flow despite a vasoconstrictor drive. As demonstrated in the present study, however, SBF was maintained similarly between hydration states (Fig. 4), thus the delivery of cutaneous blood flow is not likely responsible for alterations in cooling rate between conditions. This is further supported as there was not a difference in mean  $T_{sk}$  between hydration statuses (Fig. 5). Interestingly, there was an equally marked reduction with both hydration states in the chest  $T_{sk}$  despite no water coverage, suggesting an autonomic drive following feedback from the immersed area (Taylor et al. 2014).

Although not measured in the present study, hypohydration has been shown to decrease plasma volume, which would then decrease blood volume (González-Alonso et al. 1995, 1997, 2000). Zappe et al. (1993) demonstrated a 1.8 % decrease in body mass along with a plasma volume loss of 353 mL ( $\sim 8$  %) after completing a 24 h fluid restriction similar to the present protocol. This reduction may have altered the heat carrying capacity and thus the ability to remove heat from the skeletal muscle. It is also possible that disparities occurring in skeletal muscle microvascular blood flow with exercise (Heinonen et al. 2012; Koga et al. 2014) may persist into recovery, resulting in a mismatch between heat removal and production. The discrepancies may have been amplified by our dehydration protocol, decreasing blood flow through the microvasculature of the muscle despite marked heat production. González-Alonso et al. (1998) demonstrated reduced muscle blood flow with progressive dehydration during prolonged exercise in the heat. As CWI has also been shown to decrease muscle blood flow following exercise (Mawhinney et al. 2013), the dehydration protocol may have created a cumulative effect, enhancing muscle blood flow reductions from exercise through immersion. Thus, our hypohydration altered the ability for heat dissipation as compared to the EU state, potentially through decreases in the perfusion of the muscle tissue and reduced heat carrying capacity. This is a limited assumption, however, due to the lack of arterial blood pressure throughout the CWI procedure and no differences at the end of cooling between hydration states. Future investigations with serial blood pressures and leg blood flow measurements may elucidate this speculation.

The cardiovascular responses elicited by CWI in this study regardless of hydration state may implicate the use of CWI from exercise routines to improve recovery. It is well documented that post-exercise hypohydration attenuates MAP (Gagnon et al. 2012), HR (Charkoudian et al. 2003), baroreflex sensitivity (Charkoudian et al. 2003), and neuromuscular control (Distefano et al. 2013). The use of CWI may diminish these negative impacts following exertional hyperthermia. Immersion in thermoneutral water leads to

shifts in body fluids to increase plasma volume (Stachenfeld et al. 1997; Wada et al. 1995). Deeper levels of immersion subsequently lead to further expansions in cardiac filling and thus increased stroke volume (Gabrielsen et al. 1993). Similarly, when immersed while HY, plasma volume increases cardiac filling pressure and subsequently increases stroke volume (Stachenfeld et al. 1997). These augmentations are vital to the maintenance of cardiac output and improvements in blood pressure regulation. In the present study, this was observed immediately after cooling began, by reductions in HR (Fig. 3), as well as initial increases in SBF (Fig. 4) followed by vasoconstriction. Furthermore, increased MAP at the end of cooling indicated an improvement from the pre-cooling regardless of hydration state. These findings are further supported in the literature as CWI has been shown to elicit improvements in stroke volume, HR, and MAP (Wilcock et al. 2006).

A potential limitation to this study was the pooling of gender data in the analysis. Gender responses to CWI have been demonstrated to differ, albeit with conflicting reports. In a controlled laboratory setting, females cooled at faster rates than males following exertional hyperthermia (Lemire et al. 2009). This study only matched females and males based on body surface area to total body mass without controlling for differences in lean body mass (Lemire et al. 2009). However, a greater body surface area-to-lean body mass ratio elicits significantly faster cooling rates compared to the lower ratio counterpart (Friesen et al. 2014). Thus, anthropometric differences in cooling are driven by the body surface area-to-lean body mass ratio. Therefore, the faster cooling rates shown by Lemire et al. (2009) were likely the result of females having a lower lean body mass content, as opposed to the effect of gender itself. This is further supported by a recent study of 18 years of medical patient tent records that demonstrated there were not differences in cooling rate between males and females with EHS (Demartini et al. 2015). Additionally, since this was a repeated-measures design, issues with combining of male and female data were ameliorated as every participant was compared to their own responses. Our immersion protocol also included half the torso as well as lower extremities, likely explaining the discrepancies between the cooling rates of this and prior investigations using whole body or entire torso immersion.

Another potential limitation is the use of volitional exercise to elevate  $T_{re}$ , as this resulted in a high variation in  $T_{re}$  during cooling. The primary variable of concern in this study, however, was cooling rate, which is calculated using the change in  $T_{re}$  over time. Thus, the robustness of cooling rates reduce the concern for variability between participants regarding  $T_{re}$  since each individual was also compared to their own responses. Furthermore, the purpose

of the exercise in this study was to induce exertional hyperthermia to the level each individual would feel comfortable (i.e. potentially similar to what athletes would experience in practice settings when exercising to volitional exhaustion). Thus, we decided it was not practical to set a specific goal temperature for each individual to reach. Additionally, it has been shown that the cooling rates in exertional heat illness victims are not determined by starting  $T_{re}$  (Demartini et al. 2015). Interestingly, Flouris et al. (2014) determined that exertionally hyperthermic individuals starting at 39.5 °C cooled faster during the 39–38.5 °C portion of CWI than the 39.5–39.0 °C range. While this is not a direct conflict of findings, the non-linear response of cooling rates should be evaluated as there may be a saturation threshold for cooling rate and starting core temperature. Further, the difference between laboratory and field settings should be investigated in future research.

The use of esophageal temperature may have provided a better temporal pattern for temperature responses with CWI (Gagnon et al. 2010). It is well demonstrated that esophageal temperature provides a better indicator of core temperature than  $T_{re}$  and is quicker to respond during both exercise and recovery (Gagnon et al. 2010). However, the utilization of  $T_{re}$  in diagnosing exertional heat illnesses is a valid and reliable measure commonly used by practicing clinicians such as athletic trainers (Gagnon et al. 2010; Casa et al. 2012, 2015). Thus demonstrating the  $T_{re}$  responses in this protocol provides value to those clinical professionals operating in field settings. Furthermore, each participant was compared to their own  $T_{re}$  response, thus the temporal pattern of the thermometry utilized should have been matched between hydration states.

A lack of reliable blood pressure readings throughout the cooling procedure also provided a major limitation as this affected the ability to clarify potential mechanisms regarding differences in cooling rates. Further, this places a restraint on the utilization of skin blood flux in determining changes in cutaneous vascular conductance. However, as blood pressure was not significantly different at the end of cooling as compared to the precooling time point, it is likely that the cutaneous perfusion pressures were not different between hydration states. Additionally, it has been shown that skin blood flow is decreased with CWI despite increases in MAP, thus the vasoconstrictor response in the present study is consistent with findings in the literature (Mawhinney et al. 2013). As such, it cannot be confirmed that there were any changes in cutaneous perfusion leading to alterations in skin blood flow, thus the skin red blood cell flux values presented should be interpreted with caution.

A final limitation was the lack of randomization in trial order. As previously described, the purpose of the exercise utilized was to induce exertional hyperthermia, however,

it has been demonstrated previously that hypohydration elicits greater increases in  $T_{re}$  than in a euhydrated state. Thus, to ensure that individuals would be able to reach the same starting temperature using volitional exercise, the EU trial was conducted first. While this strategy was successful and allowed for the matching of starting temperatures, there may have an effect on cooling rates due to habituation in CWI (Tipton et al. 2013). It is possible that the adaptations in some individuals lasted slightly longer, thus the slower cooling rates elicited during HY trials may have been caused by a slight habituation to CWI. Early habituation to CWI is highlighted by decreases in the initial cold shock responses such as altered HR and reduced respiratory responses (i.e. breathing frequency and tidal volume) (Tipton et al. 2000). However, Tipton et al. (2000) demonstrated that habituation took place with repeated immersions occurring between 1 and 5 days apart. As all trials were separated by at least 7 days in the current study, this should have created a wash-out period and ameliorated any adaptations. Furthermore, the traditional HR adaptation to CWI is a dampening in the tachycardia response to the cold shock (Tipton et al. 2000; Eglin and Tipton 2005; Tipton et al. 2013). In the present study, however, CWI elicited reductions in HR regardless of hydration status rather than tachycardia. Thus, the lack of differences in HR,  $T_{sk}$ , and SBF between hydration statuses during CWI demonstrates that habituation to the cold shock response with CWI had not occurred.

## Conclusions

This study demonstrated that following exertional hyperthermia, cooling rates were modestly attenuated when participants were in a HY vs EU state. The alteration in hydration state reduced the capacity to remove heat produced during volitional exercise when enduring CWI. However, the cooling rates elicited in both hydration states still provided adequate reductions in core temperature. Clinicians should be aware that each individual cooling rate may be altered as hydration state changes, however, the primary goal in treating exertional hyperthermia should be reducing core temperature as quickly as possible, ideally covering the greatest amount of body surface area in cold water. Furthermore, the use of CWI elicited marked cardiovascular benefits as a potential recovery technique in both EU and HY states. Thus, regardless of hydration status, the cooling efficacy of CWI was preserved and should continue to be utilized in the treatment of exertional hyperthermia.

### Compliance with ethical standards

**Conflict of interest** None.

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